RECENT TRENDS IN THE BIOCHEMISTRY OF THE STEROID HORMONES

SEYMOUR LIEBERMAN AND SYLVIA TEICH

College of Physicians and Surgeons, Columbia University
Departments of Obstetrics and Gynecology and of Biochemistry
New York, N. Y.

TABLE OF CONTENTS

I. INTRODUCTION

A quarter of a century has elapsed since the first steroid hormone was isolated and characterized. Since then, the body of knowledge concerning the influence of these endocrine secretions on almost every aspect of biochemistry, physiology, and medicine has grown enormously. Paralleling this growth has been the rapid expansion of the area concerned with the metabolism of the steroid hormones themselves. It is with this phase of biochemistry that this review shall deal; it will discuss recent developments concerned with the biosynthesis of the steroid hormones, what little is known of their mode of action and their catabolic fate. In view of the significant role that methods have played in the recent progress in these fields, attention has been directed towards the advantages and limitations of the techniques currently employed for the isolation and characterization of the steroid hormones and their metabolites. In general, advances through 1952 as well as isolated reports in 1953 have been included.

The first steroid hormone to be isolated in pure form was estrone. This feat, accomplished in 1929, initiated such active interest in the steroid hormones that within a period of about seven years the three types of sex hormones, estrogens, androgens and progestins, were isolated, identified, synthesized and made available to the biologist and clinician. The structure of estrone, first isolated from the urine of pregnant women, was elucidated in 1932. The principal urinary androgen, androsterone, was obtained as early as 1931 and the androgenic hormone, testosterone, was isolated from testicular extracts in 1935. Progesterone was not isolated from the corpus luteum until 1934, although its main urinary metabolite, pregnane-3α,20α-diol, had been obtained from pregnancy urine five years previously.

In Figure 1 are shown the steroid perhydrocyclopenta[a]phenanthrene nucleus with the accepted system of numbering the carbon atoms and the formulae of three steroid hormones.

Estradiol-17β and the other natural estrogens, estrone and estriol, are phenolic,

possessing an aromatic ring A. Estradiol has a β-hydroxyl group at C_{17} (cis to the methyl group at C_{13}) and estrone has a carbonyl group at this position. The steroid nucleus is considered to be planar and, therefore, substituent groups may exist either above the plane of the molecule in which case they are designated β, or below the plane, in which case they are α. (For a detailed discussion of steroid nomenclature, see references 153, 489.) Testosterone (systematic name: 17β-hydroxy-4-androsten-3-one) and progesterone (systematic name: 4-pregnene-3,20-dione) both have the characteristic α,β-unsaturated carbonyl group in ring A. Testosterone has a β-oriented hydroxyl group at C_{17} whereas progesterone has a CH_3CO-group at this position. Investigations into the chemical nature of the active principles of the adrenal cortex were begun in 1935 and, in a short time,

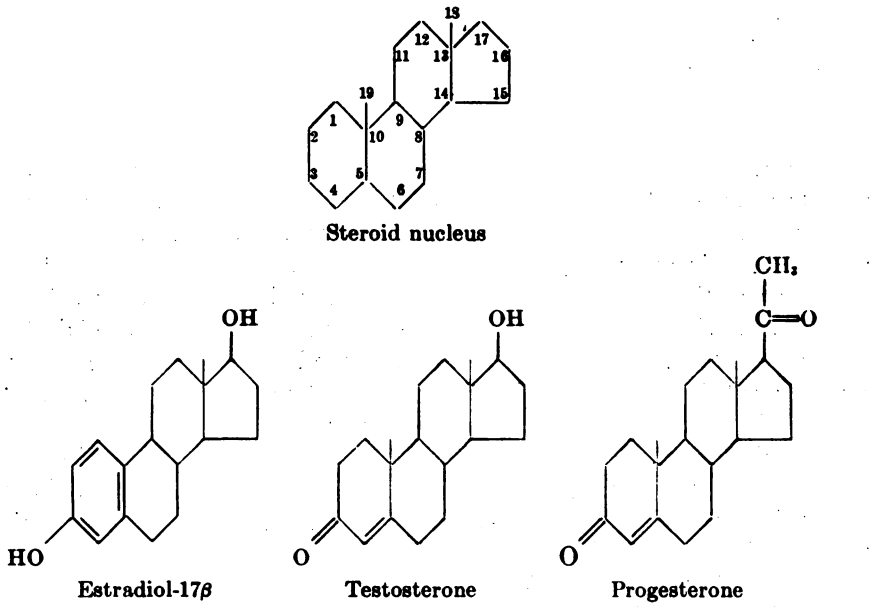


FIG. 1

led to the characterization of 28 crystalline compounds. The formulae of the six components that were found to be active in prolonging the life of adrenalectomized animals are shown in Figure 2. They all have in common the α,β-unsaturated carbonyl group in ring A and each possesses a two-carbon side chain at C_{17}. They differ from each other by the number and nature of the oxygen functions that this side chain and C_{11} bear. Desoxycorticosterone (DOC) has a side chain having a ketone at C_{20} and a primary hydroxyl group at C_{21}, a so-called "α-ketol". Corticosterone (Compound B) differs from DOC by having, in addition to these structural features, a characteristic β-oriented hydroxyl group at C_{11}. Compound A differs from Compound B by the presence of a ketone group instead of an hydroxyl group at C_{11}.

The three compounds on the bottom line of Figure 2 are alike in that they each

possess a third oxygen atom in the side chain, *i.e.*, a tertiary α-oriented hydroxyl group at C_{17}, resulting in the dihydroxyacetone side chain. Compound S, like DOC, has no C_{11} oxygen atom but differs from DOC by having the additional tertiary hydroxyl group at C_{17}. Hydrocortisone (Compound F, systematic name: 11β,17α,21-trihydroxy-4-pregnene-3,20-dione) has the dihydroxyacetone side chain and, in addition, has the 11β-hydroxyl group. Cortisone (Compound E) has, instead, a carbonyl group at C_{11}.

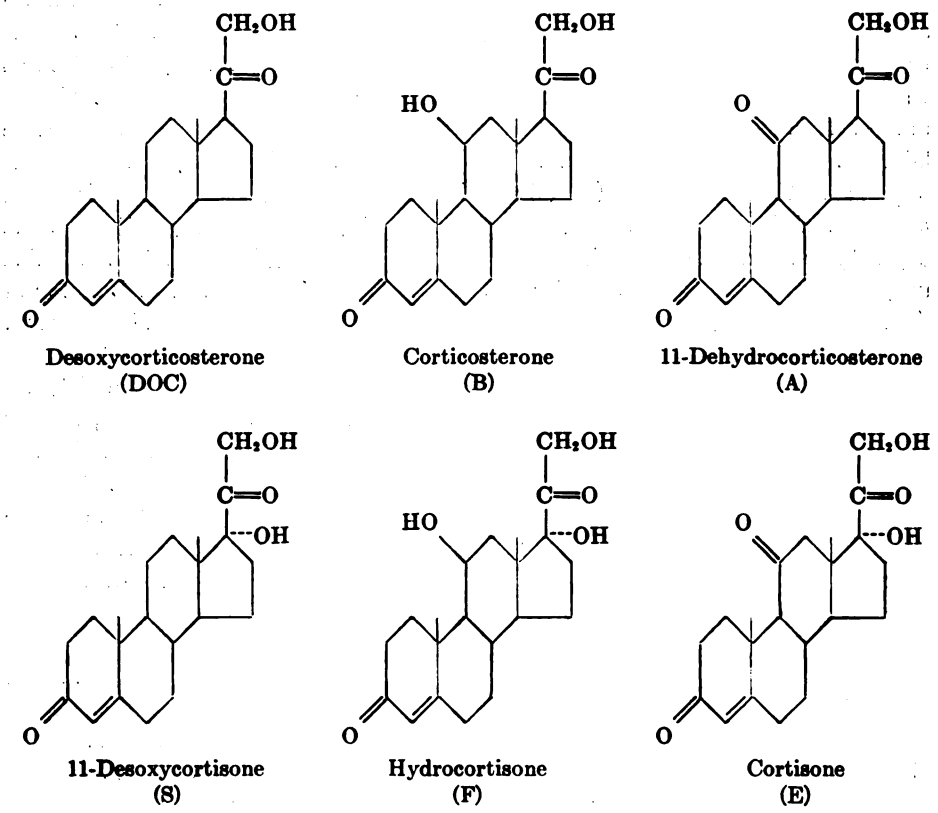


FIG. 2

The rapid progress made in this field following the isolation and structural determination of the hormones was due, to a very large extent, to the spectacular success of organic chemists of many lands in developing chemical procedures for the inexpensive commercial production of these compounds. Between the years 1935 and 1938, synthetic processes made available relatively large quantities of testosterone, progesterone, and desoxycorticosterone, using cholesterol or stigmasterol as starting materials. In recent years, improvements in procedure and the availability of a new cheap source material, diosgenin, a plant sapogenin, has made not only these hormones more abundant but also has made available many related steroids of biochemical, physiological and pharmacological interest.

Estrone, from which estradiol can easily be prepared, was isolated in large quantities from stallion urine, which served as its source for many years. For the last four years, the estrogenic hormones have also been synthesized from cholesterol, an improvement which has contributed to the more than hundredfold decrease in their cost since 1938. Desoxycholic acid obtained from bile was the first and still remains the principal starting material for the commercial production of cortisone. Newer synthetic methods have been developed which permit the commercially feasible production of the adrenocortical hormones from more abundant steroids such as cholesterol, ergosterol and diosgenin. All these synthetic processes involve at some stage the introduction of an oxygen function at C_{11}, a structural feature which characterizes some of the important adrenal hormones. This synthetically difficult process is now being accomplished in some laboratories by means of microbiological hydroxylation. Various groups of microorganisms, *Aspergillus niger* (164, 390) of the *Actinomyces*, *Rhizopus arrhizus* and *nigricans* of the *Mucorales* (149, 150, 332, 341, 342, 391, 392, 393, 394) have the capacity to introduce an α-oriented hydroxyl group at C_{11} in a variety of steroids in good yields. *Streptomyces fradiae* has been shown (94) to transform Compound S to Compound F, a conversion involving the introduction of an 11β-hydroxyl group, in yields of 1.4–5.8% as determined by glycogen deposition tests.

Finally, the ultimate in accomplishment, at least to the organic chemist, has been the total synthesis of steroids and steroid hormones from simple, readily available organic compounds. The estrogens were the first group of steroid hormones to be synthesized in this manner. Although Bachmann and his students had prepared equilenin in 1939 and later, in 1942 (11) synthesized estrone A, one of the fifteen unnatural stereoisomers of the natural hormone, it was not until 1948 that the synthesis of natural estrone was accomplished by Anner and Miescher (7). Johnson and his students, in 1951 (269), described a new total synthesis of estrone starting with anisole.

Although the non-aromatic steroids are more difficult to construct from small molecules they, too, have been synthesized in recent years. These successes, together with those that resulted in the practical transformation of the more abundant steroids into the hormones themselves, constitute one of the brightest chapters in the recent history of synthetic organic chemistry.

The ingenious synthesis of Woodward and his colleagues (553, 554), starting with a C_7 compound and involving more than twenty steps, resulted in the formation of an intermediate, steroidal in nature, from which it was possible to proceed, using known reactions, to either cholesterol, testosterone, desoxycorticosterone, cortisone or other naturally-occurring steroids. Employing another complex process, Robinson and his co-workers (73) succeeded in preparing isoandrosterone (3β-hydroxyandrostan-17-one). A different approach to the total synthesis of isoandrosterone, starting with 5-methoxy-2-tetralone, has recently been reported by Johnson *et al.* (268). Billeter and Miescher (24) and Sarett *et al.* (449) have also made important contributions to the problem of total synthesis. The latter group has accomplished the impressive task of synthesizing cortisone, Compound A and 11-keto-progesterone from simple organic molecules.

The success of the organic chemist in the synthesis and transformation of the steroid hormones has resulted, too, in the availability of isotopically labeled compounds for the study of steroid metabolism. Initially, interest centered about the synthesis of deuterated hormones and resulted in the preparation of testosterone-11,12-d_2 (292), progesterone-11,12-d_2 (292), 17α-hydroxyprogesterone-11,12-d_2 (294), Compound S-11,12-d_2 (175), etiocholanolone-11,12-d_2 (175), estrone-6,7-d_2-acetate (383), as well as Compound L (294), estrone (174), androsterone, androstanedione, Δ^4-androstenedione (175), all containing deuterium in chemically stable positions. The platinum-catalyzed exchange reaction, first applied by Bloch and Rittenberg (38) to the incorporation of deuterium into cholesterol, has been adapted to the incorporation of tritium into steroids (138). Fukushima *et al.* (172) have reported the preparation of 3α-hydroxypregnane-11,20-dione and cortisone labeled with tritium in chemically stable positions. Syntheses involving the introduction of radio-carbon into steroids have resulted in the preparation of testosterone-3-C^{14} (513), testosterone-4-C^{14} (167, 224), 17α-ethinyltestosterone with C^{14} at C_{20} or C_{21} (422), 17α-methyl-C^{14}-testosterone (328), progesterone-3-C^{14} (223), progesterone-4-C^{14} (223), progesterone-21-C^{14} (328, 423), desoxycorticosterone-3-C^{14} (223), 17α-methyl-C^{14}-5-androstene-3β,17β-diol (141), 11-dehydrocorticosterone-21-C^{14} (252), estrone-16-C^{14} (221), 17α-methyl-C^{14}-estradiol-17β (362), and 17β-estradiol-16-C^{14} (130, 311).

The usefulness of these labeled steroids for the elucidation of precursor-product relationships in the biosynthesis of the hormones as well as for obtaining information concerning their endogenous production and tissue localization is apparent. Their application to the study of steroid catabolism, preliminary as these investigations may at present be, has already yielded significant results concerning the qualitative and quantitative aspects of steroid transformations and the routes of excretion of these steroid metabolites. It seems likely, too, that investigations with labeled steroids will contribute significantly to solving the problem of what happens to the major portion of exogenously administered hormones and may shed light on the alternate catabolic pathways that steroid hormones traverse.

II. BIOSYNTHESIS OF CHOLESTEROL AND THE STEROID HORMONES

Cholesterol

For the past 20 years, ever since the chemical structure of cholesterol was definitely established, the nature of the biogenesis of this compound, so widespread in the animal kingdom, has been the subject of extensive investigation. It is pertinent to discuss these researches in some detail here, for the biosynthesis of cholesterol appears to be closely related to that of the steroid hormones. Indeed, cholesterol has been shown to be a precursor of the bile acids, pregnanediol, as well as Compound F. It has been clearly demonstrated that cholesterol can be synthesized by a variety of tissues from small molecules, but the nature of the intermediates of higher molecular weight is still a matter of much speculation.

1. Rates and sites of synthesis. The rates of synthesis of cholesterol in animals have been studied and the half-life has been found to be 6 days in adult rat liver

and 31–32 days in the carcass (396); and in man the half-life of serum cholesterol was found to be 8 days and the turnover time 12 days (322). The greatest turnover of cholesterol occurred in the adrenals and lungs; liver and kidney followed in that order (4). The liver is believed to be the primary source of plasma cholesterol (166, 183).

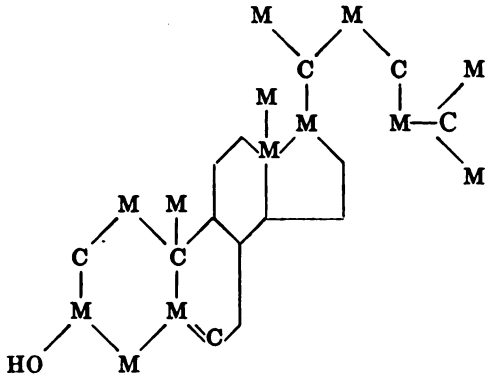
Until recently, all *in vitro* incorporation experiments involving the synthesis of cholesterol from acetate were carried out with intact cells in surviving tissue (particularly liver) slices. This transformation has now been demonstrated with homogenates of fetal rat liver (413). With homogenates of adult rat liver in a phosphate buffer containing nicotinamide, magnesium ions, ATP and DPN, Bucher and co-workers (56, 57) obtained incorporation of isotopically labeled acetate into cholesterol equivalent to that obtained with slices. In addition, the successful fractionation of rat liver homogenate and the preparation of a water-soluble enzyme system capable of incorporating C^{14}-acetate into cholesterol have recently been reported by Rabinowitz and Gurin (414). Such cell-free systems which can synthesize cholesterol may provide the means for studying the more intimate details of the mechanism of this synthesis.

Until 1948, only liver had been demonstrated to effect the transformation of acetate to cholesterol (35). However, since a hepatectomized rat can also synthesize cholesterol (484), extrahepatic sites were sought. Adrenal cortical tissue, kidney, testes, small intestine, skin, brain of a new-born, but not adult, rat (485), and aorta (474) were found to convert acetate to cholesterol *in vitro*. Since the radioactivity of the cholesterol isolated from small intestine, stomach, ovary and mammary gland in *in vivo* experiments after feeding radioactive acetate was higher than that isolated from the liver, it was concluded that these tissues, too, represent sites of synthesis (407, 408). In such *in vivo* experiments, it has been pointed out by Rittenberg and Price (425), cognizance should be taken of the fact that metabolically "active" cholesterol of high isotope concentration could be synthesized in the liver and removed from this site of synthesis before equilibrium with a metabolically "inactive" hepatic cholesterol pool was established. If the liver contained more "inactive" cholesterol than some other organ, *e.g.*, ovary, the isotope concentration of the cholesterol isolated from that organ might then be higher than that of the liver, despite the fact that the labeled cholesterol isolated from this extrahepatic tissue was synthesized in the liver.

2. Synthesis from small molecules. The conclusion that the biosynthesis of cholesterol must involve the coupling of a large number of small molecules was drawn by Rittenberg and Schoenheimer (426) from early experiments involving the incorporation of the deuterium of heavy water into cholesterol. The demonstration by Sonderhoff and Thomas (481) that isotopically labeled acetate was utilized directly for the synthesis of yeast sterols lent support to this thesis. That animal tissues can similarly incorporate acetate was subsequently proved by Bloch and Rittenberg (36, 37, 39). Both the methyl group and the carboxyl group of acetate can be utilized as a carbon source for the isooctyl side chain and for the perhydrocyclopentaphenanthrene nucleus of the cholesterol molecule (32, 321). From the results of experiments using $C^{14}H_3C^{13}OOH$, Little and Bloch (321)

determined that the ratio of C^{14}/C^{13} in the cholesterol isolated was 1.27, a value confirmed by Brady and Gurin (52). Assuming that all of the 27 carbon atoms of cholesterol are derived from acetate, it was calculated that 15 of the carbon atoms have their origin in methyl groups of acetate and 12 in carboxyl groups of acetate. The relative isotope concentration of methyl carbons of acetate to carboxyl carbons of acetate in the nuclear portion of cholesterol was found to be 1.08 corresponding to a ratio of 10 to 9, while that in the isooctyl side chain was 1.47 or 5 to 3 (321). It appears then that in the course of cholesterol synthesis 15 molecules of acetate condense and that 3 carboxyl groups are lost by decarboxylation.

That acetate could be the principal, if not the sole, precursor of a sterol was demonstrated in experiments employing a mutant strain of *Neurospora crassa* (32, 371), which cannot metabolize glucose to acetate but which requires acetate for growth. When such a mutant was grown on a medium containing doubly-



M: Acetate methyl
C: Acetate carboxyl

FIG. 3. Distribution of acetate carbons in cholesterol (103, 552,555).

labeled acetate, the isotope concentration of the isolated ergosterol was identical with that of the acetate introduced since there was no dilution from endogenous acetate. A similar conclusion that ergosterol can be synthesized solely from acetate as the carbon source was derived from experiments with *Saccharomyces cerevisiae* (201). Such direct evidence for the role of acetate in animals is, of course, lacking. In order to elucidate the extent to which acetate serves as the precursor of each carbon atom of cholesterol and also to provide clues as to possible intermediates and their mode of incorporation into cholesterol, elaborate experiments were undertaken by Bloch and his co-workers (321, 555) to determine the isotopic activity of various carbon atoms of cholesterol. Chemical degradation of the cholesterol, biosynthetically produced from doubly-labeled acetate, led to the conclusion that every carbon atom in the isooctyl side chain, in addition to the two angular methyl groups, came from acetate; C_{18}, C_{19}, C_{21}, C_{22}, C_{24}, C_{26}, C_{27} and probably C_{17} having come from the methyl group, and C_{20}, C_{23}, C_{25} and probably C_{10} and C_{13} having come from carboxyl carbon of acetate. Since almost every

carbon atom analyzed had an activity equal to that which would be predicted if acetate were the sole precursor, this series of experiments seemed to confirm the role of acetate as a major precursor. In a recent preliminary report concerning the partial degradation of cholesterol, biosynthesized from carboxyl- and methyl-labeled acetate, Cornforth *et al.* (103) inferred from their data that carbon atoms 2, 4, 6 and 10 are derived from the carboxyl carbon, while carbon atoms 1, 3, 5 and 18 are derived from the methyl carbon of acetate. Also, a recent note by Woodward and Bloch (552) reported that C_{18} has its origin in the methyl group and not, as previous reports indicated, in the carboxyl group of acetate. Preliminary degradation studies of ergosterol, synthesized by "Co-A" rich yeast from 1-C^{14}-acetic acid showed that C_{23} and C_{25} in this plant sterol likewise appeared to be derived from the carboxyl group of acetate (200).

A large number of small molecules have been tested as carbon sources for cholesterol biosynthesis, with a view towards finding precursors other than acetate some of which may be further along the biosynthetic route (32, 33, 193). In fact, the ability of compounds such as ethanol (35, 107, 426), butyrate (51, 426), valerate (426), hexanoate (51), octanoate (51), acetaldehyde (35, 52), acetone (44, 52, 410) and pyruvate (51, 426) to participate in cholesterol synthesis was comparable to their ability to form "active acetate" and this probably represents the route by which they are incorporated into cholesterol (32). On the other hand, acetoacetate is incorporated into cholesterol without prior degradation to acetate (52, 106). Butyrate, isovalerate and leucine were found to be converted to cholesterol more efficiently than was acetate (426), although all three compounds are known to be metabolized by way of two-carbon units (30, 98, 556, 557). A study using doubly-labeled isovalerate (557, 559) indicated that the terminal isopropyl moiety of this compound gave rise to a C_3-unit, leaving a residual C_2-fragment. The C_3-unit was five times more active than acetate as a precursor for cholesterol, whereas the C_2-unit was comparable to acetate. Since the terminal methyl groups of the isovalerate were incorporated to the same extent into the side chain as into the nucleus of cholesterol, it did not appear that the isopropyl group was utilized as a precursor for any particular portion of the molecule. To account for the greater efficiency with which isovalerate was incorporated into cholesterol, the suggestion has been made by Zabin and Bloch (559) that the isopropyl moiety was converted directly into a precursor which also was formed from acetate. This precursor was diluted less with endogenous material than was acetate, thus accounting for the more efficient utilization of isovalerate carbon. Acetoacetate may well be this common precursor, for the terminal methyl groups of isovalerate have been found in carbons 2 and 4 of acetoacetate (557). Carbon dioxide fixation by the isopropyl group of isovalerate (556) and of leucine (97, 98) to yield acetoacetate has been demonstrated and more recently the stoichiometric nature of this fixation was reported (404). Likewise, *in vitro* studies with doubly-labeled butyrate (558) and *in vivo* with isobutyric acid (295) seemed to implicate acetoacetate as the common intermediate in preference to acetate.

3. Mechanism of biosynthesis. It seems then that any mechanism for the biosynthesis of cholesterol must involve the incorporation of a two-carbon fragment

probably by way of a four-carbon compound, acetoacetate. There appears to be no evidence that three-carbon compounds are in the direct line of synthesis (193). The nature of the higher molecular weight intermediates has as yet not been elucidated. The distribution of radioactivity in the cholesterol side chain, as determined by Wüerch *et al.* (555), is such as to suggest a five carbon, isoprene, unit as a possible intermediate. Such a compound, which could result from the condensation of acetate and acetoacetate, has been postulated by Bonner and Arreguin (43) as a precursor in the biosynthesis of rubber. They suggested the following scheme:

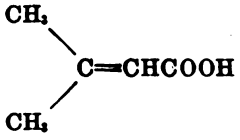
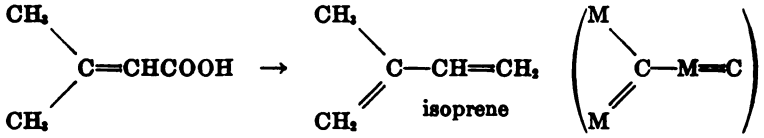
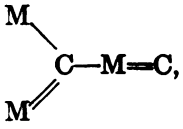
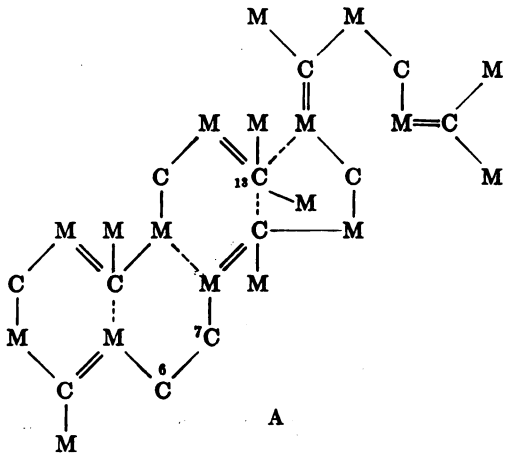
1. $2\ CH_3COOH \rightarrow CH_3COCH_2COOH$
2. $CH_3COCH_2COOH \rightarrow CH_3COCH_3$
3. $CH_3COCH_3 + CH_3COOH \rightarrow$ 
4. 

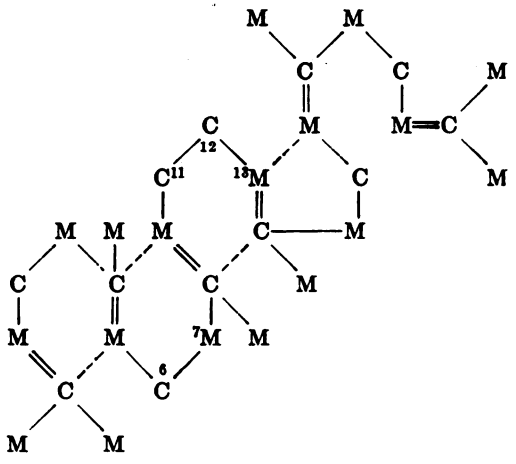
FIG. 4. Formation of isoprene from acetic acid according to Bonner and Arreguin (43).

Wüerch *et al.* (555) have suggested that a similar series of reactions could occur in steroid synthesis and have recalled the hypothesis, first proposed by Channon (79) in 1926 and later by Robinson (430), that the isoprenoid triterpene, squalene, $C_{30}H_{50}$, could be the straight chain compound which by appropriate folding, cross-linking and loss of three methyl groups would result in the perhydrocyclopenta-[a]phenanthrene nucleus. The condensation of isoprene units,  , formed as postulated in Figure 4, would result in squalene having a distribution of acetate carbons such as indicated in Figure 5. The squalene molecule is written in two ways, A and B, so as to suggest the two types of condensations which this molecule might undergo and the postulated distribution of acetate carbons in the resulting cholesterol molecules. Direct condensation, as indicated in A, involving the loss of three methyl groups, was first proposed by Robinson (430). The experimental results of Wüerch *et al.* (555) on the origin of the various carbon atoms of the side chain of cholesterol, as well as the more recent results of Cornforth *et al.* (103) on the origin of certain ring carbon atoms, are in harmony with this hypothesis. However, Woodward and Bloch (552) have recently reported that C_{13} of cholesterol, which according to scheme A would have its origin in the car-

boxyl group of acetate, actually arose from the methyl group of acetate. These authors proposed an alternative mechanism for the transformation of squalene to cholesterol, represented in B, which is in accord with the experimental findings. This scheme involves the migration of one or more methyl groups for the construction of a quaternary carbon at C_{13}.



A



B

M: Acetate methyl C: Acetate carboxyl

FIG. 5. Postulated distribution of acetate carbons in squalene (552).

Recent work by Langdon and Bloch (303, 304, 305) has been directed toward elucidating the possible role of squalene, a substance known to occur in human skin (119) and sebum (327), as a direct intermediate. Using either methyl-labeled or carboxyl-labeled acetate as a source, these workers (305) were able to isolate from squalene-fed rats a radioactive material which appeared to be squalene. When this labeled material was fed to rats, it was shown to be 10–20 times as

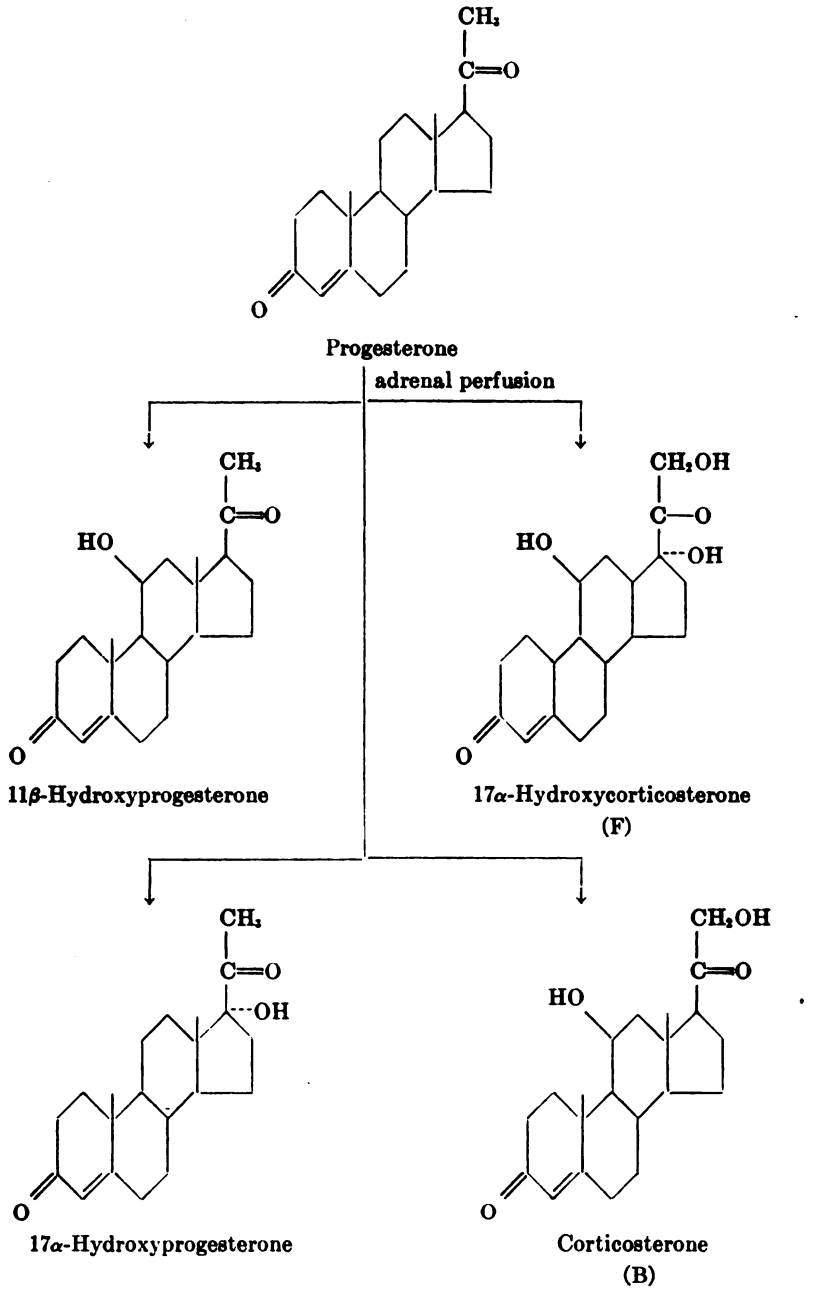


FIG. 6. Products formed from progesterone by perfusion through an adrenal gland (234).

efficient a precursor of cholesterol as was acetate. The low activity of the fatty acids isolated from these rats was taken as proof that the squalene was not converted to two-carbon compounds prior to its incorporation into cholesterol. It did

not, however, preclude the possibility that the squalene was degraded to molecules of intermediate carbon length which were efficiently utilized for this biosynthetic process. A preliminary note (306) by these workers suggests that in the conversion of squalene to cholesterol, Δ^7-cholesterol (lathosterol) is a normal intermediate.

Attempts to synthesize labeled squalene in the laboratory have so far failed to yield a single, pure substance identical with the natural compound (108, 109, 303, 305). When the labeled synthetic "squalene", which represented a mixture of isomers containing possibly some of the natural isomer, was fed to animals, no incorporation into cholesterol could be demonstrated (305, 510). As Bloch has pointed out (33), were squalene conclusively demonstrated to be an intermediate in the direct line of biosynthesis of cholesterol, it would, indeed, be a specific intermediate, for it would be difficult to account by this mechanism for other plant steroids which contain additional carbon atoms in the side chain.

Schwenk and Werthessen (469) have shown that the C^{14}-cholesterol fraction isolated by perfusing liver with labeled acetate for short periods of time (30 min.), also contained other radioactive substances. These were precipitated with cholesterol by digitonin and were separated from cholesterol only when the latter was purified through its dibromide (468). These workers suggest that since these substances have a higher radioactivity than cholesterol, they may represent precursors in the biosynthetic process from acetate.

A general approach to the problem of intermediates in the biosynthesis of plant and animal steroids has been taken by Tschesche and Korte (512) who, reasoning from a suggestion made by Conroy (95) from a consideration of the structure of picrotoxinin, proposed a C_{10}-hydroxy-acid as the biological intermediate. This, as yet hypothetical, intermediate could be visualized as condensing with a variety of compounds which results in many naturally occurring groups of steroids, differing in the carbon content of the side chain as well as in the number and position of various oxygen functions. Miescher and Wieland (344) also proposed a biosynthetic scheme which would explain the origin of many naturally occurring steroid compounds. They postulated that the combination of a large number of acetic acid molecules with oxalic acid and formaldehyde in a specific manner could give rise to a variety of structural types. Their hypothesis, however, predicts conclusions at variance with the experimental values obtained by Little and Bloch (321) for the distribution of methyl carbons and carboxyl carbons of acetate incorporated into cholesterol.

Steroid Hormones

In recent years the biosynthesis of the adrenocortical hormones has been the subject of much research. On the other hand, the mechanism of the formation of the other hormones, testosterone, progesterone and the estrogens, has received less attention. Major efforts have been directed towards elucidating the mechanism of corticosteroidogenesis, the effect of the pituitary hormone, ACTH, on this production as well as the nature of the transformations that the adrenal gland can effect on preformed steroids. Although progesterone has been postulated as a key

intermediate in the biogenesis of the adrenal hormones, very little is known about its biosynthesis in the corpus luteum (153) or placenta (117, 381, 443), the two organs besides the adrenal gland from which it has been isolated. Recent studies have revealed that testosterone can be synthesized from acetate and that this synthesis can be accelerated by chorionic gonadotrophin. The nature of the biosynthetic process which produces the only steroid hormones containing an aromatic ring A, the estrogens, which have been isolated from ovaries as well as placenta (118, 348), adrenal gland (153) and testes (180) has been virtually unexplored.

1. Adrenal cortical hormones. The effect of ACTH. With the advent of new microtechniques, such as methods for the isolation of trace amounts of steroids from blood, paper chromatography for their separation and infrared spectroscopy for their determination, investigations were directed towards elucidating the exact nature of the adrenal secretory products, to determine which of the 28 steroids which had been isolated from adrenal tissue are, indeed, elaborated by the gland and which are products of autolysis or artifacts of the method of isolation. The answer to this question is by no means complete but great strides have been made in this direction in the past few years.

The group at the Worcester Foundation for Experimental Biology have contributed much to this study by perfecting techniques for the perfusion of an isolated adrenal gland (225). This technique enabled Pincus, Hechter and their colleagues (226, 227, 229, 230, 231, 232, 234, 260, 261) to analyze by chemical and biological means the effluent from the perfused gland for secretory products as well as to study the various factors which influence the nature and quantities of these products. Moreover, by the addition of preformed steroids to the perfusate, this procedure permitted them to determine the chemical transformations which the adrenal can effect. Thus, by the determination of the corticosteroid content of the effluent blood before and after ACTH, by chemical means, the ability of the adrenal gland to synthesize corticosteroids and the rapidity with which ACTH affects the production of formaldehydogenic material was clearly demonstrated (226). Whereas the concentration of corticosteroids before ACTH was 180 μg./100 cc., the injection of ACTH intraarterially increased the titer to 406 μg./100 cc. within 30 seconds. A corticosteroid concentration of 2606 μg./100 ml. in the effluent was estimated 90 minutes after the addition of ACTH to the perfusate (398). This increase was actually due to steroid biogenesis and not to the release of steroids which had been stored in the gland (234). Since ACTH was most effective when whole blood was used as the perfusate and had little effect when plasma was employed, it was concluded that the red cells are essential for stimulation by ACTH (234).

The isolation of the neutral steroid fraction from the perfusate by adsorption on charcoal (230), subsequent elution with an organic solvent and application of paper chromatographic techniques made possible the identification of individual corticosteroids and disclosed the presence of at least 15 α-ketols formed by the ACTH-stimulated gland. In addition to the active hormones, evidence for the presence of precursors and metabolites of the hormones was obtained. In Table I

are listed the compounds which have been separated after one cycle through the adrenal gland (398). Of these, Compounds F, E, B, A and DOC have been definitely characterized and there was evidence that among the ten others may be allopregnane-3β,11,17,21-tetrol-20-one (possibly Reichstein's Compound V), allopregnane-3β,11,17-triol-20-one (possibly Reichstein's Compound P) and allopregnane-3β,21-diol-20-one. In the experiment described in Table I, the blood before perfusion contained 610μg. of steroid ketol/liter, Compounds F and B together representing 62% of the total. After perfusion for 115 minutes, all the steroids originally present were increased in amounts, but no new compounds appeared. Again Compounds F and B in equal amounts were predominant and now accounted for 55% of the total. When the blood was recycled through the gland 35–56 times, the ratio of F/B becomes 2.3–3.5/1. as opposed to the 1/1.

TABLE I

α-KETOLS	MICROGRAMS OF STEROID PER 2-LITER BLOOD SAMPLE		
	Not perfused through gland	Adrenal Perfusates	
		No ACTH	ACTH
Unknowns I–V	220	80	700
17α-Hydroxycorticosterone	360	145	1100
Cortisone	—	25	200
Unknown VI	—	40	
Unknowns VII–IX	110	45	250
Corticosterone	400	230	1100
Unknown X	—	60	300
Dehydrocorticosterone	—		250
Desoxycorticosterone	120	35	140

The α-ketols present in adrenal perfusates.
(Reproduced from Pincus, G., Hechter, O. and Zaffaroni, A. (398).)

ratio in the one cycle experiments. The reason for this preferential synthesis of Compound F upon recycling of the perfusate is obscure.

When an isolated human adrenal gland was perfused in a similar manner, the effect of ACTH was to increase the formaldehydogenic titer of the effluent. Continued recycling of the blood resulted in a loss of formaldehydogenic material. Of the compounds formed only Compound F could be isolated (433).

The *in vitro* synthesis of corticosteroids by beef adrenal slices is also accelerated by ACTH, since Haynes, *et al.* (214, 215, 216) have shown that a two-fold increase in the production of Compound F resulted from ACTH stimulation of beef adrenal gland. Likewise, ACTH had been reported to augment the corticosteroid production of rat adrenal glands (441, 442). Using paper chromatographic techniques, Brady (50) has demonstrated that dog adrenal slices incubated with ACTH produce Compounds F, E, B, A and S.

Confirmation of the results of the *in vitro* studies comes from *in vivo* experiments involving the analysis of adrenal venous blood from living animals (533). Following the intravenous administration of ACTH to a dog, Nelson, *et al.* (359) were

able to isolate from adrenal venous blood Compounds F and B and found evidence for at least two other compounds. Zaffaroni and Burton (561) have also found, by paper chromatographic methods, Compounds F and B in a ratio of about 4/3 in dog adrenal venous blood. In addition, they reported the presence of seven unidentified α-ketols.

Synthesis from acetate and cholesterol. It has now been clearly demonstrated that the adrenal gland can utilize acetate for the synthesis of the adrenal cortical hormones. Although the adrenal can utilize acetate for the synthesis of cholesterol (484), it still remains to be proved that the synthesis of the adrenal hormones must proceed via this route. Haines *et al.* (196, 197, 363) incubating slices of hog adrenal glands with C^{14}-acetate together with ACTH, were able to demonstrate the presence of labeled Compound F as the predominant product, and smaller amounts of Compound E, and what appeared to be Compounds B and S. Estimation of the quantities of the hormones in these experiments by the rat liver glycogen deposition assay showed that 91.5% of the total activity can be accounted for by Compound F whereas Compounds E and B account for 4% each.

By perfusion of an isolated whole beef adrenal gland with blood containing ACTH plus C^{14}-acetate (563, 564) and the isolation of the products by paper chromatography, the group at the Worcester Foundation reported the presence of Compounds F and B of equal radioactivity in a ratio of 3/1. In addition, the perfusion with C^{14}-cholesterol likewise resulted in the isolation of radioactive Compounds F and B. Compound S was not found in these perfusates. In view of the fact that Haines (196) employed slices of hog adrenals, whereas Hechter *et al.* (234) perfused whole beef adrenals, their results are not necessarily comparable.

In vivo experiments with labeled acetate confirm the *in vitro* studies on the precursor nature of acetate. After administration of C^{14}-acetate to a man with an adrenal tumor, Hellman *et al.* (237) found that C^{14} was incorporated into serum and fecal cholesterol as well as into urinary androsterone (3α-hydroxyandrosta-17-one), etiocholanolone (3α-hydroxyetiocholan-17-one), and dehydroisoandrosterone (3β-hydroxy-5-androsten-17-one). Similarly, Dorfman *et al.* (133) have recovered 0.5% of the radioactivity of the labeled acetate administered to a cancer patient in the form of the urinary metabolites, dehydroisoandrosterone, androsterone, etiocholanolone, and 5-androstene-3β,17β-diol.

Transformations of preformed steroids. In order to shed light upon the nature of the hormonal precursors and on the transformations which these compounds undergo in the production of the cortical hormones, numerous experiments were carried out in which known steroids were presented to the adrenal gland using either perfusion of intact adrenals or incubations with slices, breis, homogenates or cell fractions. These investigations indicated the types of reactions of which the adrenal is capable as well as the nature of the compounds upon which such transformations can be carried out *in vitro*. Although such investigations do not necessarily disclose the complete *in vivo* process by which corticosteroids are produced, they do provide the basis for present day concepts of the over-all mechanism of this process.

That desoxycorticosterone could be transformed by perfusion through an ad-

renal gland into a steroid biologically active in the glycogen deposition test was one of the earliest indications that the adrenal can convert one steroid into another (229, 230, 231). This conversion, upon which ACTH had no influence, could be effected either with blood, plasma or artificial media as perfusates. Chemical characterization of the products in the perfusate revealed only corticosterone (Compound B) and this in sufficient quantity to account for all of the observed biological activity. In a similar manner, Compound S acetate could be transformed to Compound F (230). These results demonstrated that the adrenal gland has the capacity to introduce an 11β-hydroxyl group into a preformed steroid. When progesterone was perfused, the products isolated indicated that in addition to 11β-hydroxylation, hydroxyl groups were introduced at C_{17} and C_{21} (234, 261) (Fig. 6). Following this, a large number of steroids, some possibly precursors, were perfused and the products isolated. Primarily, three types of reactions have been observed: 1) hydroxylations at C_{11}, C_{17} and C_{21}, 2) oxidation of a Δ^5-3β-ol to an α,β-unsaturated ketone, Δ^4-3-one, and 3) reduction of an α,β-unsaturated ketone to a saturated (5α configuration) C_3-carbonyl or a C_3-hydroxyl group. From these *in vitro* results certain preliminary conclusions concerning the sequence of the hydroxylations can be made. When hydroxylation occurs at C_{17} in a molecule such as progesterone, further hydroxylation at C_{11} and C_{21} can occur to yield Compound F. However, initial hydroxylation at C_{21} is apparently followed only by hydroxylation at C_{11} to yield Compound B. No C_{17}-hydroxylation has been demonstrated in Compound B under these experimental conditions (231). However, Haines (196) has reported that trace amounts of Compound F were formed from DOC by incubation with hog adrenal breis. Δ^5-Pregnenolone when perfused once through the isolated beef gland afforded only progesterone, oxidation of the Δ^5-3β-ol system to the Δ^4-3-one having occurred. Recycling of the pregnenolone eight times resulted in hydroxylation and the isolation of Compounds F and B (231).

It has now been amply demonstrated that the intact gland is not required for the hydroxylation reactions. Hayano *et al.* (209) showed that incubation with slices or homogenates of beef adrenal glands can similarly transform DOC, either free or preferentially in the form of its glucoside, into glycogenic material. This was confirmed by Savard *et al.* (452, 454) and Granata (186). McGinty *et al.* (330), by incubating Compound S with beef adrenal homogenates, were able to isolate Compound F in 50% yield. Likewise, Savard *et al.* (452) showed that Compound S can be transformed by beef adrenal homogenates into glycogenic material which paper chromatographic analysis indicated was Compound F. After the incubation of 100 mg. of DOC with hog adrenal brei containing nicotinamide, Haines (196) reported the isolation of 0.2 mg. of 11-dehydrocorticosterone (Compound A), 26.5 mg. of corticosterone (Compound B) and 0.4 mg. of 17-hydroxycorticosterone (Compound F). There was evidence, too, of small amounts of 11-desoxy-17-hydroxycorticosterone (Compound S) and cortisone, in addition to 3.6% of 6β-hydroxy-11-desoxycorticosterone. He also reported that incubation of Compound S resulted in 39% conversion to Compounds E and F with the latter usually found as the predominant component. The preparation of Compounds

B and F on a commercial scale by incubation of hog adrenal breis has been shown to be feasible (196).

Further studies have been made to characterize the enzyme systems involved in these hydroxylations, particularly 11β-hydroxylation. Again, in the transformation of DOC to glycogenic material, Hayano *et al.* (211) demonstrated that oxygen was required and that the per cent conversion could be increased by the addition of fumarate, ATP and magnesium ion. These findings are in agreement with the results of McGinty *et al.* (330). When the homogenate was fractionated by centrifugation to give a supernatant (1500 $\times$ *g*) and a residue (5000 $\times$ *g*) about equal activity was reported in both fractions (211). Sweat (498) reported that all the enzymic activity necessary for the transformation of Compound S to F resides in the granules, presumably the mitochondrial fraction (separated at 19000 $\times$ *g*); none was in the supernatant or loosely packed layer. Although he found that fumarate was required, apparently ATP did not enhance the conversion. In one experiment, crystalline Compound F was isolated and characterized.

Kahnt and Wettstein (271) reported that enhancement of the conversions of Compound S to Compound F and of DOC to Compound B by hog and beef adrenal homogenates could be effected by ascorbic acid and oxidizable acids of the Krebs citric acid cycle, other than fumarate, *e.g.*, cis-aconitic, citric, lactic, etc. These increases were reported in only a qualitative manner. Further increases in activity were found when nicotinamide was added or in its stead, adenine, adenosine, adenylic acid and nicotinic acid. The authors concluded that this evidence implicates the codehydrases and cyclophorase system in this reaction. Confirmation for the increase in the amounts of F and B when ascorbic acid was added also came from experiments using adrenal pulp (248).

Further studies by Hayano and Dorfman (208) of 11β-hydroxylation of free DOC to Compound B by a purified beef adrenal homogenate residue (5000 $\times$ *g*) led to the demonstration of the specificity and absolute necessity for fumarate and magnesium ions and the stimulatory capacity of ATP and DPN. On the basis of the compounds incubated and the products characterized, in large scale runs by isolation, in others by paper chromatographic methods and color tests, these authors proposed a classification of the structural requirements of a substrate for 11β-hydroxylation. Three groups of compounds, in decreasing order of their reactivities as precursors, are: a) C_{21} steroids containing a C_{20},C_{21}-ketol and a Δ^4-3-one system, b) similar compounds with the double bond in ring A reduced, in either the normal or allo series, and c) C_{19} steroids containing a Δ^4-3-one group and a C_{17}-ketone. A Δ^5-3β-ol system, *e.g.* pregnenolone, gave only poor conversion. With the purified enzyme preparation, progesterone and 17α-hydroxyprogesterone were inactive as substrates, indicating that a C_{21}-hydroxyl group was required for 11β-hydroxylation. Earlier findings (207, 401) that incubations of these compounds with whole homogenates yielded Compounds F and B lend support to the idea of Plager and Samuels (402, 403) that the enzyme system which effects the C_{21}-hydroxylation has been removed in the supernatant fraction.

Recently, other transformations have been demonstrated by incubation with

beef adrenal homogenates. Studying C_{21}-hydroxylation in addition to 11β-hydroxylation, Hayano and Dorfman (207) isolated Compound B from progesterone, Compound F from 17α-hydroxyprogesterone, and Compound E from 17α-hydroxy-4-pregnene-3,11,20-trione (Compound 21-desoxy E). No intermediates having a C_{21}-hydroxyl group but lacking a C_{11}-hydroxyl (such as DOC or Compound S) could be found. Zaffaroni and Hendrichs (565) showed that even when the configuration of the hydroxyl group at C_{11} is α, the unnatural configuration, further hydroxylation at C_{17} and C_{21} can occur. Thus, incubation of 11α-hydroxyprogesterone with adrenal breis resulted in the isolation of two compounds: 11α,17α,21-trihydroxy-4-pregnene-3,20-dione, and C_{11}-epimer of Compound F as well as a compound hydroxylated only at C_{21},11α,21-dihydroxy-4-pregnene-3,20-dione, the C_{11}-epimer of Compound B. Similar incubation of 11α,17α-dihydroxyprogesterone gave the corresponding 21-hydroxylated product. Plager and Samuels (401) reported that ATP activates the oxidation of a C_{21}-methyl group to a C_{21}-hydroxyl as well as hydroxylations at C_{11} and C_{17}. In a preliminary note, Plager and Samuels (402) have reported that C_{17}-hydroxylation must apparently occur prior to C_{21}-hydroxylation if both groups are to be introduced and that the enzyme systems for these reactions occur in the supernatant fractions of centrifuged beef adrenal homogenates. The enzyme effecting 6β-hydroxylation, a reaction previously reported by Haines (196) with hog adrenal breis, has recently been reported to occur in the corpus luteum of cow ovary (213).

Conflicting reports concerning the ability of tissues other than adrenal gland to effect hydroxylations have been made. Hechter *et al.* (234) reported that rat liver or human placenta could not hydroxylate DOC at C_{11}. However, Seneca *et al.* (471) claimed to have evidence for the transformation of DOC to Compound E by liver, kidney, testes and ovarian slices in addition to adrenal slices. Kahnt and Wettstein (271) demonstrated activity with homogenates of liver and kidney but none with those of heart and brain tissue. The results of Nissim (364), from the incubation of DOCA followed by testing for the production of glucocorticoids, showed activity with beef adrenal tissue and corpus luteum but none with liver, placenta, ovary, testes, brain, muscle or plasma.

The capacity of the adrenal gland to oxidize a Δ^5-3β-ol system to a Δ^4-3-one has already been mentioned. Thus, the transformation of Δ^5-pregnenolone to progesterone was demonstrated by perfusion techniques (234). Samuels *et al.* (445) have studied this conversion using *in vitro* techniques with tissue slices and homogenates. They found the structural requirements of a steroid substrate to be: 1) an oxygen at C_{17}, or if there is a side chain, an oxygen at C_{20}, 2) a double bond between C_5 and C_6, and 3) a 3β-hydroxyl group. So, for example, Δ^5-pregnenolone was oxidized to progesterone and dehydroisoandrosterone gave rise to Δ^4-androstenedione, but cholesterol was apparently unaffected. Both tissue slices and homogenates were found to be active and the co-factors DPN, adenosine-3-phosphate and adenosine-5-phosphate accelerated this reaction. A variety of tissues other than adrenal cortex has been demonstrated to effect this oxidation. These include corpus luteum, interstitial cells of testes and placenta (445), a find-

ing also reported by Nissim and Robson (365) on the basis of increased progestational activity resulting from the incubation of Δ^5-pregnenolone with beef corpus luteum, adrenal tissue and placenta. No activity was found in liver, kidney, uterus or spleen tissue (445). The failure to detect this oxidative capacity in the liver or kidney may be due to the further rapid reduction of the Δ^4-3-ketone which is known to occur in these tissues (549) and not to the absence of the necessary Δ^5-3β-ol dehydrogenase.

The in vivo *synthesis of the corticosteroids.* In the light of the various transformations which the adrenal gland can effect, we should return to the question posed at the outset of this discussion, namely, what is the most probable course

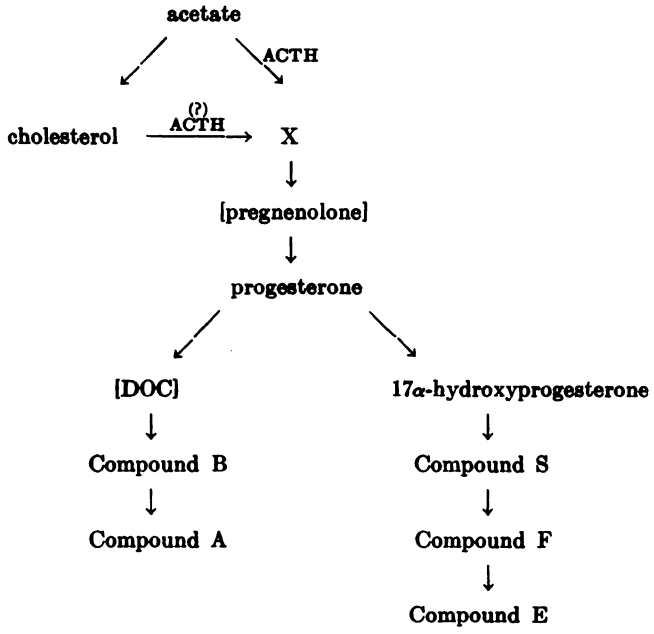


FIG. 7. Tentative scheme of the biogenensis of adrenal steroid hormones.

of corticosteroidogenesis *in vivo?* A tentative scheme, incorporating the results of Hechter *et al.* (234) and Haines (196) is outlined in Figure 7.

Progesterone is postulated as a key intermediate which can be transformed to 17α-hydroxyprogesterone and further hydroxylated to Compound F (234) via Compound S (196). Since Compound B is apparently not further hydroxylated to Compound F in perfusion experiments, it has been represented here as a side route arising possibly from progesterone via desoxycorticosterone. Haines, however, did find small amounts of Compound F when DOC was incubated with hog adrenal breis. Compounds E and A, also isolated from incubations of DOC with hog adrenal breis (234) are here represented as secondary derivatives of Compounds F and B, respectively, resulting from the oxidation of an 11β-hydroxyl group to an 11-ketone group. It is apparent that there are a number of postulated

steps which have yet to be demonstrated *in vitro*. More extensive study is needed in order to distinguish between the compounds formed when the gland is presented with an exogenous steroid and the endogenous precursors formed in the biosynthesis of steroid hormones from acetate.

The role of cholesterol as a possible obligatory intermediate in the corticosteroidogenetic process has still to be clearly elucidated. It has been amply demonstrated (323, 324) that a decrease in adrenal cholesterol content is accompanied by an increase in the formation of corticosteroids (532) following injection of ACTH. In addition, the conversion of cholesterol to pregnanediol (31), cholic acid (34), and Compound F (563) has also been demonstrated. However, these experiments provide no information as to whether cholesterol is an essential intermediate in the path of synthesis.

In the case of the adrenal hormones, Zaffaroni *et al.* (563, 564) demonstrated by perfusion experiments with ACTH that cholesterol was 60 times as efficient a precursor of Compounds F and B as was acetate (228). Thus in the transformation to cortical hormones, cholesterol need not be degraded completely to C_2 units. However, as Chaikoff *et al.* (77, 475) have shown, the side chain of cholesterol can give rise to small carbon compounds and these may be utilized for the biosynthesis of the hormones. Hechter (228) has mentioned some results which led to the conclusion that there are alternative pathways for converting acetate to the corticosteroids other than that which proceeds via cholesterol. Thus, when labeled acetate was perfused through an adrenal, the Compounds F and B isolated from the perfusate had 6.4 times as much radioactivity as did the adrenal free-cholesterol fraction. If cholesterol were in the direct line of synthesis of Compounds F and B from acetate, he reasoned, the specific activities of the hormones should not be greater than that of the adrenal free-cholesterol fraction. When radioactive cholesterol was perfused, the activity of the corticosteroids was 0.4 that of the adrenal free-cholesterol. These data do not rigorously exclude cholesterol as an obligatory intermediate of Compounds F and B. In interpreting results from such experiments, the possibility of the existence of metabolically "active" and "inactive" cholesterol pools in the adrenal gland must be taken into consideration (425). It is possible that the rate at which "active" labeled cholesterol synthesized from labeled acetate is equilibrated with the "inactive" pool of cholesterol in the gland is slow compared to the rate of its conversion to Compounds F and B. Under such conditions only, the isotope concentration of the "active" cholesterol would be comparable to that of the adrenal hormones. However, dilution of the labeled "active" cholesterol during the isolation procedure would be much greater than that of the labeled F and B, since unlabeled cholesterol is present in much higher concentration in the adrenal gland than are the adrenal hormones. Were such the case, the isolated adrenal hormones would exhibit a higher isotope concentration than the isolated cholesterol, despite the fact that cholesterol represents an obligatory intermediate in the synthesis of Compounds F and B from acetate.

In a preliminary report, Hellman *et al.* (237) concluded that urinary steroids were derived from serum cholesterol although initially, the radioactivity of the

urinary dehydroisoandrosterone isolated was such as to indicate that in part, it might arise more directly from acetate. These studies were carried out by the administration of labeled acetate to a man with an adrenal tumor. The incorporation and disappearance of radioactivity from the serum cholesterol and urinary dehydroisoandrosterone, androsterone and etiocholanolone were then estimated. From his work with testicular tissue, Brady (49) concluded that cholesterol may not be an intermediate in the biosynthesis of testosterone from acetate.

That ACTH can enhance the production of corticosteroids from acetate has been mentioned throughout this section. However, the specific role that it plays has yet to be clarified. A recent report by Stone *et al.* (496), confirming previous suggestions (234), indicated that ACTH had no effect on the conversion of progesterone to Compounds F and B. ACTH would then appear to exert its effect at some earlier stage in the biogenetic process, *i.e.*, in the conversion of acetate to some intermediate, X, or in the conversion of cholesterol to X (Fig. 7). The possible role of ACTH in the biosynthesis of cholesterol from acetate has not been elucidated.

2. Testosterone. The biosynthesis of testosterone from acetate by testicular tissue has been demonstrated both for animals (49) and humans (49, 451). Brady (49) first reported the transformation of radioactive acetate to radioactive testosterone by incubation with hog, rabbit or human testicular tissue slices. The perfusion of human testes with blood containing C^{14}-acetate and gonadotrophin to yield labeled testosterone and 4-androstene-3,17-dione was reported by Savard *et al.* (451).

From the results of his experiments, Brady (49) concluded that cholesterol need not be a direct intermediate in the biosynthesis of testosterone from acetate. The incubation of C^{14}-acetate with testicular tissue resulted in the incorporation of isotope into both testosterone and cholesterol. When, however, chorionic gonadotrophin was added to the incubation mixture,the incorporation of C^{14} into testosterone was enhanced by a factor of 10 whereas the incorporation into cholesterol was unaffected.

The enzyme system studied by Samuels *et al.* (445) which oxidizes a Δ^5-3β-ol to an α,β-unsaturated ketone has been found to occur, as has been mentioned earlier, in the interstitial cells of testes. The effect of chorionic gonadotrophin on this oxidation has also been observed (238). When hypophysectomized rats were injected with gonadotrophin for 6 days prior to the removal of the testes, the organs showed enhanced activity in their ability to oxidize Δ^5-pregnenolone to progesterone compared to uninjected controls. Neither pre-incubation with chorionic gonadotrophin nor simultaneous addition of the hormone increased the enzymatic activity. Thus, it was concluded that the effect on this enzyme system was associated with changes in the interstitial cells rather than with direct activation of the enzyme. It is interesting to note in this connection that the *in vitro* conversion of acetate to testosterone could be increased by the direct addition of gonadotrophin to the incubation mixture (49). The role of the Δ^5-3β-ol dehydrogenase in the biogenesis of testosterone has yet to be clarified.

3. Progesterone. The conversion of cholesterol into pregnanediol, a urinary

metabolite of progesterone, was first demonstrated by Bloch (31). Deuterated cholesterol, prepared by a platinum-catalyzed exchange reaction with D_2O (40), was administered to a pregnant woman and the labeled pregnanediol isolated from the urine. The concentration of the deuterium of the isolated product was such as to lead to the conclusion that at least 65–70% of the pregnanediol was derived from the cholesterol, if the assumption was made that the cholesterol of the circulating blood was the immediate precursor and corrections were made for the losses of deuterium during the transformation. Recent work by Fukushima and Gallagher (170, 171) resulted in localizing the deuterium in the cholesterol prepared by the platinum-catalyzed exchange and recalculation of the earlier data in terms of these findings gave a minimum value of 68% for the pregnanediol derived from blood cholesterol. Earlier experiments by Bloch and Rittenberg (34), involving the administration of deuterated cholesterol to an animal, led to the conclusion, based on similar assumptions as mentioned above, that at least ⅔ of the cholic acid was derived from cholesterol and Fukushima and Gallagher recalculated this to be a minimum value of 87%.

The recent studies of the role of progesterone in the biosynthesis of the adrenal cortical hormones, particularly Compounds F and B, would lead to the conclusion that, at least in the adrenal gland, progesterone can be synthesized from acetate and from cholesterol. Whether the route of synthesis in other tissues from which progesterone has been isolated (corpus luteum and placenta) are parallel, remains to be shown.

That Δ^5-pregnenolone can be transformed by the adrenal gland to progesterone has already been discussed. It has been demonstrated by Samuels *et al.* (445) that this transformation can also be effected *in vitro* by corpus luteum and placenta. The *in vitro* transformation of pregnenolone by beef corpus luteum to a more active progestational substance, presumed to be progesterone but not chemically characterized, has also been reported by Nissim and Robson (365).

4. Estrogens. Virtually nothing is known concerning the mechanism of the biosynthesis of the estrogens.

III. STEROID HORMONES IN BLOOD

Nature of the Circulating Hormones

To this day little is known about the chemical form in which the hormones circulate in the blood. It is well known that cholesterol and other lipids are bound to blood proteins and this, together with other evidence cited below, suggests that the steroid hormones likewise circulate in the blood in the form of "protein complexes". This may indeed be true; however, it must be pointed out that the concentrations in which the hormones have been found in the blood do not exceed their solubilities in water.

The question of whether a steroid is truly "bound" to a protein is not easy to decide because, on one hand, protein-lipid complexes, especially the lipoproteins, are, as has recently been pointed out by Folch-Pi (161), easily dissociable at room temperature by the action of organic solvents such as chloroform-methanol or ethanol-ether. Thus, attempts to separate the *free* from the *bound* steroids

of the blood by extraction with an organic solvent may tend to obscure the true nature of the complex. On the other hand, the possibility exists that steroids are adsorbed non-specifically on proteins. Estrogens are known (309, 319) to be adsorbed easily and tenaciously by tissue proteins (prostatic and liver). Boiled, non-viable rat liver slices adsorb estrogens which can be freed only by extensive degradation of the protein, preferably by vigorous hydrolysis. In spite of the difficulties, however, it appears likely that more precise information about the exact nature of the steroid hormones in blood will be forthcoming in the near future since methods, not previously available, for the analysis of trace amounts of steroids and for the study of plasma proteins are now at hand.

Evidence that estrogens are bound to blood proteins has been presented by Muhlbock (352) and Rakoff *et al.* (416) who demonstrated that only a fraction of the estrogen present in serum could be extracted by alcohol-ether. The latter group reported that the bound estrogens could not be separated from the protein by dialysis; only by vigorous acid hydrolysis could they be liberated.

Since this work, three types of proteins have been implicated as "carriers" for steroids.

Serum albumin has been shown by Bishoff and Pilhorn (25, 26, 27), to form complexes with testosterone and progesterone. Of all the protein-steroid complexes reported, the evidence for the existence of these steroid-albumin complexes appears to be the best documented. Lumry *et al.* (326) proved that these complexes were in true equilibrium with the free hormone dissolved in the serum. They also found (456) that the ease of formation of the albumin complexes was determined to a large extent by the number and nature of the polar groups present in the steroids. On the basis of these observations, Rothschild (436) has recently been able to prepare human serum albumin solutions of testosterone and progesterone which are suitable for intravenous administration to human subjects.

The *α-lipoprotein* fraction of human plasma apparently also binds steroids. Cohn (93) has reported that cholesterol and lathosterol (7-cholesten-3β-ol) have been isolated from this fraction. He suggested that the interaction between the plasma proteins and steroids may be highly specific, since cholesterol, lathosterol and certain other steroids were associated with the α-lipoproteins while still others were recovered from the β-lipoprotein fraction

The *β-lipoprotein* fraction (III-O) was examined by Szego and Roberts (427, 503) who found that almost all the estrogen present in human plasma could be isolated from this fraction. They reported that some of the estrogen could be extracted from the protein by cold ether. In contrast to previous observations (416), they observed that all the estrogenic activity could be separated from the protein by dialysis through a collodion membrane. Suprisingly, the estrogen in the dialysate was still not in free form. Acid hydrolysis of the dialysate was necessary to liberate the estrogenic compound which was considered to be possibly estriol. These findings led Szego and Roberts to postulate that the dialyzable estrogenic compound was a conjugate of estriol, probably the glucuronoside, and that this estriol conjugate was bound to the β-globulin.

From the data of Szego and Roberts, Oncley and Gurd (368) have estimated that 1 mg. of estriol is linked to 100 g. of β-lipoprotein of molecular weight 1,300,000. This indicates there is one molecule of estriol associated with 50 molecules of β-lipoprotein. Similar calculations for unesterified cholesterol indicated that there are 280 molecules of the sterol for every molecule of protein.

The significance of these results is somewhat unclear because they bear not upon the nature of the circulating estrogenic hormone in the blood, but rather upon the nature of its main urinary excretory product, namely, estriol glucuronidate. Moreover, it is not certain that the dialyzable estrogen conjugate is truly bound to the β-lipoproteins, since the only criterion for its being bound was its insolubility in ether, a solvent in which the conjugate, either bound or free, would not be soluble. In addition, the amounts obtained do not agree with those of Werthessen *et al.* (537) who, using a biological assay method for the determination of estrogens, succeeded in extracting from blood, directly without hydrolysis, ten to a hundred times as much hormone.

Other than the previously mentioned testosterone-albumin complexes, little is known about the form in which the androgenic hormone is transported in the blood. Regarding the nature of progesterone in blood, Hooker and Forbes (251) have come to the conclusion that in the rabbit, mouse, monkey and women, the blood progesterone is probably entirely "free". The total blood levels of progesterone, as determined by bioassay, amounted to 4–8 μg./ml. of whole blood, a quantity which does not exceed the solublity of this substance in water. After precipitating the proteins with acetone, they found 90% of the progesterone in the supernatant. This, they concluded, represented the unbound hormone; however, from what is known of the instability of protein-lipid complexes (161) this conclusion may be open to question. The remaining progesterone, which was biologically inactive until liberated, could be freed from the precipitated proteins only by acid hydrolysis.

Savard *et al.* (453) have reported some experiments which indicated that some of the adrenal cortical hormones present in blood occur in the form of glucuronosides. Using paper chromatographic techniques, they were able to detect Compound F in the free form in the dialysates from the artificial kidney. Additional amounts of Compound F and Compound B were liberated from these dialysates by the action of glucuronidase.

Hormonal Levels

The detection and estimation of the steroid hormones and their metabolites in blood has recently received a great deal of attention. In spite of the formidable difficulties due to the extremely low concentrations of the hormones in peripheral blood, much effort has been expended in this direction with the hope that a knowledge of the hormones and their levels in blood would circumvent some of the limitations of urinary studies and would permit a better evaluation of the actual hormonal environment. Some success in this direction has been achieved, especially in the determination of the concentrations of the hormones present either in the peripheral blood following the intravenous administration of a

steroid or one of the trophic hormones, or in the venous effluent from the endocrine gland. The peripheral blood of untreated individuals contains only trace amounts of the hormones and this imposes serious methodological difficulties. Only in the case of the adrenal hormones has the level of the peripheral blood been successfully estimated by chemical means.

The most satisfactory method appears to be that of Nelson and Samuels (358) and Nelson *et al.* (360) which estimates the type of adrenal hormones known as "17-hydroxycorticosteroids" or those possessing a dihydroxyacetone side chain. After extraction and preliminary purification by partition between hexane and 70% ethanol, followed by adsorption chromatography, these steroids were measured by the Porter-Silber test. The normal values thus obtained were 3–10 μg./100 ml. of peripheral blood. In Addison's disease the values were too low for measurement. ACTH, stress or imminent death raised the values. Using the glycogen deposition biossay, Paschkis *et al.* (375) reported peripheral blood levels somewhat higher than those of Nelson and co-workers. Done *et al.* (131) measured the circulating 17-hydroxycorticosteroid concentrations in rabbits, rats, guinea pigs and humans using the method of Nelson and Samuels and found 0 μg., 2 μg., 33 μg., and 11 μg. per 100 ml. of peripheral blood, respectively.

In a recent preliminary report, Sweat *et al.* (499) described a micro-chromatographic technique which permits an analysis of the concentrations of Compound B and Compound F present in 5 ml. of human peripheral blood plasma. Average normal values were 4.9 μg. of Compound B/100 ml. and 11.0 μg. of Compound F/100 ml. Three-to-five-fold increases in the levels of these compounds were observed within 15 minutes after the administration of 15 mg. of ACTH. Weichselbaum and his co-workers (535) have also reported a chromatographic method for the estimation of blood corticosteroids.

The blood concentrations of the other hormones have been determined only by bioassay methods. No chemical technique has succeeded in detecting the minute quantities of the estrogens, progesterone, testosterone or desoxycorticosterone present in blood. According to Cantarow, *et al.* (72) and Szego and Roberts (427, 503), the estrogenicity of the serum of non-pregnant women corresponds to 0.2–2.0 μg. of hormone per 100 ml. as measured by bioassay. However, as mentioned previously, 10–100 times these amounts were reported by Werthessen *et al.* (537) who took special pains to minimize oxidation of the estrogen by peroxides or air during the processing and these precautions may have been responsible for the higher figures. Although estrogenic activity has been detected in the spermatic vein blood of stallions, only trace amounts were found in the venous effluent from dog ovaries even after the administration of gonadotrophin (415).

The progesterone content of human pregnancy blood has been estimated by Hooker and Forbes (251) and by Forbes (162) who used a highly sensitive bioassay method. The variable levels (average 5.3 μg./ml.) they obtained, however, were within the range of those of the normal menstrual cycle and could not be correlated at all with the pregnanediol excretion. With a polarographic method sensitive to 0.1 μg./ml., Butt *et al.* (67) failed to detect progesterone in human pregnancy blood at a time when the urinary pregnanediol probably could have

been measured in milligram amounts. Likewise, Haskins (204) failed to detect progesterone in the plasma of women by means of an ultraviolet spectroscopic method of high sensitivity. Edgar (137), using a chemical method that could detect 4 μg. of progesterone, failed to find the hormone in as much as 160 ml. of peripheral blood of pregnant or non-pregnant women. Similarly, West *et al.* (545) could demonstrate by chemical means no 17-ketosteroids, either free or conjugated, in the blood of normal individuals. Minute amounts of androgens have been detected in human blood by bioassay methods (283, 329).

When the venous effluent of an endocrine gland is examined, the higher blood levels permit a more specific and accurate determination of the steroids present. For example, from the spermatic vein blood of a dog, West *et al.* (541) succeeded, after extensive purification, in isolating testosterone, 4-androstene-3,17-dione and 7-ketocholesterol. With their polarographic method, Butt *et al.* (67) detected small amounts of progesterone in two samples of human placental blood obtained from the umbilical cord. Bush (61), using elegant paper chromatographic methods, found that Compound F was present in larger amounts than Compound B in the adrenal effluent of a dog. He previously reported (63) that unknown compounds more polar than F could also be isolated from this source. Bush also has observed (62) species differences in adrenocortical secretions. Whereas the ratio of Compound F to Compound B might be less than 0.05 in rats, it was greater than 20 in the rhesus monkey. This agrees with the result of Sweat and Farrell (500) who could find no F in the adrenal vein blood of rats although they did detect Compound B and an equal amount of an unidentified compound. Later, Sweat *et al.* (499) were able to examine by their chromatographic technique the adrenal effluent from the human gland. During surgery, the apparently normal adrenal secretes, according to these workers, 80 μg. of Compound B and 290 μg. of Compound F per 100 ml. of adrenal effluent blood. Adrenal venous blood from a patient with Cushing's syndrome contained 236 μg. of Compound B and 850 μg. of Compound F per 100 ml.

After the administration of ACTH, still larger amounts of adrenal cortical steroids are found. Nelson *et al.* (357) and Reich *et al.* (419) isolated for the first time crystalline Compound F and Compound B from adrenal venous blood of dogs after the administration of ACTH. Using paper chromatography, Zaffaroni and Burton (561) estimated from the venous effluent, that the dog's left adrenal, after stimulation with ACTH, secreted 6 mg. of Compound B, 8 mg. of Compound F and 2 mg. of seven unidentified α-ketols in 24 hours or, expressed differently, secreted 0.2–0.7 mg. of α-ketols per hour per gram of gland.

There are many reports on the levels of the hormones in blood following the administration of various steroids either intravenously or intramuscularly. For example, Hertz *et al.* (241) administered single doses of estradiol, both intravenously and intramuscularly, and observed, by bioassay, that the hormone completely disappeared from the blood stream within a few hours. Likewise, Butt *et al.* (67) injected large doses of progesterone into rats intravenously and found that within 5 minutes over 95% of the injected dose had disappeared from the circulation. Sommerville and Bigler (479) studied the fate of 30 mg. of pro-

gesterone administered intravenously to men. At the end of the infusion period, which lasted between 15 and 30 minutes, progesterone levels were already low; within 15 minutes after administration, no progesterone could be detected in the blood. Blood levels of pregnanediol, determined simultaneously, reached a maximum value of 100 μg./ml. of blood at the termination of the infusion period. Within 20 minutes after the infusion ended, its level fell to about 30 μg./100 ml. Half of the blood pregnanediol appeared to be in the free form and half conjugated with glucuronic acid.

Testosterone injected intravenously also disappears quickly from the circulation (204). West *et al.* (544, 545) have shown that within 1 hour after the intravenous administration of testosterone, the blood level fell to normal (below 20 μg./ml.). West (540) found that the blood level of testosterone fell rapidly during the first few minutes after the intravenous injection of testosterone into rabbits or rats; thereafter, it fell at a slower logarithmic rate. Furthermore, West and his colleagues (545) observed that within 5 minutes after injection of intravenously administered testosterone to man, the blood level of this hormone was less than 10% of the expected blood level. No free 17-ketosteroids could be detected in the blood. Following acid hydrolysis, however, they were demonstrable. No 17-ketosteroids could be found in the blood of untreated individuals by the method used, either before or after hydrolysis. Twenty minutes after injection of the testosterone, the 17-ketosteroid level in the blood reached its highest value, approximately 500 μg./100 ml. of plasma.

Nelson *et al.* (361) have measured the blood corticosteroids following the administration of various adrenal steroids. Oral administration of Compound E and Compound F or their acetates to normal individuals regularly resulted in the elevation of the blood levels of 17-hydroxycorticoids. On the other hand, intramuscular administration gave irregular responses. There was no increase following the oral or intramuscular administration of Compound S.

It is evident from the available data that the steroid hormone levels in peripheral blood are infinitesimally small. In this respect, they are similar to the concentrations that apparently exist within the endocrine glands themselves. Studies with intravenously administered steroids have proved that the hormones disappear quickly from the circulation. Moreover, the evidence at hand indicates that alterations in endocrine gland secretions, frequently deducible from physiological considerations or from urinary excretion levels, are not always reflected in changes in blood levels, as measured either by biological or chemical assays. These considerations, it would appear, limit the practical value of the estimation of the hormone levels in peripheral blood. At the present time, only estimations of the corticoids (360, 499, 535) provide useful information which apparently can be correlated with the existing physiological state. In general, it should be recognized that were the blood assays accurate and specific, they would reveal at best the status of the endocrine secretion prevailing at the moment the blood sample was withdrawn. The important temporal relationships disclosing an over-all picture of the status of production of the endocrine gland would still be unknown.

IV. METABOLIC EFFECTS OF STEROIDS AT THE ENZYMATIC LEVEL

Almost every aspect of metabolism is at some time influenced intimately and extensively by the steroid hormones. These biologically potent compounds exert an influence upon carbohydrate, fat, protein, water, electrolyte and energy metabolism. Such biochemical entities as citric acid, ascorbic acid, uric acid, histamine, histidine, plasma cholesterol levels, phospholipids, antibodies—to choose only a few—appear to be under some control of one of the steroid hormones. The multiple biochemical and physiological actions of these hormones constitute a major portion of the science of endocrinology. In spite of the availability of an enormous body of descriptive information, the intimate chemical mechanism by which any one of these actions is mediated is as yet unknown. In general, the hormones are considered to act as catalysts (or inhibitors) and as such control the rate of various biochemical processes but do not initiate any that are not already existent. Naturally enough, the biochemists have considered that the most direct approach to this problem is to search for possible sites of action of the steroid hormones on the enzyme systems known to be involved in intermediary metabolism. Such studies have the virtue of being readily amenable to experimental study and several excellent reviews have described the formidable quantity of data already obtained by this line of attack (132, 206, 528). Although the study of the steroid-enzyme relationships has not yet succeeded in elucidating the mode of action of any steroid hormone, this approach still appears, at this time, to be most promising.

1. *Interactions between steroids and enzymes.* Dorfman, in a recent comprehensive review (132), has formulated the problem of steroid-enzyme interactions in the following way. The hormones may exert their influence on enzyme systems by: 1. acting as a component (prosthetic group) of an enzyme system, 2. accelerating or inhibiting an enzyme system, 3. affecting, directly or indirectly, accelerators or inhibitors of enzyme systems, and 4. changing tissue-enzyme concentrations. There is no evidence available at present that supports the hypothesis that steroid hormones act as prosthetic groups of enzymes. Nevertheless, the fact that steroid-protein complexes can exist (25, 93, 210, 545) makes this thesis at least possible.

There is evidence that steroids may inhibit isolated enzyme systems. Hayano *et al.* (210) observed that DOC strongly inhibited the D-amino acid oxidase system, the inhibition resulting from reaction of the steroid with the apoenzyme of the oxidase and not by the steroid in competition with the substrate or prosthetic group. Incubation of the enzyme with the steroid for 5–10 minutes before addition of the substrate completely inactivated the enzyme. Addition of large amounts of the substrate, alanine, after the inhibition had been established did not remove the suppressing effect of the steroid, although complete protection of the enzyme could be obtained if the amino acid was present at the start of the incubation. Similar results were obtained when FAD (isoalloxazine adenine dinucleotide) replaced the excess alanine. Recovery of the enzyme activity after inhibition could be accomplished by acetone precipitation of the enzyme which removed the DOC and regenerated the active catalyst. Other steroids also

caused this inhibition but DOC was the most potent. Hochster and Quastel (247) discovered that steroids inhibit the α-glycerophosphate dehydrogenase system *in vitro*. Ketosteroids, especially those possessing a Δ^4-3-keto grouping, were most active, while C_8 (saturated), C_{17} and C_{20} ketosteroids caused less inhibition.

Phosphate esters of some estrogens have been shown to inhibit rabbit kidney alkaline phosphatase (3), and the sulfate ester of estrone has been shown to inhibit the succinoxidase system of rat liver (343). Kalman (272) and Bargoni and DiBella (14) have also reported the inhibition of succinoxidase by steroids. In order to dissolve the water-insoluble steroids in the enzyme reaction mixture, the inorganic esters of steroids (sulfates, etc.) have been used by some workers. The results of such experiments must be interpreted with caution since these esters may be acting merely as detergents and not necessarily as specific steroids inhibiting a given enzymic reaction.

Some synthetic estrogens such as diethylstilbesterol also effectively inhibit the succinoxidase system, probably through the cytochrome oxidase component. However, 4,4'-dihydroxystilbene (76) inhibited the complete system but neither the cytochrome oxidase nor the succinic dehydrogenase alone. Inhibition by this compound would then appear to be at the cytochrome level.

Choline acetylase was shown to be inhibited by testosterone and other steroids (511), whereas DOC strongly inhibited tyrosinase and partially inhibited urease, ascorbic acid oxidase, lipase and transaminase (210). Some adrenal steroids were found to inhibit hyaluronidase (370).

The above reports are all examples of inhibitory actions of various steroids upon enzyme systems. Of greater significance for the problem of mode of action would probably be examples of activation of enzymes by steroids. Unfortunately, the data supporting this possibility are at present meager. Hayano et al. (210) found that DOC increased the activity of glutaminase, the decarboxylation of pyruvate by yeast cells and the hydrolysis of denatured hemoglobin by trypsin. Dirscherl and Hauptmann (123) reported the activation of enolase, hexokinase and carboxylase by various androgens and estrogens. As Dorfman has pointed out, it must not be assumed that these inhibitions or accelerations actually occur *in vivo*. It might be added that no direct relationship between these effects of steroids on enzymes *in vitro* and the biological properties of the steroids has developed from these studies. Hence, the bearing of these experiments on *in vivo* processes remains to be clarified.

The recent work of Umbreit and Tonhazy (519) provides evidence for the third possible mechanism of hormone effects on enzyme systems. *i.e.*, the influence on accelerators or inhibitors. These investigators found that homogenates of kidney tissue from adrenalectomized animals oxidized the substrates, α-ketoglutarate, fumarate and oxaloacetate, at diminished rates. Addition of ATP or adenylate restored the rate to normal. *In vivo*, the administration of cortisone to an adrenalectomized rat also prevented the decrease in the rate of oxidation of these substrates.

The problem of whether steroid hormones influence the rate of enzyme reactions, either diectly or indirectly, is related to the last of the possibilities pro-

TABLE II

In vivo *influence of steroids on enzyme concentrations in tissues*

ENZYME	SPECIES & TISSUE	TREATMENT OR STEROID HORMONE	INFLUENCE ON ENZYME CONCENTRATION	REFERENCE
Succinic dehydrogenase	Rat seminal vesicles & prostate	Castration	Decrease	111
		Castration + testosterone	Restored to normal	
Succinoxidase	Rat liver	Adrenalectomy	Slight decrease	509
		Adrenalectomy	Unaltered	551
		Hypophysectomy	Increase	
Arginase	Mouse kidney	Androgen	Increase	285
		Cortisone	Increase	290
		Estradiol-17β	Increase	288
		Progesterone	Decrease	284
	Rat liver	Cpd. A, Cpd. B, or Cpd. E	Increase	163
D-amino oxidase	Mouse kidney	Castration	Decrease	86
		Castration + testosterone proprionate	Restored to normal	
	Rat liver	Fed amino acids	Increase	262
		Fed amino acids + adrenalectomy or hypophysectomy	No effect	
		Fed amino acids + adrenal cortical extract	Increase	
	Rat liver	Adrenalectomy	Decrease	517
	Rat kidney	Adrenalectomy	No effect	
Cytochrome oxidase	Rat seminal vesicles & prostate	Castration + testosterone propionate	Increase	111
Choline esterase	Rat serum	Ovariectomy	Decrease	152
		Castration	Increase	
		Castration + estrogen	Increase	
		Castration + testosterone propionate	Decrease	
	Guinea pig serum	Castrated male	Decrease	534
		Castrated male + testosterone propionate	Restored to normal	
Peptidase	Mouse serum	Adrenal cortical hormone	Increase	250
	Rat or mouse serum	Adrenal cortical hormone or ACTH	No effect	467
	Human (?) serum	ACTH	Decrease	267

TABLE II—*Continued*

ENZYME	SPECIES & TISSUE	TREATMENT OR STEROID HORMONE	INFLUENCE ON ENZYME CONCENTRATION	REFERENCE
Acid phosphatase	Human serum	Castration or estrogen	Decrease	254
		Androgen	Increase	253
β-glucuronidase	Mouse uterus	Ovariectomy	Decrease	158, 159
		Ovariectomy + estrogen	Restored to normal	
	Mouse kidney, liver, spleen & vagina	Ovariectomy	No effect	203
		Estrone + progesterone	Decrease	
	Mouse liver	Ovariectomy + estrogen	Increase	277, 278
	Human serum	Estrogen	Increase	160
		Cortisone	Increase	
		Cushing syndrome	Increase	
		Pregnancy	Increase	
		Female + testosterone	No effect	
	Rat uterus	Estrogen	Increase	504
		Estrogen + progesterone	No effect	
	Rat preputial gland	Estrogen	Increase	
		Estrogen + Cpd. F (E)	No effect	
Esterase	Mouse uterus or vagina	Ovariectomy	Increase	203
		Ovariectomy + estrogen	Restored to normal	
		Estrone alone	No effect	
Proline oxidase	Rat kidney	Adrenalectomy	Decrease	518
		Adrenalectomy + cortisone	Restored to normal	
Adenosine triphosphatase	Rabbit uterus	Ovariectomy	Decrease	105
		Ovariectomy + estrogen	Restored to normal	
Choline acetylase	Rat brain	Hypophysectomy	Decrease	511
		Hypophysectomy + ACTH	Restored to normal	
Catalase	Male mouse liver	Castration	Decrease	1
		Castration + testosterone	Restored to normal	
		Adrenalectomy	Decrease	
		Adrenalectomy + cortisone	Restored to normal	
Histaminase	Hog kidney & placenta	Estrogen	Increase	273

posed by Dorfman, namely, that the hormones alter the concentration of tissue enzymes. That steroids can, *in vivo*, produce such changes has been well established in many instances. Some of these are listed in Table II.

While many of these *in vivo* effects are undoubtedly of great significance for the endocrine control of various phases of metabolism, it is difficult to evaluate the pertinence of these results to the problem of the mode of action of the steroid hormones. In many cases, these results illustrate Schoenheimer's proverbial slot machine experiment with all its logical hazards. That great caution must be observed in interpreting some of these results can be exemplified by a recent observation of Cohn and Kolinsky (92). Altered enzyme levels observed in adrenalectomized animals may be due to inanition effects rather than to lack of cortical hormones, since qualitatively different results were obtained when food intakes were equalized. Cohn and Kolinsky have shown this to be the case for glucose utilization by isolated diaphragm from adrenalectomized and normal rats.

2. *In vitro effects upon oxidative metabolism.* Certain steroids influence the oxidative metabolism of isolated tissues. In 1947, Gordan and Elliott (181) observed that testosterone, DOC and other steroids depressed the oxidation of glucose by rat brain slices. Castration increased the oxygen uptake of rat brain tissue and testosterone treatment of castrated rats reversed this phenomenon (139). Hayano *et al.* (212) have extended these observations by demonstrating that DOC, dehydroisoandrosterone, methyltestosterone and testosterone can inhibit the oxygen consumption of rat liver, kidney and brain slices. In general, steroids with ketonic groups were more active than those lacking this function. In experiments with cellular fractions from rat brain homogenatess it appeared that the inhibitory action of DOC on oxidative metabolism occurred through a reaction between the steroid and the enzyme system present in the insoluble particulate matter. The results further suggested that the site of suppression was a reaction involving transfer of hydrogen ions or electrons. However, the respiration of primary Brown-Pearce epithelioma is not inhibited by testosterone (286). The action of various steroids upon the respiration and glycolysis, both aerobic and anaerobic, of various tissues such as liver slices, vesicle glands, etc. has been extensively studied by Dirscherl and coworkers (122, 123).

Within the last year several other studies of the *in vitro* effect of steroids on respiration have been reported. Guidry *et al.* (192) demonstrated that estrogens decreased the oxygen consumption of rat liver homogenates in the presence of substrates which were oxidized through coenzyme I or II; this effect was not observed when succinate was the added substrate. Thus estrogen inhibition appeared to be specific for coenzyme I- and II-linked oxidations. On the other hand, when human endometrium or term placenta was incubated with estradiol, the oxygen uptake was increased (194, 195). Estradiol also increased glycogenolysis, glycolysis and pyruvate utilization by placenta (194, 195). Similarly, estradiol administration stimulated respiration and glycolysis in surviving rat uterine tissue, a stimulation which was abolished by the simultaneous administration of Compound F (504). Fatty acid (octanoate) utilization by mouse liver

homogenates was decreased by adrenalectomy and could be restored to normal by treating the animals with cortisone (320). By the administration of C^{14}-glucose, Welt *et al.* (536) found that the rate of formation of glucose was seven times greater when cortisone was administered than it was in untreated rats. In alloxan-diabetic animals, however, cortisone treatment caused only a two-fold increase in the rate of gluconeogenesis. The rate of oxidation of glucose to CO_2 was not affected by Compound E (536).

Marked effects of cortisone and DOC on *in vitro* oxidations of various tricarboxylic acid intermediates in adrenal, cardiac muscle and kidney homogenates were observed by Sourkes and Heneage (482). Of the two steroids, DOC was the more potent inhibitor of the oxidation of those substrates by adrenal homogenates. With kidney and heart muscle homogenates the effects were different, cortisone increasing the rate of oxidation of some substrates and decreasing the rate of others. In general, DOC depressed the oxidation of the substrates and markedly inhibited the respiration of cardiac muscle. The effects of steroid hormones on the formation of citrate from pyruvate and malate by isolated mitochondria have been studied. Grant and Taylor (190) found that *in vitro* citrate synthesis was inhibited by addition of testosterone, progesterone, 17α-hydroxyprogesterone, Compound S and DOC, but was apparently increased by addition of Compounds E and B.

3. In vitro *effects on other aspects of metabolism.* The influences that steroids, especially the adrenal cortical hormones, exert on the carbohydrate metabolism of isolated tissues have received considerable attention and have recently been extensively reviewed by Verzár (529). In 1940, Seckel (470) demonstrated that the addition of adrenal cortical extracts to rat liver slices diminished the disappearance of glycogen. Chiu and Needham (82) confirmed this observation and presented evidence that the hormones affect the synthesis of glycogen and not its breakdown. DOC and Compounds A and E were all effective in diminishing glycogenolysis (81). In 1948 Verzár and Wenner (530), using surviving rat diaphragm, reported that DOC increased glycogen disappearance and could counteract the effect of insulin on this tissue. Various other steroids were active, although Compound F, which possesses the highest *in vivo* activity on carbohydrate metabolism, was not (531). Using C^{14}-glucose and isolated rat diaphragm Bartlett *et al.* (16, 17) confirmed the finding of Verzár that DOC prevents glycogen formation from glucose. It should be noted that the results obtained with isolated diaphragm by Verzár and by Bartlett *et al.*, are contrary to those obtained with liver slices by Seckel and by Chiu and Needham. Cortical hormones appear to increase glycogen deposition in liver, while increasing the loss of glycogen from muscle.

The adrenal cortical hormones appear to influence both the production of glucose and its further metabolism; in this way the hormones are intimately concerned with the energy production of the cells. They apparently are not concerned solely with glycogen formation, nor with the regulation of the blood sugar level. Since adrenalectomy diminished the rate of absorption of glucose, Verzár has suggested that the adrenal hormones play a role in phosphorylating mechanisms.

Thus, the glycogenolysis produced by cortical hormones in isolated muscle systems may be due to a stimulation of phosphorylase activity. Montigel and Verzár studied the effects of many steroids upon various enzymes concerned with phosphorylation (349, 350). The phosphorylase activity of minced muscle was increased *in vitro* by DOC, and decreased in tissues from adrenalectomized animals. The latter effect was reversed by the addition of DOC to the mince. However, this activity could also be restored by the addition of cysteine or glutathione. The phosphorylase activity of rat liver was also decreased by adrenalectomy (128, 486). *In vivo*, DOC has much less effect on carbohydrate metabolism than have the 11-oxygenated adrenal hormones, Compounds F and B. The *in vitro* actions of these compounds do not parallel their *in vivo* effects. Hence, the significance of the results from isolated tissue experiments is unclear.

Conway and Hingerty (96) obtained evidence which indicated that the phosphoglucomutase activity of muscle decreased after adrenalectomy. Thus, in normal muscle there was more than twice as much glucose-6-phosphate as glucose-1-phosphate; in the muscle of adrenalectomized animals the ratio was reversed. *In vitro* studies of growing embryonic tissue, by the roller tube technique, have demonstrated that cortisone alone did not affect the total synthesis of ribonucleic acid phosphorus (RNAP), nor of desoxyribonucleic acid phosphorus (DNAP). Compound E plus growth hormone increased the cell number by 75% with increases in lipid phosphorus (LP) and RNAP. Compound E plus insulin increased LP, RNAP and DNAP. All three hormones together caused marked stimulation of growth (310).

The study of steroid-enzyme relationships by means of isotopically-labeled steroids, only recently made available, has not been explored. However, other isotopically-labeled compounds have been used to elucidate the effects of steroid hormones on specific phases of metabolism. To the extent that these labeled compounds have facilitated and advanced the study of such aspects of metabolism as the rates of turnover, incorporation, formation or destruction, so too will they contribute to the study of the effects of the steroid hormones on metabolic reactions. Mention has already been made of the work of Welt *et al.* (536), who, by the administration of C^{14}-glucose, showed that cortisone markedly increased the rate of glucose formation. However, the oxidation of glucose to CO_2 was not influenced by cortisone. Isotope incorporation studies have also demonstrated that cortisone could inhibit the *in vitro* synthesis of fatty acids in rat mammary gland slices (13).

Clark (84) has studied the effect of adrenal cortical hormones on the protein synthesis of animals fed N^{15}-glycine. The findings indicated that the increased excretion of urinary nitrogen due to the administration of Compound E to both intact and adrenalectomized rats resulted from a diminished rate of protein synthesis and not from an increased rate of catabolism of the protein. Similarly, using isolated diaphragm, Sinex (473) observed that Compound E added *in vitro* decreased the incorporation of labeled alanine into tissue proteins.

Several workers have measured the *in vitro* uptake of labeled amino acids by isolated sexual tissues from animals treated with steroid hormones. Mueller and

Yanagi (351) demonstrated that estradiol, administered intravenously, enhanced the capacity of surviving rat uteri to incorporate glycine-2-C^{14} into protein. The uteri, removed 20 hours after the administration of the hormone, showed a two- to three-fold increase in rate over that of the controls. DOC and progesterone inhibited incorporation of the glycine while Compound E had no effect. On the other hand, Szego and Roberts (428) found that concomitant administration of Compound F counteracted the stimulatory effect of estradiol on the incorporation of alanine-C^{14} into rat uterine protein. Frieden *et al.* (165) investigated the incorporation of labeled glycine into proteins of the tissues of the reproductive tract. Symphyseal slices from guinea pigs treated with estrogens for 4–7 weeks incorporated glycine-1-C^{14} five to ten times as rapidly as did liver slices from these animals. Symphyses from untreated castrates showed only slight activity. The uterus of the castrate rat incorporated glycine *in vitro* approximately five times as rapidly as did rat liver. After injection of estradiol, the rate of incorporation into isolated uterine tissue increased, reaching a maximum in 48 hours. Incorporation of labeled glycine and alanine in uteri of castrate rats treated with progesterone was somewhat greater than in castrate controls (165).

V. CATABOLIC FATE OF STEROID HORMONES

Up to this point we have summarized the available knowledge on the biogenesis of the steroid hormones, the transportation in the blood following the secretion by the endocrine gland and the little that is known about the chemical mechanism by which steroids mediate their physiological and biochemical effects. It is evident from the foregoing that enormous gaps in our knowledge of these areas still remain. Paradoxical as it may seem, more information is available about the catabolic fate of the steroid hormones than about any other phase of steroid biochemistry. This is so, in spite of the fact that the catabolic fate of the hormones may be completely unrelated to their unique biological functions. The investigations of the degradative metabolism of the steroid hormones that have been carried out may be divided into two categories: 1) the effect of purified enzymes or isolated *in vitro* systems upon steroids, and 2) the analysis of the steroid metabolites found in the excreta. Much effort has been extended in these two directions because of the relative ease with which they can be approached experimentally.

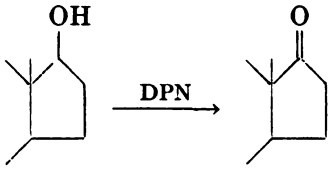
Role of the Liver

As mentioned above, any hormone administered intravenously disappears from the blood stream with celerity. There is some evidence that some of this disappearance may be due to factors within the blood itself (397, 538). At present, however, factors in other nonendocrine tissues are assumed to be responsible for the major catabolic alterations which the steroid hormones suffer. As early as 1934 Zondek demonstrated that enzymatic factors in the liver destroyed the biological activity of the estrogens. Since that time attention has been centered on that organ and, indeed, the liver is now considered to be the most important organ for the catabolism of the steroids, although other organs such as kidney may also be active.

The role of the liver and kidney in the metabolism of the steroid hormones has been reviewed by many workers within the past few years and no attempt will be made here to present a detailed picture of the available data. Interested readers will find excellent reviews by Glass (178) on *in vitro* inactivation of estrogens, by Samuels (444) on androgens, and by Samuels and West (448) on androgens, progesterone and adrenal cortical steroids.

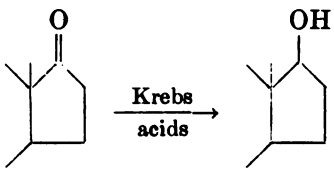
1. In vitro metabolism. The known types of metabolic conversions of steroids that enzymes present in various organs can effect are summarized in the following paragraphs. Those reactions concerned with hydroxylation at C_{11}, C_{17}, and C_{21} and that concerned with the dehydrogenation of the Δ^5-3β-ol grouping to the Δ^4-3-ketone group have been omitted, since they are more likely related to anabolic phases of steroid biosynthesis and have been discussed previously.

Reaction 1



The ability of the liver and kidney to oxidize a steroid alcohol to a ketone has been demonstrated with a variety of molecules. Thus, the conversion of testosterone to 4-androstene-3,17-dione by rabbit liver slices was first observed by Clark and Kochakian (85). The DPN-dependence of this reaction was demonstrated by Samuels and co-workers (446). Sweat *et al.* (501) have concentrated the enzyme responsible for this transformation and found that it occurs in the cytoplasm, contains neither a metal nor cytochrome, has an estimated molecular weight of less than 43,000 and a Michaelis constant, $K_m = 3.3 \times 10^{-5}$ mole/liter. Although enzymes in the kidney can also effect this transformation, they appeared to be present in lower concentration in this organ (289, 543). Samuels and his colleagues (447) have explored the phylogenetic aspects of this reaction as well as that of Reaction 3. Analogous conversions with other steroid substances have also been observed. Thus, Schneider and Mason (466) incubated 5-androstene-3β,17β-diol with liver slices and isolated the 17-ketone, dehydroisoandrosterone (see also 287). Estrone, the 17-ketone, was likewise isolated by Ledogar and Jones (308) by the incubation of estradiol with acetone powders prepared from beef liver. This conversion has been confirmed using liver slices and breis (69).

Reaction 2

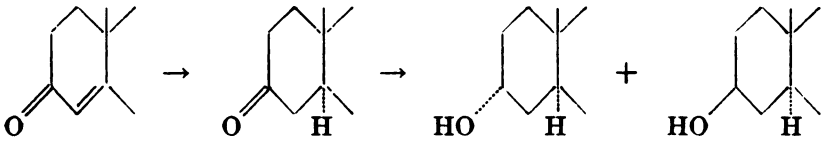


This conversion in over-all effect is the reverse of Reaction 1. Thus, from the incubation of dehydroisoandrosterone with rabbit liver slices, Schneider and

Mason (465) isolated 5-androstene-3β,17β-diol. Similarly, these investigators isolated androstane-3α,17β-diol (about 15% yield) after the incubation of androsterone, and etiocholane-3α,17β-diol (about 33% yield) after the incubation of etiocholan-3α-ol-17-one. In the latter experiment, Schneider and Mason also isolated the 17α-epimer, etiocholane-3α,17α-diol (8.5%). That Reaction 2 may be accelerated by acids of the Krebs cycle has been demonstrated by West and Samuels (543). The interconversion of estrone and estradiol by various normal and neoplastic tissues has been reported by Engel and Ryan (145).

Dehydrogenation (Reaction 1) and hydrogenation (Reaction 2) can occur with oxygen functions located at C_3, in addition to those at C_{17}. For example, Schneider and Mason (466) isolated the diketone, androstane-3,17-dione as well as androstan-3β-ol-17-one (isoandrosterone) after incubating androsterone with liver slices. They also found etiocholane-3,17-dione (1.5%) after the incubation of etiocholanolone.

Reaction 3



The *in vitro* reduction of the α,β-unsaturated carbonyl group, a characteristic grouping of the biologically active hormones, has been observed with testosterone (446), Δ^4-androstenedione (543), progesterone (549) and desoxycorticosterone (463). The enzymatic destruction of the Δ^4-3-keto group of testosterone by liver tissue has been shown to be specifically catalyzed by citrate. However, the cofactor necessary for the hydrogenation of Δ^4-androstene-3,17-dione was not citrate and is as yet unidentified (543). The capacity of the kidney for such hydrogenation has been demonstrated in the dog and rat but not in guinea pig or rabbit (543).

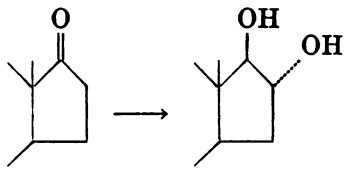
The metabolic destruction, presumably reduction, of the conjugated system of ring A of progesterone by rat and rabbit liver was studied in detail by Wiswell and Samuels (549). This transformation, unlike the analogous one in testosterone, proceeded at normal rates in the virtual absence of oxygen. It was not influenced by dicarboxylic acids nor DPN but was accelerated by the presence of one of the tricarboxylic acids as well as cysteine and cyanide. The product(s) of the reduction was not isolated. Incubation experiments with progesterone-21-C^{14} likewise did not lead to identifiable products but the results indicated that the radioactivity was present mainly in the fractions which would contain the reduced products, the pregnanolones and pregnanediols.

When desoxycorticosterone was incubated with surviving rat liver, Schneider and Horstmann (463) observed that the α,β-unsaturated ketone group was reduced by an enzymatic reaction which was independent of glucose, citrate, pyruvate or DPN. Later, Schneider (462) isolated some of the products of this reaction and proved them to be four allo (5α) compounds: a) the C_3-ketone, 21-hydroxyallopregnane-3,20-dione (1.4%) (only the double bond had been

reduced), b) the two saturated C_3 alcohols, 3α,21-dihydroxyallopregnan-20-one (4%) and 3β,21-dihydroxyallopregnan-20-one (22%), and c) the saturated triol, allopregnane-3α,20α,21-triol (0.8%) (see Reaction 6).

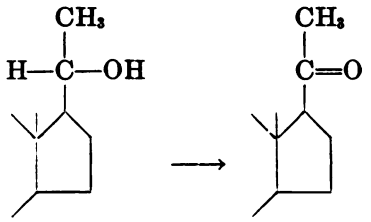
The *in vitro* metabolism of cortisone by liver minces and breis was investigated by Clark (83) who was able to demonstrate a similar disappearance of the conjugated system in ring A. That incubation of Compound E with surviving tissue slices could also destroy its glycogenic activity was shown by Paschkis *et al.* (373) for liver, brain and diaphragm and by Louchart and Jailer (325) for spleen and kidney as well. Later, Schneider and Horstmann (464) incubated Compounds E, F, S and A with rat liver and observed rapid reduction of the α,β-unsaturated ketone group. Kidney slices also carried out this reaction but to a lesser extent. Extensive alterations also occurred in the side chains of these compounds but neither 17-ketosteroids nor other products could be isolated. Hechter *et al.* (233) reported that although the perfusion of corticosteroids through a rat liver resulted in a rapid disappearance of the α,β-unsaturated ketone, 40–50% of the transformed product still retained the ketol side chain after 5–10 cycles.

Reaction 4



Dehydroisoandrosterone has been converted by incubation with rabbit liver slices under aerobic conditions into 5-androstene-3β,16α,17β-triol in 2.5–8.9% yield (465). Since this and several other 16α-hydroxylated products have been isolated from urine, this hydroxylation reaction appears to represent a detoxification mechanism, probably in preparation for further oxidative degradation of ring D of the nucleus.

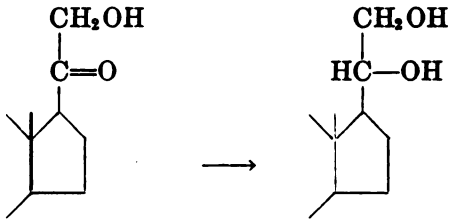
Reaction 5



The *in vitro* conversion of a C_{20}-ketone to a $C_{20\alpha}$-alcohol has not yet been accomplished. However, the reverse reaction has been demonstrated. Grant and Marrian (189) observed that incubation of pregnanediol (as the hemisuccinate) with rat liver preparations resulted in the rapid conversion of the steroid to unkonwn products. DPN was required and cyanide did not inhibit the conversion. Sub-

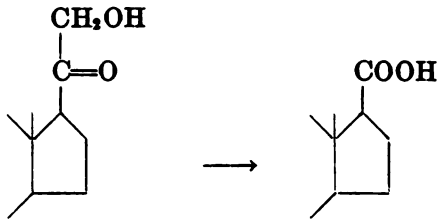
sequently, in a large scale experiment, the 20-ketone, 3α-hydroxypregnan-20-one was isolated in 10% yield along with 50% of the unchanged diol (188).

Reaction 6



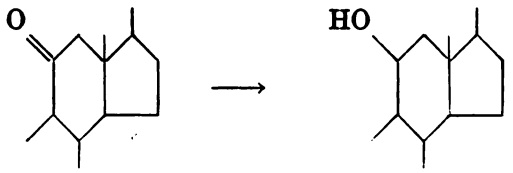
As mentioned above, Schneider (462) has isolated the glycol, allopregnane-3α,20α,21-triol in about 1% yield following the incubation of desoxycorticosterone with rat liver slices.

Reaction 7



The perfusion of desoxycorticosterone through rat livers enabled Picha *et al.* (395) to prove the conversion illustrated above; Δ^4-3-keto-etiocholenic acid was obtained in 3.3% yield from the perfused desoxycorticosterone.

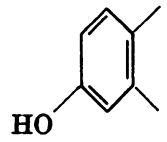
Reaction 8



When cortisone was incubated with rat liver slices and the resulting products chromatographed on paper, Eisenstein (140) detected hydrocortisone (Compound F), in addition to recovered starting marterial. The conversion of Compound E to Compound F by pig, rat and beef liver homogenates in 30% yield has recently been reported by Fish *et al.* (157). This confirms the *in vivo* results reported by Mason (338) who isolated Compound F from the urine of patients receiving Compound E and the results of Burstein *et al.* (59) who isolated Compounds E, F, 3α,17α,21-trihydroxypregnane-11,20-dione (tetrahydrocortisone), and 3α, 11β,17α,21-tetrahydroxypregnan-20-one (tetrahydro F) from the urine of patients receiving Compound E. Since tetrahydrocortisone is a urinary metabolite

of Compound F, it is apparent that the reverse of Reaction 8 may also occur *in vivo*.

Reaction 9



⟶ unknown non-phenolic products

The *in vitro* destruction of estrone or estradiol both by incubation with liver slices or minces (236, 568) and by perfusion through the liver (459, 539) or kidney (457) has been amply demonstrated. While the nature of the inactive products remains obscure, the mechanism undoubtedly involves deep-seated changes. It is likely that these include alterations in the phenolic ring and not only interconversions among estradiol, estrone and estriol. Conjugation to the sulfate or glucuronoside was not responsible for this inactivation. Oxidative systems appeared to be involved in these transformations, since cytochrome oxidase—cytochrome C and an unknown dehydrogenase can cause a loss of estrogenicity (312). That the phenolic ring A is the point of oxidative attack was suggested by the ease with which estrone and estradiol were acted upon by tyrosinase and laccase (191). Likewise, recent results on human as well as rat liver slices lent support to this suggestion. Lieberman *et al.* (319, 505) studied the *in vitro* metabolism of estrogens by means of a colorimetric method which is dependent upon the presence of an intact phenolic ring. By the use of this colorimetric assay, the products of metabolism were shown not to be phenolic and therefore could not consist solely of estrone or estradiol. From their results, they estimated that a 1500 g. human liver had the capacity to inactivate 1.5 g. of estradiol per day. Biological evidence that human liver can inactivate estrogens has been presented by several other investigators (151, 280). The inactivation of estrone by liver homogenates has also been studied, using a fluorimetric method (154). The D ring is also a likely site for further reaction. *In vivo* experiment with estrone-16-C^{14} (307) resulted in the degradation of this ring and the formation of radioactive CO_2.

The intracellular distribution of the estrogen-inactivating system in rat liver and other tissues has been studied by Riegel and Meyer (424). Using bioassay as a test method, they found that neither nuclei, mitochondria, microsomes nor the supernatant alone could inactivate estradiol. When the microsomes were combined with the supernatant or with riboflavin monophosphate, the activity originally present in the homogenate was restored.

In spite of the fact that nearly all, if not all, of the steroid metabolites are excreted in the urine in conjugates form, as glucuronosides, sulfates or possibly other modes, the ability of liver or other tissue to conjugate steroids *in vitro* has yet to be demonstrated. However, isolated liver systems are known to effect the conjugation of phenols (23, 113) and other molecules to produce glucuronosides or sulfates.

2. Excretion studies in hepatic disease. The role of the liver in the *in vivo* metabolism of the steroids in humans can be surmised indirectly by studying the urinary steroid excretion of patients with hepatic disease. In evaluating the results of these experiments, it must be borne in mind that the liver probably has an enormous capacity for metabolizing steroids and therefore, only in cases of extensive hepatic damage or in instances of specific metabolic defects is information concerning the role of the liver in the catabolism of the steroids likely to be secured. It has long been appreciated that the metabolism of the steroids in humans is considerably different from that in animals and therefore there is little reason to expect that the human liver *in vivo* necessarily causes the same alterations in the steroid hormones that animal tissues effect *in vitro*. Urinary excretion studies indicate that some of the metabolic transformations that the human liver can carry out *in vivo* do coincide with those demonstrated by *in vitro* experiments using animal tissues. On the other hand, there are some alterations, suggested by excretion studies, that have not been observed in *in vitro* experiments and contrariwise, there are some reactions known to occur *in vitro* that have not been observed *in vivo*.

It is evident from an analysis of excretion studies that in hepatic disease certain pathways by which steroids are normally metabolized are impaired. These may be summarized as follows: The unspecified reactions leading to the conversion of the estrogens to unidentified (probably non-steroidal) metabolities are depressed, resulting in an excessive excretion of intact estrogens in the urine of cirrhotics. The capacity of the liver to convert the 17-hydroxyl group, present in testosterone, to a 17-ketone is likewise diminished. This deficiency may be responsible, in part, for the low 17-ketosteroid excretion observed in these patients. The capacity of the damaged liver to conjugate the steroid metabolites, especially with glucuronic acid, appears also to be impaired. This, too, may result in abnormally low values of urinary 17-ketosteroids. Another reason for low ketosteroid values may be the failure of the diseased liver to convert adrenocortical hormones to 17-ketones, a reaction involving oxidative fission of the side chain. As far as the metabolism of progesterone is concerned, its conversion to pregnanediol appears to occur in a normal manner. However, the further metabolism of pregnanediol to as yet unknown and probably non-steroidal products is impaired.

That the ability of the liver to convert estrogens to further products is impaired was suggested by the excretion of excessive amounts of estrogens in individuals with hepatic disease (177). Recent studies by Gyorgy *et al.* (129, 420) seemed to indicate that a decreased biliary excretion of estrogens may be responsible for their increased urinary excretion in this disease. Both free and conjugated estrogens were excreted in excessive amounts and the degree of increase could be correlated with the bromsulphalein retention and serum bilirubin estimates. In patients with gynecomastia, the total excretion of estrogens was high, but the excretion of estriol (both free and conjugated) was increased to a greater extent than that of either estrone or estradiol.

Another indication of the diminished ability of the liver to catabolize steroids

is the depressed 17-ketosteroid excretion by patients suffering from hepatic disease, although individual values frequently were normal (177). In 14 out of 17 patients with liver disease, the urinary 17-ketosteroids were reported by Gyorgy *et al.* (129) to be less than normal. Bongiovanni and Eisenmenger (42) observed that the greatest diminution in these low 17-ketosteroid values occurred in the α-ketonic steroid fraction. They found that adrenal failure was not responsible for the low ketosteroid values, for stimulation of the adrenal by the administration of ACTH did result in an increased ketosteroid excretion. There were indications that the cirrhotic liver was less able to degrade adrenal hormones to ketosteroids. Butt et al. (65) came to a similar conclusion for when studying cortisone metabolism in two cirrhotics they found less conversion to 17-ketosteroids than in the normal.

That patients with cirrhosis can transform the 17-hydroxyl group of a C_{19}-steroid to a 17-ketone was shown by Bongiovanni and Eisenmenger (42) who found elevated ketosteroid values after the administration of testosterone propionate. However, the magnitude of this elevation was found by Cantarow *et al.* (68) and West *et al.* (542, 545) to be less than the one encountered in normal persons to whom testosterone or testosterone propionate was given. Within one hour after the intravenous injection into normal persons of testosterone dissolved in albumin, West *et al.* observed that, on the average, 16.4% of the hormone was converted into 17-ketosteroids. On the other hand, patients with liver disease who were similarly treated could transform only 4.6% of the administered testosterone. Because the testosterone in the blood disappeared equally rapidly in both groups but the 17-ketosteroid levels in the blood appeared more slowly in cirrhotics, West *et al.* concluded that some intermediate between testosterone and the 17-ketosteroids accumulated in patients with liver disease. Since a considerably smaller fraction (about $\frac{1}{3}$ compared to $\frac{3}{4}$ in normals) of the administered testosterone was excreted into the urine as 17-ketosteroids by patients with liver disease, a predominance of metabolic pathways other than those leading to 17-ketosteroids was indicated. Testosterone itself was not excreted in the urine of either group.

An interesting feature of the experiment of West *et al.* (545) was the fact that the greater portion of the intravenously administered testosterone had been excreted in the urine as 17-ketosteroids before there was any measurable effect upon nitrogen balance. The metabolic effect of a single dose continued for several days after the hormone ceased to be identified in the blood or excess 17-ketosteroids appeared in the urine. On the fifth to eighth day after injection, there again appeared a slight increase in the ketosteroid excretion. This was interpreted to mean that the first rapid excretion of the 17-ketosteroids represented the metabolites of excess and not active testosterone; the subsequent rise may represent the eventual metabolism of the active hormone (544). The renal plasma clearance of the 17-ketosteroids in normals and cirrhotics was about the same. The results indicated that the 17-ketosteroids were filtered rapidly through the glomerulus, were not reabsorbed and were excreted (545). In kidney disease, the rate of formation of 17-ketosteroids following the intravenous administration of

testosterone was rapid, as measured by their presence in blood, although the amount of 17-ketosteroids in the urine was very low.

Since larger amounts of free steroids were found in patients with liver disease, Cantarow *et al.* (68) concluded that hepatic damage also impairs the process of conjugation. Although Bongiovanni and Eisenmenger (42) could isolate almost no free ketosteroids from the urine of cirrhotics, they concluded, too, that the conjugation mechanism was impaired, because the fraction of ketosteroids excreted as glucuronosides was considerably less than that found in normals. Whereas normals excrete about 50% of the total ketosteroids as glucuronosides, the value in cirrhotics was 10–30%. These authors concluded that since the liver has a diminished capacity to conjugate, the steroid metabolities are probably not cleared by the kidney and can follow alternative pathways of metabolism. This further degradation, possibly to non-steroidal products, may account, in part, for the decreased ketosteroid excretion. Contrary results were obtained from patients with liver disease to whom testosterone propionate had been administered. Thus, Engstrom *et al.* (148) reported no impairment of the process of conjugation in these patients. Also, West *et al.* (545) found that those steroids which were excreted in the urine of patients after testosterone administration were mainly in conjugated form.

The metabolism of progesterone in patients with severe liver disease has been studied by Paschkis *et al.* (372). They measured the excretion of pregnanediol after administering 100 mg. of progesterone per day to such patients for three to four weeks. More pregnanediol was recovered from the urine of the diseased patients than from that of normal individuals and therefore, the conclusion was reached that the mechanism for the conversion of progesterone to pregnanediol was unimpaired but the further metabolism of pregnanediol (to unknown products) was depressed in these patients. Parenthetically, it may be pointed out that arthritics showed the same excretion pattern as did the cirrhotics.

An interesting observation concerning a possible relationship between liver function and progesterone metabolism has been made by Karnofsky *et al.* (274). These workers found that newborn mice were highly susceptible to the lethal action of progesterone. However, within 2–3 days resistance developed, and by the 7th day a mouse could tolerate 100 times the amount of progesterone it could when 1 day old. This suggested to the authors that a mechanism appeared (probably in the liver) during this time which enabled the mouse to metabolize progesterone in a normal manner.

That the liver can metabolize the adrenal hormones *in vivo* was proved by Nelson *et al.* (360) who compared the level of 17-hydroxycorticosteroids present in the blood of the arterial system with that of the hepatic vein. In normals, the difference in the arterial and venous values was about 30% and after the intravenous injection of cortisone as much as 60% difference was discernible. Butt *et al.* (65) studied cortisone metabolism in two cirrhotics and found that less cortisone was converted to 17-ketosteroids when the liver was damaged. The excretion of corticoids was normal before treatment with cortisone; after treatment, the rise in the excretion of corticoids was not significantly different from that in

similarly treated normals. Bongiovanni and Eisenmenger (42) found that patients with Laennec's cirrhosis excreted only slightly elevated amounts of corticoids (measured as reducing steroids, formaldehydogenic steroids and by glycogen deposition bioassay). However, a group of young women with undetermined cirrhosis which is characterized by many of the same features seen in Cushing's syndrome did have elevated corticoid excretion values. Administration of ACTH increased the reducing steroids of both groups of patients.

Steroids in Bile and Feces

Numerous experiments using isotopically labeled steroids have pointed to the bile and feces as important excretory routes for the metabolites of the steroid hormones (175, 220, 514). Unfortunately, most of the published data record the situation that obtains for small animals and it seems highly unlikely that the metabolism of steroids in small animals parallels that in humans. The available evidence suggests that the human metabolizes steroids in a unique manner and, therefore, until human experiments employing labeled steroids are reported, the importance of bile and feces as excretory pathways in this species must be based upon meager evidence.

1. Bile. Estrogens. Estrogens have been recovered from the bile of dogs and cows and have been detected in the bile of pregnant women (382). Estrone, for example, has been isolated from cows' bile (388). The existence of an enterohepatic circulation for estrogens has been postulated by Cantarow *et al.* (70, 71) from experiments performed on dogs. This thesis hold that the estrogens are rapidly removed from the circulation by the liver and excreted by way of the bile into the duodenum. From there, like the bile salts, they are reabsorbed and enter into an enterohepatic circulation, small amounts being lost in the feces each time. When estrone was injected into bile-fistula dogs (382, 389), only 35% of the activity was recovered in the bile in 4 days; the ratio of estrone to estradiol recovered being 4.4/1. Only 1–3% of the activity was accounted for in the urine and feces.

Both free and conjugated estrogens were found in the bile of pregnant women by Pearlman and Rakoff (387); although they could not isolate estriol, its presence was suggested. Stimmel (492) has analyzed the bile of patients to whom estrogens had been administered exogenously. In one case, 3% of the ingested estriol was recovered in the bile and 11.5% in the urine. He reported that both free and conjugated forms of estriol (in the ratio of 1/2) were present in the bile.

Androgens. Etiocholane-3α,17β-diol has been isolated by Pearlman and Cerceo (379) from the bile of pregnant cows. Although this compound, like testosterone, is composed of 19 carbon atoms, it seems unreasonable, in view of its source, to suppose that it is derived from that hormone. Paschkis *et al.* reported the excretion of androgens in the bile of dogs after the intravenous administration of testosterone, androsterone and methyltestosterone (374). An interesting study has recently been reported by Rubin and her colleagues (437) who gave huge doses of testosterone propionate orally to three patients. No increase in the biliary excretion of either 17-ketosteroids or androgens, as measured by the bioassay, could be found, although there was considerable increase in the excretion of

17-ketosteroids in the urine. In contrast, when estradiol propionate was fed to one of these patients, the biliary excretion of the estrogens, estrone, estradiol and estriol, rose 8–10 times. The urinary excretion during the same time increased more than 30 times.

Progesterone and corticosteroids. Several compounds, apparently related to progesterone, have been isolated from bile. From ox bile Pearlman (378) isolated allopregnane-3β,20β-diol and from unhydrolyzed bile of pregnant cows, Pearlman and Cerceo (379) isolated 3α-hydroxypregnan-20-one and pregnane-3α,20β-diol. From the bile as well as the urine of post-menopausal women given Δ^5-pregnenolone, Pearlman and Pincus were able to isolate pregnane-3α,20α-diol (385). The isolation of pregnane-3α,20α-diol from the bile of patients to whom progesterone had been administered has also recently been reported (431).

No steroid has been isolated from the bile that can be related unequivocally to the adrenal corticosteroids. But as will become apparent from a consideration of the metabolic pathways, it is conceivable that some of the above mentioned compounds, especially allopregnane-3β,20β-diol and etiocholane-3α,17β-diol, could be derived from adrenal steroids.

2. Feces. Of the four types of steroid hormones, only the estrogens have been detected in human fecal extracts (276). In recent years estrone, estradiol and estriol in the free form have been detected in the feces of pregnant women (493). After acid hydrolysis, additional amounts of estriol but not of the other two compounds were recovered. Simultaneous analysis of the urinary estrogens revealed that much higher concentrations of estrogens were present in the urine, the ratio of urinary to fecal estrogens being: for estrone 8.5, for estradiol 3.7, and for estriol 4.2.

In extensive studies with isotopically labeled C_{19}-steroids administered to rats or mice, Barry *et al.* (15) as well as others (175), have found appreciable amounts of the radioactivity in the feces. When, however, a search was made for testosterone metabolites in the feces of normal men, none were found, in spite of the fact that the best available isolation methods and infrared spectral analysis were used (175). Later, Gallagher (174) commented that when C^{14}-labeled steroids were administered to humans, no significant amounts of radioactivity appeared in the feces. In spite of the fact that the feces represents the most important excretory route in some species, the available data suggest that in humans it is a minor pathway.

Steroids in Urine

1. *Urinary steroid conjugates and their hydrolysis.* Practically all of the steroid metabolites excreted in the urine occur in conjugated form, either as sulfates, glucuronosides or other forms as yet unidentified. Since the isolation and characterization of these water-soluble derivatives has proved difficult, it has been found necessary to liberate the steroid metabolites from their conjugates in order to identify them. A completely satisfactory method for the hydrolysis of any class of the conjugates of urinary steroids has still to be described, and this has been a great deterrent to progress in this field.

Boiling with acid was the first hydrolytic procedure used and for some fifteen

fifteen years has remained the principal if not the sole method. It has long been recognized, however, that this drastic procedure not only cleaved the conjugates but frequently caused undesirable alterations in the steroid metabolites themselves. These alterations frequently gave rise to artifacts which often, however, could be related by virtue of their chemical structures to the steroid metabolites from which they were probably formed. Thus the presence of a chloro group where a hydroxyl (or sulfate) probably existed before, or the occurrence of a double bond (*e.g.*, between C_3 and C_4 or between C_9 and C_{11}) at a carbon atom where hydroxyl was presumed to have been, did not alter the significance of these compounds for the elucidation of the metabolic pathways. More serious, however, were those conversions that produced such deep seated alterations (*e.g.*, in the estrogens by oxidation or in corticoids by reactions involving the acid-labile side chains) that the artifacts were completely lost to estimation or isolation. Thus, the corticoids are quantitatively converted by acid hydrolysis at elevated temperatures to unrecognized, biologically inactive products. Were it not for the fact that Compounds E and F are partially excreted in free form, permitting their detection by bioassay (526), knowledge of this class of metabolites might be considerably more meager than it is at present.

In order to interpret properly the many reports on urinary steroids, cognizance must be taken of the limitations of the methods of hydrolysis. Failure to bear these in mind has embarrassed many investigators and negates many time-consuming investigations. One example will serve to illustrate this point. As late as 1950, the excretion by normal individuals of the adrenal metabolite, dehydroisoandrosterone, was usually considered to be of the order of 0.2 mg./day. This value was obtained after hydrolysis of the urine with acid at reflux temperature. When, however, milder methods of hydrolysis were employed (435) the true values were shown to be as much as 2–5 mg./day. Thus dehydroisoandrosterone has been transformed from a minor constituent of the 17-ketosteroid fraction into one of the most abundant.

Of the many steroid metabolites known to occur in urine, only a limited number have been isolated in the form of their conjugates. The following steroids have been isolated from the urine as their sulfates: dehydroisoandrosterone (353), estrone (455), androsterone (527), 3β-hydroxyallopregnan-20-one, 3β-hydroxy-16-allopregnen-20-one and uranediol (281). In addition to these, there is suggested evidence that the following steroids may also be present in the urine as sulfates or other easily hydrolyzable conjugates: estradiol and estriol (88), androstanedione, etiocholanedione, etiocholanolone and 11-ketoetiocholanolone (314, 337).

Of the two known types of steroid conjugates, the sulfates are much the more easily cleaved by acid hydrolysis. In fact, it is merely necessary to extract continuously an acidified solution (pH 1) of these conjugates with ether to liberate the free steroids (314). Using an acetate buffer at pH 4.7, Bitman and Cohen (29) have hydrolyzed sulfates of neutral steroids having a double bond β to the sulfate group (*e.g.*, sodium dehydroisoandrosterone sulfate); sulfates of saturated alcohols were not split by this method. It has also been demonstrated (508) that this procedure may lead to the formation of a 3,5-cyclosteroid. Dioxane has also been shown to split steroid sulfates (187) but unfortunately

this technique cannot be applied directly to urinary sulfates, since water is one of the substances which interferes with the cleavage. Cohen and Oneson (91), using residues from the concentration of butanol extracts of urine (pH 2–3) which contained the ketosteroid conjugates, reported that HCl in dioxane effected the hydrolysis of the total ketosteroids in urine. On the other hand, dioxane-trichloroacetic acid mixtures hydrolyzed only 40%, presumably only the sulfates of the ketosteroids. Jensen and Tötterman (265, 266) have suggested a fractional acid hydrolysis of 17-ketosteroids which they claim gives higher values than several other methods tried by them. A scheme of differential hydrolysis based upon the ease with which sulfates are cleaved, pH 1 at room temperature and continuous extraction of the liberated steroids into ether, was suggested by Lieberman and Dobriner (124, 314). Under these conditions, the glucuronosides were also extracted into the ether, probably in the form of lactones. Since these lactones are alkali soluble they could be separated from the free steroids and could be subsequently hydrolyzed by glucuronidase or acid at elevated temperature. Still other steroid conjugates appeared to be present in the urine since boiling with acid liberated additional quantities of free steroids. In a more recent paper, Buehler and his colleagues (58) have demonstrated that when urine is made 7.2 N with respect to hydrochloric acid and then continuously extracted at room temperature with ether, equal or higher yields of 17-ketosteroids were obtained than could be recovered by a combination of processes involving both acid (pH 1 at room temperature) and enzymatic hydrolysis. It is apparent that under these conditions the steroid glucuronosides were also being cleaved. Estrogen sulfates may be split by phenolsulfatase (87) but as yet no alcohol sulfatase has been reported that will hydrolyze ketosteroid sulfates.

Although no sulfate ester of a corticoid has been isolated, acidification of the urine to pH 1 has been shown to yield increased corticoid titers (314, 525). Paterson *et al.* (376) have studied the liberation of formaldehydogenic steroids at various time intervals after acidification of the urine to pH 1 and concluded that there were two modes of corticoid conjugation: 1) acid-labile conjugates (presumably sulfates) from which free corticoids are obtained by chloroform extraction of acidified but not neutral urine, and 2) acid-stable steroid conjugates (presumably glucuronosides) which cannot be extracted by chloroform from neutral or acidified urine. Subsequently these investigators (377) showed that the first type was also hydrolyzed at pH 3–4 at room temperature, but was not split by glucuronidase. The chloroform-insoluble fraction was split by the enzyme and, therefore, was considered to be conjugated with glucuronic acid.

The glucuronosides represent the second major mode of conjugation by which the steroids are known to be excreted in the urine. The following steroids have been isolated from the urine in the form of their glucuronosides or have been liberated in free form by the enzyme, β-glucuronidase: estriol; estrone (369); pregnane-3α,20α-diol; 3α-hydroxypregnan-20-one; pregnane-3α,17α,20α-triol; 3α,17α-dihydroxypregnan-20-one (340); 3α,17α,21-trihydroxypregnane-11,20-dione; androsterone; etiocholanolone (337, 542); etiocholane-3α,17α-diol (337); and 16-androsten-3α-ol (55) (for earlier references, see 153).

Although many steroid conjugates have been isolated from urine, the difficul-

ties involved are such that few attempts have been made to recover these derivatives systematically. As mentioned earlier, Jayle *et al.* (258) have investigated intensively the steroid glucuronoside content of urine. They have examined the glucuronoside fractions extracted into butanol at pH values varying from 2–13. Those extracted at pH 13 (acetone insoluble) were considered to contain the pregnanediol conjugates; those extracted at pH 11 (acetone soluble) were thought to be related to testosterone metabolites; and those obtained at pH 6–11 appeared to be the corticoid metabolites. The extraction of the conjugates of the 17-ketosteroids from urine has been accomplished using cationic resins or reversed phase chromatography (6).

For the hydrolysis of the glucuronosides by acid, elevated temperatures are required and this, as mentioned previously, may have deleterious effects on many urinary steroids. Reference has already been made to the results of Buehler *et al.* (58) which indicated that these conjugates may be cleaved by concentrated acid solutions (7.2 N) at room temperature. In addition, much effort has been expended on studying the hydrolysis of these conjugates enzymatically with spleen glucuronidase (506) or bacterial glucuronidase (275). However, as far as the estrogens, 17-ketosteroids and pregnanediol are concerned, the employment of glucuronidase, while probably superior in a qualitative way, does not afford increased recoveries of these substances over that obtained from acid-hydrolyzed urine.

In a brilliantly critical discussion of the hydrolysis of conjugated estrogens in human urine, Marrian and Bauld (335) recommended boiling with acid for 1 hour as the optimum conditions for the liberation of the estrogens. The enzyme hydrolysis of the estrogen glucuronosides has been reported by two groups. Katzman *et al.* (275), using a potent bacterial glucuronidase, reported the liberation of amounts of estrogen from human pregnancy urine equal to or greater than that obtained by acid hydrolysis. Oneson and Cohen (369) have reported that most of the estrone present in human pregnancy urine was liberated by spleen glucuronidase.

A significant contribution to the problem of the hydrolysis of estrogen conjugates has been made by Beer *et al.* (22). These investigators studied various methods of hydrolysis of the conjugated urinary estrogens resulting from the intravenous administration of estrone-16-C^{14} and 17β-estradiol-16-C^{14} to human females. Following enzymatic hydrolysis with calf spleen β-glucuronidase, most of the radioactivity in the urine was found in the phenolic fraction.

As far as the enzymatic hydrolysis of the 17-ketosteroid glucuronosides are concerned, Bitman and Cohen (28) and Cohen (89) found that 50% of the "total" 17-ketosteroids (the amount liberated by acid hydrolysis at reflux temperatures) was liberated by spleen glucuronidase; only three percent of the β-ketosteroids (principally dehydroisoandrosterone) was freed by this enzyme. By combining this procedure with their method of buffer hydrolysis (pH 4.7) (29), these workers obtained $\frac{2}{3}$ to $\frac{3}{4}$ of the "total" ketosteroids. Katzman *et al.* (275) reported that the major portion of the 17-ketosteroids present in normal male urine was liberated by bacterial glucuronidase and therefore was conjugated with glucuronic acid. This is not true for the ketosteroids present in the

urine of patients with adrenal tumors, a condition in which large amounts of dehydroisoandrosterone are excreted conjugated almost completely with sulfuric acid (29).

Sodium pregnanediol glucuronidate was hydrolyzed rapidly and apparently completely by spleen glucuronidase (54, 90). Cohen (89) found that this glucuronidate was hydrolyzed 20 times more rapidly than were the glucuronosides of the corticoids and these, in turn, were cleaved faster than those of estriol and the 17-ketosteroids. Since heat-labile factors (bacteria, enzymes, etc.) in the uring interfered with the enzymatic hydrolysis, Cohen recommended that urine be sterilized before incubation with glucuronidase.

Although acid hydrolysis appears to be as effective as enzyme for the fission of the conjugates of several structural types of urinary steroids, such is not the case with the urinary corticoids. Acid hydrolysis at elevated temperatures is required to cleave the corticoid conjugates (predominantly glucuronosides) and this treatment results in the destruction of the corticoids themselves. Many investigators have observed increases in the quantities of corticoids liberated by spleen β-glucuronidase as compared to those obtained by acid hydrolysis at pH 1. Thus, a 10- to 20-fold increase in formaldehydogenic titer was reported by Corcoran *et al.* (101), 12- to 27-fold increase by Cohen (89), 10- to 20-fold increase by Venning (524) and a 10-fold increase by Paterson and Marrian (377). A 2- to 4-fold increase as determined by bioassay was observed by Venning (524). Using bacterial glucuronidase, Kinsella *et al.* (279) reported that 3 to 5 times more reducing steroids could be obtained by this enzyme hydrolysis than could be recovered from unhydrolyzed urine.

Although there can be no question of the efficiency of glucuronidase hydrolysis for the liberation of the urinary corticoids, the proper evaluation of the various methods of hydrolysis of the conjugates is completely dependent upon the specificity of the corticoid assays. Since the significance of the formaldehydogenic and reducing steroid determinations as true and specific measures of corticoids is unclear, the exact appraisal of the hydrolytic procedures becomes difficult. Excellent discussions of the problems involved in the hydrolysis and extraction of formaldehydogenic corticoids have been given by Bayliss (21) and Marrian (334).

2. *Analysis of the urinary steroids.* In order to evaluate properly the results obtained from the study of steroid metabolites in terms of their physiological significance, some attention must be given to the methods of analysis of urinary steroids. Since many valuable reviews have been written discussing in detail the available methods, their advantages and limitations, only the general aspects of the analytical procedures as well as any pertinent advances made in recent years will be considered here.

The techniques used for the analysis of urinary steroids may be divided into three categories: a) bioassay, b) chemical or colorimetric estimation and c) isolation and identification of the individual components by typical organic chemical procedures. Since there are many misunderstandings and misstatements in the literature from the failure to recognize that these three types of analyses do not necessarily give the same answers, it may not be amiss to reiterate this here. Thus,

a chemical analysis is not a measure of a biological property nor need a bioassay measure a specific chemical grouping or compound.

Methods of bioassay were, historically speaking, the first techniques used for the detection and estimation of steroids in urine. Now, however, these methods are less popular, not only because they are more time-consuming and less precise, but because it is recognized that the active hormones themselves are rarely excreted in the urine. Testosterone and progesterone have never been found in the urine and only small amounts of estradiol and Compounds E and F are usually present. In addition to these, what is customarily measured by bioassay methods are metabolites of the hormones which, perhaps fortuitously, are biologically active. As significant is the fact that many, and frequently the most revealing urinary excretory products of the hormones, are biologically inactive; *e.g.*, etiocholanolone from testosterone, pregnanediol from progesterone and desoxycorticosterone, and tetrahydro E from Compound F appear to be devoid of biological activity. Another limitation of the bioassay technique is derived from the fact that the biologically active compounds are excreted in the urine in the form of their conjugates, most of which are biologically inactive. Since the bioassay methods are sensitive to minor alterations in structure, the failings of the hydrolytic procedures for the cleavage of the conjugates are here intensified and further curtail the usefulness of this type of analysis.

Although it is well-known that bioassay techniques can disclose nothing about the patterns of urinary steroid metabolites, it is less well recognized that the biological activity often reveals little about specific glandular precursors. So, for example, androgenicity need not be an index of testicular function. Androsterone, one of the most important androgens found in urine may be derived from adrenal as well as testicular secretory products. Similarly, dehydroisoandrosterone, which is known to be an index of adrenal and not testicular function, is also androgenic.

On the other hand, there are important advantages to be gained from the bioassay methods. Their extreme sensitivity cannot be duplicated by any other known method of analysis. Moreover, their high degree of specificity, while not confined to a single compound, characterizes unequivocally the biological property in question.

Although the chemical methods are usually less sensitive than the bioassay methods, they are generally cheaper, simpler, more rapid and more precise. Consequently, colorimetric assays have all but replaced biological methods. It must be pointed out, however, that a chemical analysis does not measure a biological activity and, in those instances where a biologically active material of unknown structure is sought, the bioassay techniques are indispensable.

The chemical or colorimetric estimations are also not without limitations. Although these techniques are designed to measure a specific chemical grouping, the presence of interfering substances, often encountered in the urinary extracts, limits the reliability of the methods and complicates the interpretations made from them.

Estrogens. In normal urine the estrogens, estradiol, estrone and estriol, are

excreted in only minute amounts so that all colorimetric or fluorimetric methods are extended to the limits of sensitivity. A serious interference to accurate estimations appears to be the so-called "background material" which has received some attention from Garst and Friedgood (176). They report the isolation of an interfering material which may possibly be an estriol catechol or quinone. The presence of other phenolic substances in urinary extracts may also create difficulties. When the estrogen content of urine is above 1 μg./ml., the modified Kober colorimetric assay gives satisfactory results (259, 490). The smaller amounts present in non-pregnant states require still more sensitive fluorimetric methods which, however, must be preceded by extensive preliminary purification of the extracts. Engel (143) has reviewed this subject and Bates and Cohen (18, 19) as well as others (179) have carefully studied the variables involved in the fluorimetric assay of the estrogens. Braunsberg (53) has made a comparison of five fluorimetric methods which have been suggested for the determination of estrogens and concluded that the most sensitive method was that of Bates and Cohen (18) and the method least influenced by impurities that of Finkelstein *et al.* (155).

Fractionation of the urinary phenolic steroids has been accomplished by counter-current distribution (143, 144, 146, 476), by adsorption chromatography (491), partition chromatography (20, 74, 502) and paper chromatography (10, 46, 47, 235, 367, 562). Stimmel *et al.* (494) have recently described a technique for the concomitant separation and estimation of pregnanediol and the three urinary estrogens in human urine. Stimmel has also measured the amounts of the three estrogens in the feces of pregnant women (493). Because estrogens are present in minute amounts in the urine of men and women, few investigations of these substances have been made. It is quite likely, however, that with the newer refined methods of estimation and fractionation of these trace substances, a revival of interest will ensue.

Ketosteroids. The original Zimmermann determination, or modifications thereof, still remains the most widely used and undoubtedly the best method for the estimation of the 17-ketosteroids present in biological extracts. Many modifications and refinements have been developed in recent years (135, 198, 411, 566, 567) and these have facilitated the manipulations involved, and enhanced the reliability and the sensitivity. The specificity of the test, however, is not limited to those substances containing a 17-ketone group. 20-Ketosteroids, saturated 3-ketosteroids and α,β-unsaturated-3-ketosteroids all respond to the test but give less color than do the 17-ketosteroids. Moreover, all 17-ketosteroids do not react to the same extent (546), for example, 11-keto-etiocholanolone gives 17% more color than androsterone.

Attempts to obtain more specific tests for individual steroids have succeeded in the case of dehydroisoandrosterone which can be measured by the Pettenkofer reaction (354) or the Allen test (5, 263, 264).

Another significant advance in the analysis of the 17-ketosteroids has been the development of methods for the fractionation and separation of the individual components of the mixture of substances known as the neutral 17-ketosteroids. These procedures all involve some form of chromatographic analysis. The older

techniques (127, 315) which were so successful in isolating and identifying many of the urinary steroids now known, required large amounts of material, and laborious, time-consuming methodology. The recent trend has been to develop standardized micro-methods which are applicable to small volumes of urine and which will give a qualitative and quantitative analysis of the major 17-ketosteroid components. A harbinger in this field has been Dingemanse and her coworkers (120, 121) who devised a systematized chromatographic analysis using alumina as an adsorbent and benzene containing increasing amounts of ethanol as eluent. Modifications of this procedure have been introduced by Devis (114), Zygmuntowicz *et al.* (569), Pond (405, 406) and Robinson and Goulden (429). Further improvements have been reported by Lieberman and Lakshmanan (300) which involve the replacement of the earlier technique of increasing the polarity of the eluate in discrete steps by a method consisting of continuous variation of the solvent polarity (gradient elution). In addition, the application of infrared spectroscopy to the identification of the individual components offered greater reliability than did the previous method of depending solely upon the position in the chromatogram for the identity of the eluted steroids. Silica gel (439) has also been employed as an adsorbent in the chromatographic analysis of the 17-ketosteroids. Displacement analysis of the steroids has been suggested by Hamilton and Holman (199) and partition chromatography has been used by Butt *et al.* (66). Another promising line of attack appears to be the separation of 17-ketosteroids by paper chromatography; several usable systems have already been described (9, 291, 293, 296, 297, 298, 356, 450, 472). The technique of paper chromatography is particularly applicable when only microgram amounts of materials are available for analysis.

Infrared spectral analysis has become almost indispensable for the unequivocal identification of steroids isolated from natural sources (270). The infrared spectra, frequently obtained from trace amounts of non-crystalline but purified materials, serve as fingerprints for the characterization of the substances. When this technique is used in conjunction with any of the above methods of separation, the reliability of the results is greatly enhanced.

The technical progress already achieved permits a qualitative and quantitative analysis of the most important 17-ketosteroids in a day's urine, or a fraction thereof and, thereby, makes possible the study of the 17-ketosteroid excretion in a wide variety of physiological and pathological situations. Not only can those extreme endocrinopathies as Addison's disease, Cushing's syndrome or adrenal hyperplasia be extensively studied but disorders such as mental disease, hypertension, and many others can now be investigated for any subtle relationships that may exist between the disease and steroid hormones.

Pregnanediol. Several methods, none entirely satisfactory, are available for the estimation of pregnanediol, the most important urinary metabolite of progesterone. The classical method of Venning (522) consists of the isolation and measurement of sodium pregnanediol glucuronidate, the conjugated form in which this steroid is found in the urine. Among other difficulties, this method suffers from lack of specificity, for in addition to pregnanediol, four other compounds have been isolated from the glucuronidate precipitate (NaPG): 3α-hydroxy-

pregnan-20-one (497); 3α,17α-dihydroxypregnan-20-one (340); pregnane-3α, 17α,20α-triol (339); and 16-androsten-3α-ol (55). Since some of these products are related to the metabolism of progesterone, some to the metabolism of the adrenal cortical hormones and the last is of unknown derivation, great care must be taken in interpreting any results based on this determination.

Smith and Barker (478) have recently studied the increase in the NaPG complex which occurred when stilbesterol was administered to pregnant women. This increase, they claimed, was due to an unknown metabolite of progesterone and was not caused by the excretion of the glucuronosides of pregnanolone, stilbesterol, pregnane-3α,17α,20α-triol, pregnane-3α,20α,21-triol nor allopregnanediol.

A colorimetric method for the determination of pregnanediol depends upon the yellow color it develops in concentrated sulfuric acid (480). For reliable results highly purified fractions are necessary. Allopregnane-3α,20α-diol and allopregnane-3β,20β-diol (205) also respond to this test. An improvement has been made by deWatteville *et al.*, (116) who have purified the fraction to be assayed colorimetrically by preliminary chromatography on alumina (45, 115). Chaney *et al.* have recently described a simplified procedure for the determination of free pregnanediol (78, 156). Jayle and his associates in France (257) have made extensive studies of the glucuronidate fractions of human urine and have related these to progesterone, adrenocortical and testosterone metabolites.

Corticoids. Corticoids are urinary metabolites of adrenocortical hormones which are characterized by highly oxygenated side chains (α-ketols, dihydroxyacetone, glycol or glycerol types), similar to those which distinguish the hormones themselves. They have been referred to by other names, such as 11-oxy-steroids, reducing steroids and formaldehydogenic steroids, depending upon the method of analysis. The value of the interpretations based upon these methods, it must be emphasized, is determined and limited by the specificity of the method employed. The great variety of methods that have been proposed discloses the dissatisfaction that workers in the field have with existing techniques. Several reviews which discuss these problems in detail have appeared (143, 432, 488, 523). Since some of these metabolites are reducing substances, various oxidants such as alkaline copper (99, 507) or phosphomolybdic acid (222, 487) have been suggested but for the most part have fallen from favor due to lack of specificity. Another oxidant which is employed is periodic acid. This cleaves the corticoids to yield, as one of the products, formaldehyde which is then measured colorimetrically. This method (110) estimates the so-called formaldehydogenic steroids and is at present the most popular corticoid assay (102, 136, 240, 412). It gives what appear to be the most precise values, although there is still some doubt as to its reliability. Wilson (547) has recently compared the formaldehydogenic titers of corticoid extracts before and after fractionation by paper chromatography, and found enormous discrepancies. Only a small fraction of the corticoid material originally assayed could be accounted for after purification by paper chromatography. Considerable amounts of formaldehydogenic material are liberated by hydrolysis of the urine with the enzyme, β-glucuronidase, but it still remains to be proved that all this enzyme-liberated-formaldehyde-producing material is steroidal.

Another colorimetric method of corticoid analysis which is finding wide use

is the Porter-Silber technique (409) which is based upon the development of the yellow color produced from the reaction of steroids with dihydroxyacetone side chains with phenylhydrazine in sulfuric acid solution. Although Carrol *et al.* (75) applied this method to urinary extracts, they found the presence of interfering substances in the urine to be troublesome. In fact, Cope and Hurlock (100) could use this method only after preliminary chromatography on paper. Using crude extracts, Krupp *et al.* (299) could not detect steroids with dihydroxyacetone side chains in normal urine by this method. Reddy *et al.* (417, 418) have reported that the Porter-Silber determination can be applied to the butanol extracts of urine which purportedly contain the conjugates of the corticoids. Further work will have to be done before the reliability of the Porter-Silber method of analysis for urinary corticoids is insured. A similar statement can probably be made about the methods employing "blue tetrazolium" (BT,3,3'-dianisole bis-4,4'(3,5-diphenyl)tetrazolium chloride) (80, 256, 331) or 2- (p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride (239) or phenylhydrazine (182).

An ingenious suggestion has been made by Cox (104) who measured the "acetaldehydogenic" steroids, that is, those compounds which liberate acetaldehyde when oxidized with periodic acid. Compounds which fall into this category are those containing two hydroxyl groups on vicinal carbon atoms, C_{17} and C_{20}. After hydrolysis of the urine by glucuronidase, he found that the total excretion of such substances by a normal male, expressed in milligrams of pregnane-3α,17α,20α-triol per 24 hours, is 1–3 mg. Norymberski (366) attempted to determine the corticoids by the oxidation of the dihydroxyacetone side chain with sodium bismuthate and the measurement of the 17-ketosteroids so formed by the Zimmermann determination. In this way, he estimated the corticoid content of normal urine to be 7–8 mgs./day.

The widely discrepant values reported for urinary corticoid excretion by the use of different methods suggest lack of specificity in these estimations. Moreover, these methods are limited by the fact that they do not measure those important adrenal metabolites which lack the characteristic side chains of the hormones themselves and hence do not fall into the class of corticoids.

Some success has been achieved in the further fractionation of the urinary corticoids, especially by paper chromatography. Burton *et al.* (60) have analyzed a 72-hour sample of urine and have detected cortisone and hydrocortisone in amounts of 15–40 μg./day. Bush *et al.* (64), using a different chromatographic system (61), also found these substances in addition to other, unidentified, compounds. Larger amounts of corticoids were isolated by Baggett *et al.* (12) following the hydrolysis of the urine with bacterial glucuronidase and chromatography of the extracts on paper. They found that 3–6 mgs. of tetrahydrocortisone was excreted by adults in 24 hours, which was 10–50 times the amount recovered when enzyme hydrolysis was not employed. The increased recovery of tetrahydrocortisone following enzyme hydrolysis has also been demonstrated by Schneider (461). Hofmann and Staudinger (249) have proposed another system of paper chromatography which may find some use in the analysis of urinary corticoids. Romanoff and her colleagues (434), using a silica gel chromatogram followed by

paper chromatography, found it possible to separate a 48-hour urinary corticoid extract into approximately 20 steroidal components.

Since similar rates of movement of an unknown and a standard compound on a paper chromatogram represent a necessary but not sufficient criterion for their identity, it becomes necessary to invoke various other tests which are applicable to ultra-micro amounts of material for adequate characterization. In addition to using spot tests and mixed chromatograms of several derivatives, Zaffaroni (560) has measured the characteristic ultraviolet spectra that trace amounts of corticoids, as well as other steroids, exhibit in concentrated sulfuric acid solution. This technique, while not as specific as infrared analysis, is more sensitive and is finding wide use in the identification of minute amounts of steroids.

Isolation of urinary steroids. It is obvious that in order to relate a urinary steroidal metabolite to its hormonal precursor, the precise chemical structure of the metabolite must be determined. In the past, this was best done by the application of classical organic chemical methods to the characterization of the urinary metabolites. This approach is considerably limited by the necessity for a relatively large amount of material and the time-consuming and laborious procedures usually involved. For newly discovered metabolites, this still remains the sole method of chemical identification and, indeed, until recently was the preferred method of analysis even for steroids of known structure. However, recent advances in microtechniques and particularly in spectroscopic means of analysis (ultraviolet spectra of steroids in sulfuric acid solution and infrared spectra) have reduced considerably the amount of substance required for unequivocal identification. Although extensive fractionation and purification, usually involving chromatography, are still necessary, these identifications can be achieved with noncrystalline materials. It should be emphasized here that, from the metabolic point of view, the chemical characterization of a urinary metabolite does not always enable one to relate it to its hormonal precursor.

3. *Urinary steroids and their glandular precursors.* What is known concerning the *in vivo* catabolic fate of the steroid hormones in humans has been derived, for the most part, from detailed studies of the urinary metabolites. Of the various excretory paths available for the catabolic products of the steroids, the urinary route appears to represent the major mode of excretion in humans. The sparse data that are presently available suggest that the feces do not represent an important excretory route in man, a result in contrast to the situation usually found to obtain in small animals. Furthermore, there are no indications at present that the nucleus of the steroid hormones suffers complete degradation, a pathway which would result in the formation of small molecules that would enter the body pool of Krebs cycle intermediates. Thus, although the urine represents the primary route of excretion, the exact nature of the major portion of the urinary metabolites has never been established. In metabolic studies involving the administration of large doses of steroids, never has more than half of the substance given been recovered from the urine as intact steroid. In fact, in most instances, it has been possible to account for only a small fraction of the administered compound. Whether these poor recoveries of steroids are simply a reflection of in-

adequate procedures for isolation or whether they indicate the degradation of steroids to as yet unknown metabolites, remains an unsolved problem. Isotopically labeled steroids are ideally suited for the study of such problems but unfortunately, at present, few of the reported results of such investigations are concerned with the metabolic fate of these labeled substances in humans. When tritium-labeled cortisone was administered to human subjects, Bradlow *et al.* (48) found that 90% of the radioactivity was recovered within seven days from the urine as neutral ether-soluble material. Similar results were obtained by Fukushima *et al.* (173) who reported that 50–70% of the administered testosterone-3-C^{14} was recovered within one day from the urine in neutral ether-soluble form. The difference between these high recoveries of radioactivity as compared to the poor recoveries of intact steroids that follow the administration of unlabeled hormones can only be resolved when the isotopically-labeled, ether-soluble urinary metabolites are identified.

Neither the qualitative nor the quantitative aspects of urinary steroid excretion depend solely upon the nature and rate of the secretion from the endocrine gland. Many non-endocrine factors, *e.g.*, the liver, the kidney, the volume of urine, may influence the character and the amount of the steroid metabolites excreted in the urine. The levels of urinary steroids are determined to a great extent by the metabolic transformations which the hormones undergo following their secretion from the endocrine glands, and therefore it is evident that these excretion products, at best, are only an indication of the existing endocrine situation. Nevertheless, in many instances, they apparently do reflect an accurate picture of the hormonal environment and, as a result, the estimation and determination of these urinary constituents represent, at present, the most useful procedures for securing an insight into the internal environment. This is especially true in studying the human where, needless to say, many experimental procedures are excluded. Whether the known urinary steroids are truly representative of all the hormones produced by the endocrine glands remains an unanswered question. The steroids in the urine represent an accumulation, over a period of time, of metabolites of a fraction of the hormones synthesized by the endocrine glands, and thus may provide a more integrated and a more representative picture of the status of endocrine function than can otherwise be achieved. Even though the fraction appearing in the urine may be small, perhaps only a few per cent of the total secretion, at least the neutral urinary steroids are made available in milligram amounts. Such quantities make possible the analysis of excretion patterns which can be correlated to a significant extent with what is known of the anabolic and catabolic phases of steroid metabolism.

About one hundred steroids have been isolated from urine. From their structure, it is apparent that during metabolism the hormones are usually chemically transformed to a more highly reduced state. The evidence obtained from excretion studies indicates that the hormones are metabolized in accordance with certain general rules. By understanding these general transformations, it is possible to deduce the most probable parent hormone from which a given urinary steroid is derived. Urinary metabolites may frequently be related to their hormonal precursors by their structural similarity to a known hormone. Although such

reasoning, based simply on chemical analogy, may have its pitfalls, this approach has nevertheless yielded significant results. At present, it is possible to correlate most of the characterized urinary steroids with their precursors. To summarize the evidence as simply and completely as possible, two approaches have been used. The first (Fig. 8) outlines the general pathways by which the steroid hormones appear to be metabolized and the second presents in chart form (Figs. 9–15), the structures of the urinary steroids in a manner which suggests the glandular precursor(s) of each. This latter arrangement is predicated not only on the close chemical resemblance between the hormone and the metabolite but also upon the entirely reasonable and, in many cases, known reactions by means of which one may be converted into the other. It must be emphasized that the lines joining the individual urinary steroids are not meant to establish that one is necessarily derived from the other. The lines are included for convenience and guidance. In many cases, these lines could be replaced by arrows, since the transformations indicated by them have been demonstrated by experiment, either *in vivo* with exogenously administered steroids or *in vitro* with tissue preparations. In other cases, the indicated relationship is at present only conjectural.

The apparent metabolic fate of the structural characteristics of the representative hormonal precursors is summarized in Figure 8. The α,β-unsaturated carbonyl group in ring A appears to be reduced first to two intermediate saturated ketones which are then further reduced to saturated alcohols. These alcohols may occur in all of the four possible stereochemical configurations that exist at the asymmetric centers at C_3 and C_5. The secondary hydroxyl group at C_{17} in testosterone or estradiol appears in the metabolites as either a 17-ketone or a 17-hydroxyl group. The carbonyl group at C_{20} can be found unaltered but more often, it is converted to a secondary α-hydroxyl group. Metabolic experiments employing desoxycorticosterone (for earlier references see 124) and compound A (336) have shown that the organism is capable of reducing the primary hydroxyl group at C_{21} to a methyl group. The 21-hydroxy-20-keto side chain has been found intact in one urinary steroid, *viz.*, 3β,21-dihydroxy-5-pregnen-20-one (126). The dihydroxyacetone side chain, such as occurs in Compound F, may be found in the urine intact or may be converted to the 21-desoxy compounds, containing either the 17-hydroxy-20-keto or the 17,20-dihydroxy functions. Steroids with a tertiary C_{17}-hydroxyl group and a vicinal oxygen function at C_{20} are usually absent from the urine of normal persons and therefore, in the course of normal metabolism, they are apparently converted to substances that appear in the urine as C_{19}-17-ketosteroids. The fact that several 11-oxygenated C_{19} metabolites have been isolated from the urine favors this interpretation, for the only known hormonal precursors with 11-oxygen groups are C_{21} compounds of adrenal cortical origin. Since there is no evidence for the existence of a metabolic pathway which would result in the elimination of an oxygen function at C_{11}, the conclusion may be drawn that if the hormonal precursor possesses an oxygen function at C_{11} its urinary steroidal metabolites will retain this grouping. Whether the other C_{21} hormones which lack the tertiary hydroxyl group at C_{17} can be metabolized to C_{19} compounds in the human, remains to be established.

It is evident from the data presented in Figure 8 that the alterations that occur

during metabolism often tend to eliminate the structural differences that exist between the steroid hormones and thus tend to obscure the true precursor(s) of

FIG. 8. The possible metabolic fate of the structural characteristics of representative steroid hormones

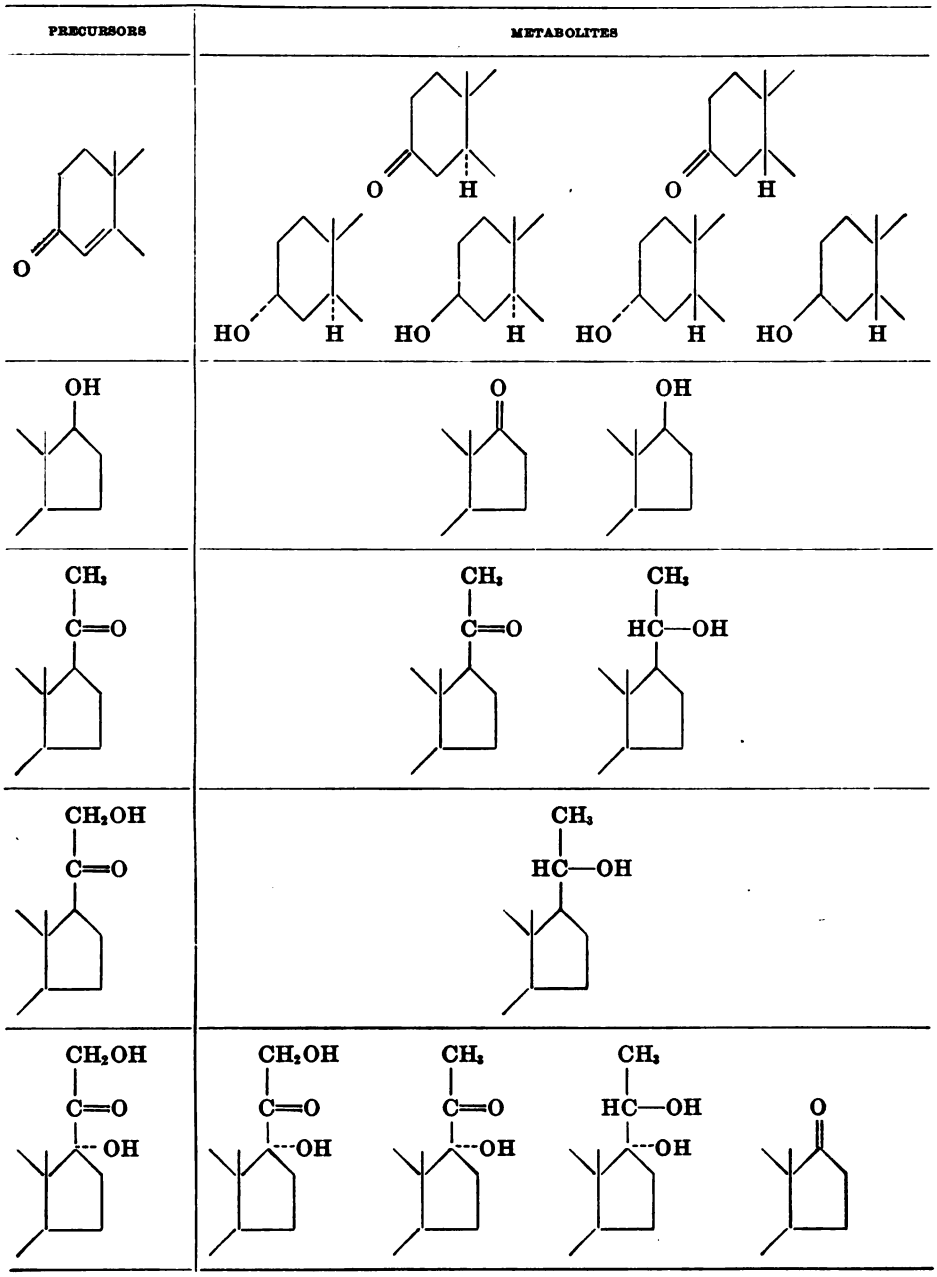
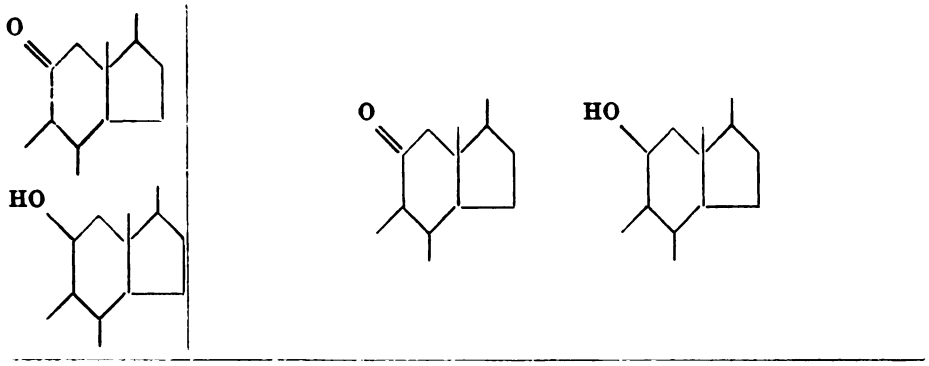


FIG. 8—*Continued*



any given urinary metabolite. In addition, these considerations make it clear that the precursors of a given urinary steroid cannot always be unequivocally deduced by superficial chemical analogy.

C_{19}-Metabolites related to testosterone. One way of attempting to relate the urinary steroids to their hormonal precursors is to arrange the metabolites in order of increasing state of reduction. In this way, certain regularities in metabolism become evident. The urinary steroids which contain nineteen carbon atoms and have no oxygen function at C_{11} are shown in Figure 9. Testosterone (*1*) is placed at the top to indicate the close chemical relationship between it and these urinary steroids. Testosterone itself has also been isolated from the urine but only in trace amounts after the parenteral administration of huge amounts of this hormone (124). The fact that the substances in Figure 9 are chemically similar to each other might lead to the conclusion that they are all derived from a common precursor, testosterone. However, as later discussion will make evident, this conclusion is not always warranted.

Two C_{19}-α,β-unsaturated ketones have been isolated from urine: 4-androstene-3,17-dione (*2*), which has been found in trace amounts in the urine of normal persons (315), and the rather unusual isomeric substance, 1-androstene-3,17-dione (not shown), the origin of which is obscure. The two possible isomeric saturated diketones, *3* and *4*, are consistently found in urine in small amounts (315). At the next level of reduction another asymmetric center is introduced at C_3 and all four resulting isomeric hydroxyketones (*5*, *6*, *7* and *8*) have been isolated from the urine. The absence of metabolites possessing the 3-keto-17-hydroxyl group in the urine is another indication of the existence of definite metabolic pathways. Androsterone (*6*) and etiocholanolone (*7*) are two of the most important urinary 17-ketosteroids; they are excreted in approximately equal amounts in both men and women. The ratio of *6* to *7* appears to vary from one individual to the next although the ratio is characteristic for each individual. Obviously, since women and eunuchs excrete *6* and *7*, these metabolites do not arise solely from the testicular hormone. In fact, it is impossible to determine from the excretion values of these 17-ketosteroids and others whether a subject is a male or female. Dingemanse *et al.* (121) believed that these two metabolites account for 30–66% of the

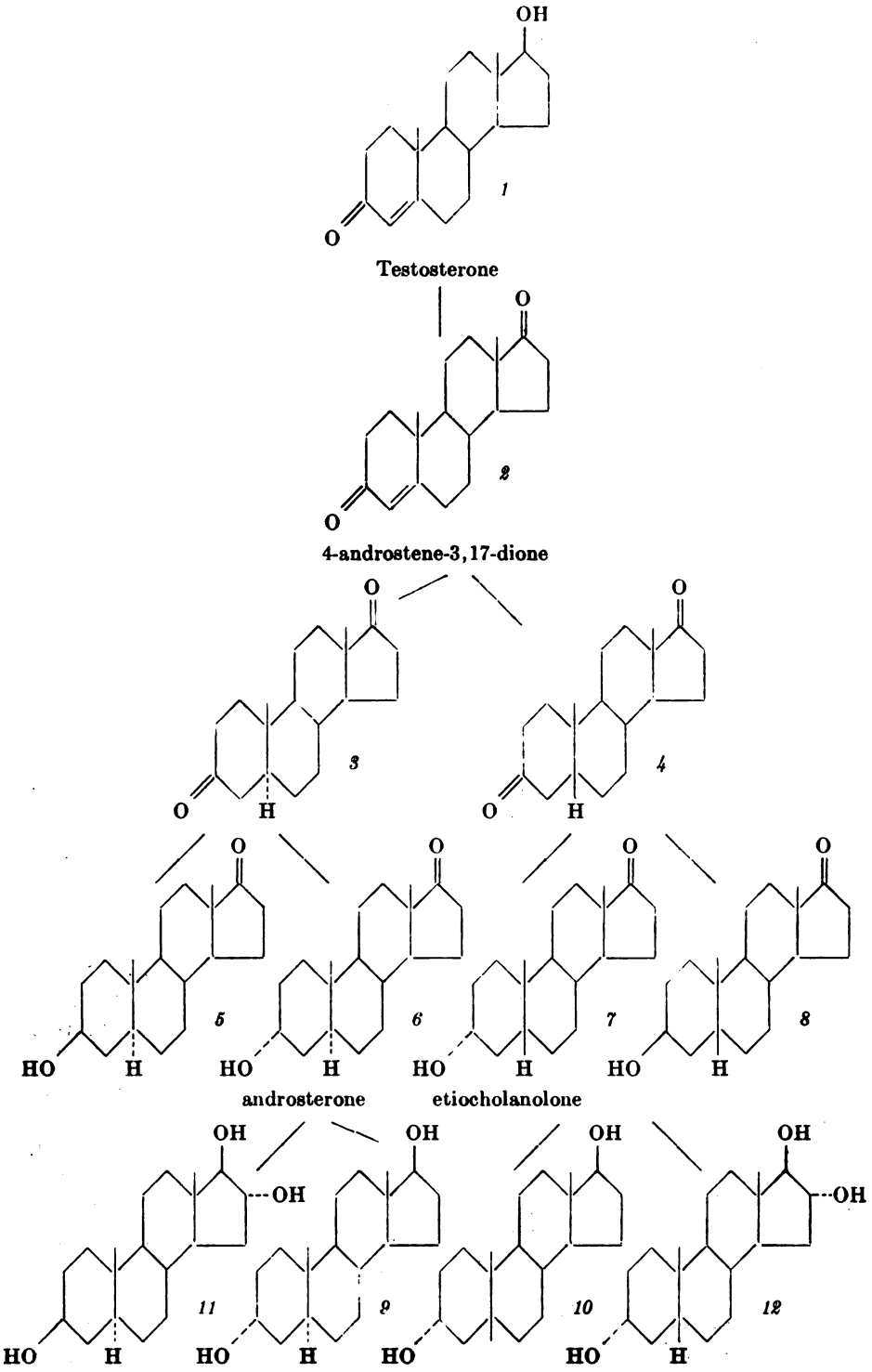


Fig. 9. C_{19}-Metabolites related to testosterone.

total 17-ketosteroids. Dobriner and Lieberman (124) have reported 1–2 mg. as the daily excretion of androsterone and 2–4 mg. as the daily excretion of etiocholanolone.

The study of the metabolism of testosterone in man by means of deuterium-labeled testosterone as well as testosterone-3-C^{14} by the Sloan-Kettering group has provided specific quantitative information about the metabolites of testosterone and shed some light on the endogenous production of the hormone and some of its metabolites. When 100 mg. of deuterated testosterone (174) was administered intramuscularly to a man and the major urinary metabolites, androsterone and etiocholanolone, were determined, it could be concluded that the endogenous production of androsterone in this man was 2.9 mg./24 hours and that of etiocholanolone was 9.0 mg./24 hours. It could be calculated from the isotope concentrations of the metabolites that 78% of the excreted androsterone and 74% of the excreted etiocholanolone had been derived from the testosterone administered, assuming that no loss of isotope occurred during the metabolic transformation. In addition to etiocholanolone and androsterone, which account for 26% and 10%, respectively, small amounts of labeled 2-androsten-17-one, androstane-3,17-dione (*3*), etiocholane-3,17-dione (*4*) were recovered from the urine accounting for a total of 37% of the administered testosterone. The β-ketonic fraction contained negligible amounts of isotope indicating the stereospecificity of the reduction of the 3-keto group. In another experiment by this group, a sample of labeled testosterone was administered intravenously (169) and the amount metabolized determined by isotopic dilution and isolation; 37% of the administered dose was found to have been metabolized and excreted during 9 hours and 48% in the first day. It was estimated from the data that the maximum amount of testosterone produced in man was 30 mg./day, assuming that all of the etiocholanolone and androsterone came from testosterone. This value is known to be too high, for etiocholanolone and androsterone are known to arise, too, from adrenal precursors one of which has been shown to be 17α-hydroxyprogesterone (168). The influence of the exogenously administered hormone upon its endogenous production may also alter this value.

In experiments using deuterated etiocholanolone and androsterone, Gallagher *et al.* could demonstrate that the exogenous dose did not suppress the endogenous production of these metabolites. When 100 mg. of deuterated etiocholanolone was administered intramuscularly to a man, these workers (175) recovered from the urine 49% of the administered material in the first 24 hours. Similarly, with deuterated androsterone, 25% was recovered in the urine in the first day. Also, when labeled 4-androstenedione was used, etiocholanolone and androsterone containing deuterium were isolated in about the same amounts as when testosterone was given. This was considered confirmatory evidence for the idea that androstenedione is a metabolic intermediate between testosterone and the urinary metabolites, etiocholanolone and androsterone. Labeled androstanedione was shown to give rise only to androsterone.

Since in some experiments the isolation of labeled material from urine afforded only about 25–50% of the administered compound, the feces was investigated for

steroidal materials. However, contrary to the case of the mouse and rat (15), no C_{21}- or C_{19}-steroids could be detected even after using a hydrolytic procedure which presumably would cleave any conjugates that might be present.

In mice, testosterone-3-C^{14} was found to be rapidly excreted, and in 24 hours 30–40% of the radioactivity was found in the urine and 60% in the feces, with no activity in the tissues and none in the expired CO_2 (15, 174, 175). Similar results were found by the St. Louis group (8, 141, 255) using testosterone-4-C^{14} administered intramuscularly to rats as well as methyl-C^{14}-androstenediol and methyl-C^{14}-testosterone. A major fraction of the radioactivity appeared in the urine only in bile-duct ligated animals. When bile-fistula animals were used, it could be shown that the bile represented a major pathway of excretion. Hydrolysis of the biliary metabolites of testosterone showed them to be mainly in the form of glucuronosides (141).

Of the eight possible 3,17-dihydroxy C_{19}-metabolites only two, *9* and *10*, have been discovered, and these both possess the β-configuration at C_{17}. Recntly, Brooksbank and Haslewood (55) have reported the isolation of another C_{19}-urinary steroid, 16-androsten-3α-ol, after enzyme hydrolysis of the "pregnanediol glucuronidate" complex. The true origin of this unsaturated substance is obscure. Among the several examples of 16α-hydroxylated urinary steroids isolated are the two triols, *11* and *12* (318). Such metabolites appear to represent further steps in the catabolism of steroids, preliminary to the formation of more highly degraded products.

An important type of investigation for the elucidation of the metabolic pathways involves the identification of the steroids excreted into the urine following the administration of possible precursors or intermediates. Thus, a number of C_{19}-compounds related to testosterone have been administered to humans and the urinary steroids isolated and characterized. Dorfman and his co-workers have studied the metabolism in humans of 4-androstene-3,17-dione (134), androstane-3,17-dione, androstane-3α,17β-diol, dehydroisoandrosterone acetate (347), 17α-testosterone (521), androstane-3β,17β-diol, and 1-androstene-3,17-dione (520). The metabolism of testosterone in man has been studied by Dobriner and Lieberman (124) and by West *et al.* (542). In general these studies, in spite of their obvious limitations, have served to clarify and confirm many of the postulated pathways of metabolism.

C_{21}-Metabolites related to progesterone. When the urinary metabolites containing 21 carbon atoms and two other oxygen functions, one at C_3 and one at C_{20}, are arranged in a manner analogous to that used for the metabolites of testosterone, similar relationships become apparent (Fig. 10). Many of the compounds in Figure 10 have been isolated after the administration of progesterone (*13*) to human subjects (124), although the major portion of the injected material could not be accounted for. Both possible diketones, pregnane-3,20-dione (*15*) and allopregnane-3,20-dione (*14*) (315) have been isolated from human urine. Of the four possible 3-hydroxy-20-ketones, only three have been isolated thus far: 3α-hydroxyallopregnan-20-one (*16*), 3β-hydroxyallopregnan-20-one (*17*), and 3α-hydroxypregnan-20-one (*18*) (124, 315). The fourth possibility, 3β-hydroxypregnan-

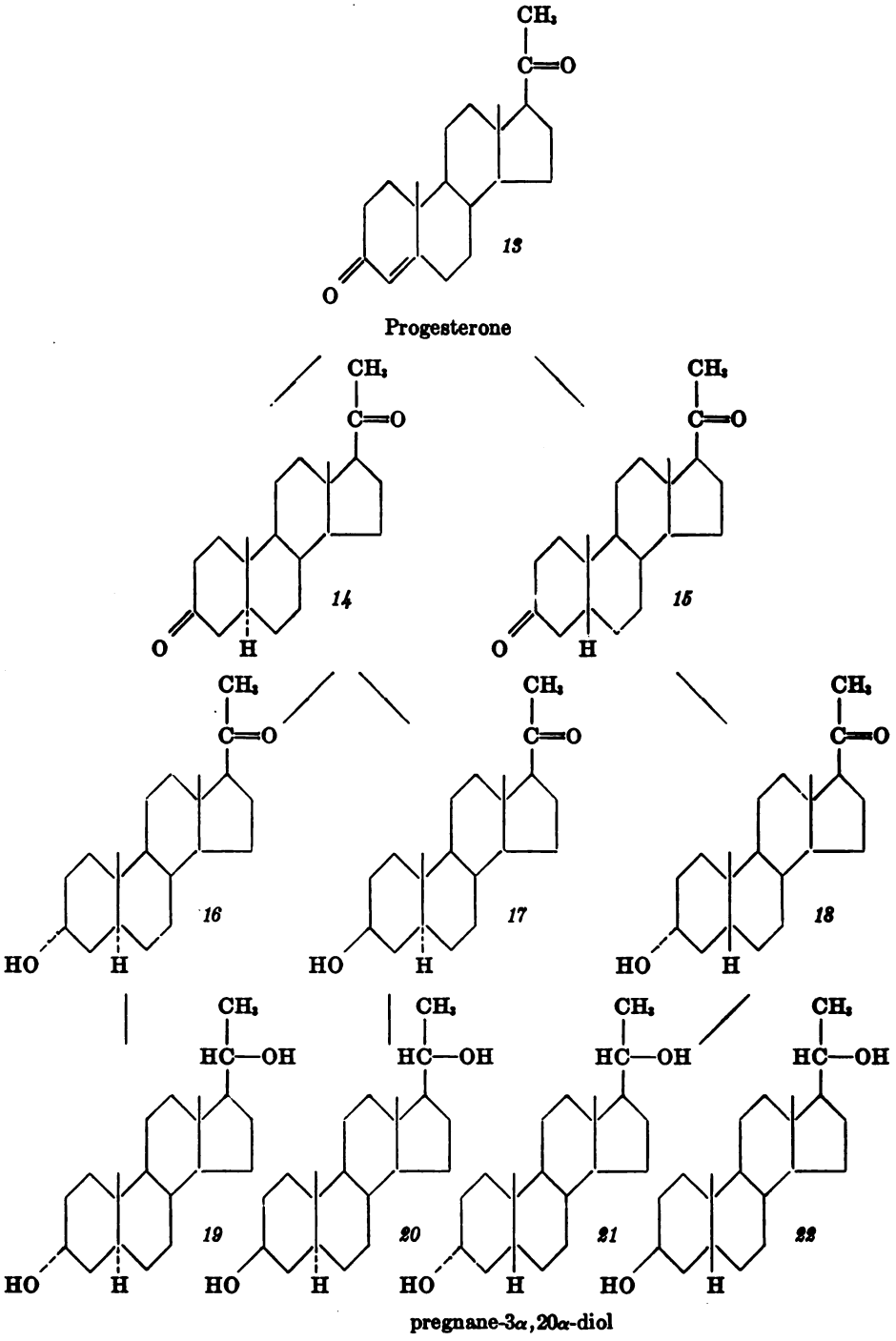


FIG. 10. C_{21}-Metabolites related to progesterone.

20-one, has not been discovered nor have any of the 3-keto-20-hydroxy compounds been detected. Of the eight possible pregnanediols, four have been isolated: allopregnane-3α,20α-diol (*19*), allopregnane-3β,20α-diol (*20*), pregnane-3α,20α,-diol (*21*), and pregnane-3β,20α-diol (*22*) (for references, see 124). In spite of the close chemical resemblance of these compounds to progesterone, it is not always possible to relate their presence in the urine to the progestational hormone. Pregnane-3α,20α-diol and possibly 3α-hydroxypregnan-20-one are regularly encountered in the urine of women, both pregnant and non-pregnant, and these apparently reflect the level of the corpus luteum hormone. However, the occurrence of these compounds, as well as that of the others in Figure 10, is frequently associated with disorders of the adrenal gland. Since this aspect is better considered in the section concerned with the metabolites of the adrenal hormones, it will be elaborated upon there.

Studies using radioactive progesterone have so far been confined to rodents and in view of the great species differences, the conclusions cannot be extended to man. In mice and rats, the administration of progesterone-21-C^{14} led to the excretion of at least 50 % of the radioactivity in the neutral, unsaponifiable fraction of the feces (185, 421). Twenty per cent appeared in the expired CO_2. Although the material did not appear to be stored in any tissue or organ, a high concentration was found in the pituitary and liver. As in the case of testosterone, Doisy and his colleagues (130, 142, 184, 185, 252) found that labeled progesterone injected intramuscularly into rats resulted in 22% of the radioactivity in the urine and 66% in the feces. Bile-duct ligation resulted in 90% of the activity in the urine and only 30% in the feces, whereas in animals with bile fistulas 73% of the activity could be found in the bile, 2% in the feces and 2.2% in the urine (142, 185). The excretion of the administered material could be shown to be virtually complete in 24 hours (185, 252). Similar results on rapidity of excretion, distribution of activities in urine, feces and CO_2 were obtained in rodents by Barry *et al.* (15).

The results reported by Leblond *et al.* (307) on one tumor-bearing mouse injected intramuscularly with progesterone-3,21-C_2^{14} showed, too, that the biliary route was a major pathway of excretion, but they found more radioactivity in the urine than in the feces. As in the case of estrone, most of the radioactivity remained in the water-soluble portion of the urine even after hydrolysis. Two and a half per cent of the administered radioactivity appeared as CO_2 within 12 hours. In contrast to the *in vivo* investigations on mice and rats, the incubation of rat liver homogenates with progesterone-21-C^{14} resulted in no significant amount of radioactivity in the expired CO_2 (448).

Urinary metabolites related to the adrenal cortical hormones. From the structural point of view, the C_{21}-adrenal cortical hormones are unique because they possess unlike the other hormones previously discussed, oxygen functions at C_{11}, C_{17} and/or C_{21}, in addition to those at C_3 and C_{20}. Just as these oxygen functions serve to characterize the hormones, so they label their urinary metabolites. Therefore, C_{21}-urinary steroids bearing oxygen at any or all of these positions (C_{11}, C_{17}, C_{21}), as well as C_{19}-metabolites having a carbonyl or hydroxyl group at C_{11}, are

known to be adrenal metabolites. By an examination of the urinary steroids that fall into these catagories, it is possible to indicate many of the pathways through which the adrenal hormones pass. This approach, however, is somewhat unsatisfactory since it fails to clarify the origin of many urinary steroids which do not possess any of the unique structural characteristics of the adrenal hormones but are nevertheless unquestionably derived from them. Among these metabolities are three of the most important urinary ketosteroids, androsterone (*6*), etiocholanolone (*7*), and dehydroisoandrosterone (*28*), which together comprise about three-quarters of the total 17-ketosteroids.

In an attempt to give an over-all view of the complex maze which the adrenal hormones and their metabolites traverse during catabolism, the possible metabolic precursors of urinary dehydroisoandrosterone have been discussed first. This substance has been chosen not only because it represents an important index of normal as well as abnormal adrenal cortical activity but because the considerations which are used to direct attention to its progenitors are helpful in deducing the precursors of many other urinary adrenal metabolites, especially those that do not bear oxygen atoms at C_{11}, C_{17} or C_{21}. In analyzing this problem it is necessary to consider the biogenetic phases of adrenal hormone biochemistry in addition to the evidence accumulated from urinary excretion studies. The approach which we have applied in Figures 11–15 for the analysis of the data on the urinary metabolites of the adrenal cortical hormones is one designed primarily to present the information in a chemically logical manner. It must not be assumed that these schemes necessarily represent the *in vivo* pathways by means of which these transformations are effected.

Since it has recently been shown that dehydroisoandrosterone (*28*) is excreted in normal urine in much larger amounts than hitherto recognized, the question of its precursor has assumed added importance. Recent results by Landau *et al.* (302), Dingemanse *et al.* (121) and Ronzoni (435) have demonstrated that this metabolite may represent 10–60% (1–12 mg./day) of the total ketosteroids excreted by normal individuals.

Although attempts have been made to examine certain steroids as possible precursors of urinary dehydroisoandrosterone, the nature of these precursors remains obscure. In spite of the superficial similarity in chemical structure between the hormone, testosterone, and the metabolite, dehydroisoandrosterone, it was recognized early that this hormone was not the precursor of the urinary steroid. The exogenous administration of huge doses of testosterone intramuscularly (124, 521) or intravenously (542) does not increase the concentration of the urinary dehydroisoandrosterone.

There exist in the literature two conflicting statements regarding the precursor of dehydroisoandrosterone. Munson and co-workers (355) administered Compounds E and F to adrenalectomized and gonadectomized men and found an increase in the excretion of dehydroisoandrosterone as measured by the Pettenkofer test. On the contrary, Wolfson *et al.* (550) reported that these two compounds did not augment the excretion of the Pettenkofer chromogen although they found that corticosterone did. The bearing of these reports on the precursors

of dehydroisoandrosterone must await the resolution of these opposing results and the determination of the exact nature of the chromogenic material.

Since the excretion of large amounts of dehydroisoandrosterone in the urine has always been found in association with adrenal hyperactivity, *e.g.*, following stimulation of the adrenals with ACTH (301, 435), the precursors of this metabolite must be sought among the steroids elaborated by the adrenal cortex. The exact chemical nature of this precursor can be surmised from an examination of the chemical nature of some of the urinary steroids together with a consideration of the mechanism of biogenesis of the adrenal hormones. A clue to the answer appears to rest with the Δ^5-3β-hydroxy grouping in rings A and B of the dehydroisoandrosterone molecule. In the past attention has been focused upon the elucidation of a mechanism by which an active hormone, possessing as they all do an α,β-unsaturated carbonyl group in ring A, can be metabolized to the β,γ-unsaturated alcohol structure present in dehydroisoandrosterone. Although this conversion has recently been effected chemically, there is no evidence that this metabolic pathway exists in a living system. Indeed, a consideration of the variety of compounds that have been isolated from human urine which contain a Δ^5-3β-hydroxy system (see Fig. 11) leads to the conclusion that it is more likely that the immediate precursor of dehydroisoandrosterone is a substance containing such a chemical grouping. This consideration is not based solely on the number of such urinary steroids isolated, but more importantly, on the fact that they represent compounds reflecting different levels of the biogenetic and metabolic processes.

In addition to dehydroisoandrosterone (*28*), the Hirschmanns (242, 243, 244, 245, 246) have isolated from the urine of a seven year old boy with carcinoma of the adrenal cortex a number of Δ^5-3β-hydroxy steroids among which were the C_{19}-compounds *30* and *31*. Since the transformation of *28* to *30* and *31* has been shown to occur *in vitro* with liver slices by Schneider and Mason (465), it is likely that *30* and *31* are also derived *in vivo* from *28*; in any case, they provide little information concerning the precursor of dehydroisoandrosterone. From this same urine the Hirschmanns succeeded in isolating and identifying *25* (about 10 mg./liter) and *26* (1.3 mg/liter), along with 315 mg./liter of dehydroisoandrosterone. It was recognized by these workers, and has since been proved several times, that a C_{21}-steroid containing oxygen functions at C_{17} and C_{20} (such as *25* and *26*) can be metabolically degraded to a C_{19}-17-ketone (such as *28*). Administration of cortisone to humans has resulted in the increased excretion of 11-oxygenated 17-ketosteroids (317, 483) and recently Fukushima *et al.* (168) have reported the isolation of deuteroandrosterone and deuteroetiocholanolone after the administration of isotopically labeled 17α-hydroxyprogesterone. Thus, it seems likely that the immediate precursor(s) of dehydroisoandrosterone is a C_{21}-steroid containing oxygen functions at C_{17} and C_{20}, as well as the Δ^5-3β-hydroxy system.

Substances *24* and *29* (6 mg./liter) were isolated by the Hirschmanns from the same urine described above (243, 245). Compound *24* was also isolated by Miller and Dorfman (346) from the urine of a patient with adrenal hyperplasia (0.2 mg./liter) and by Marker and Rohrmann (333) from pregnant mares' urine.

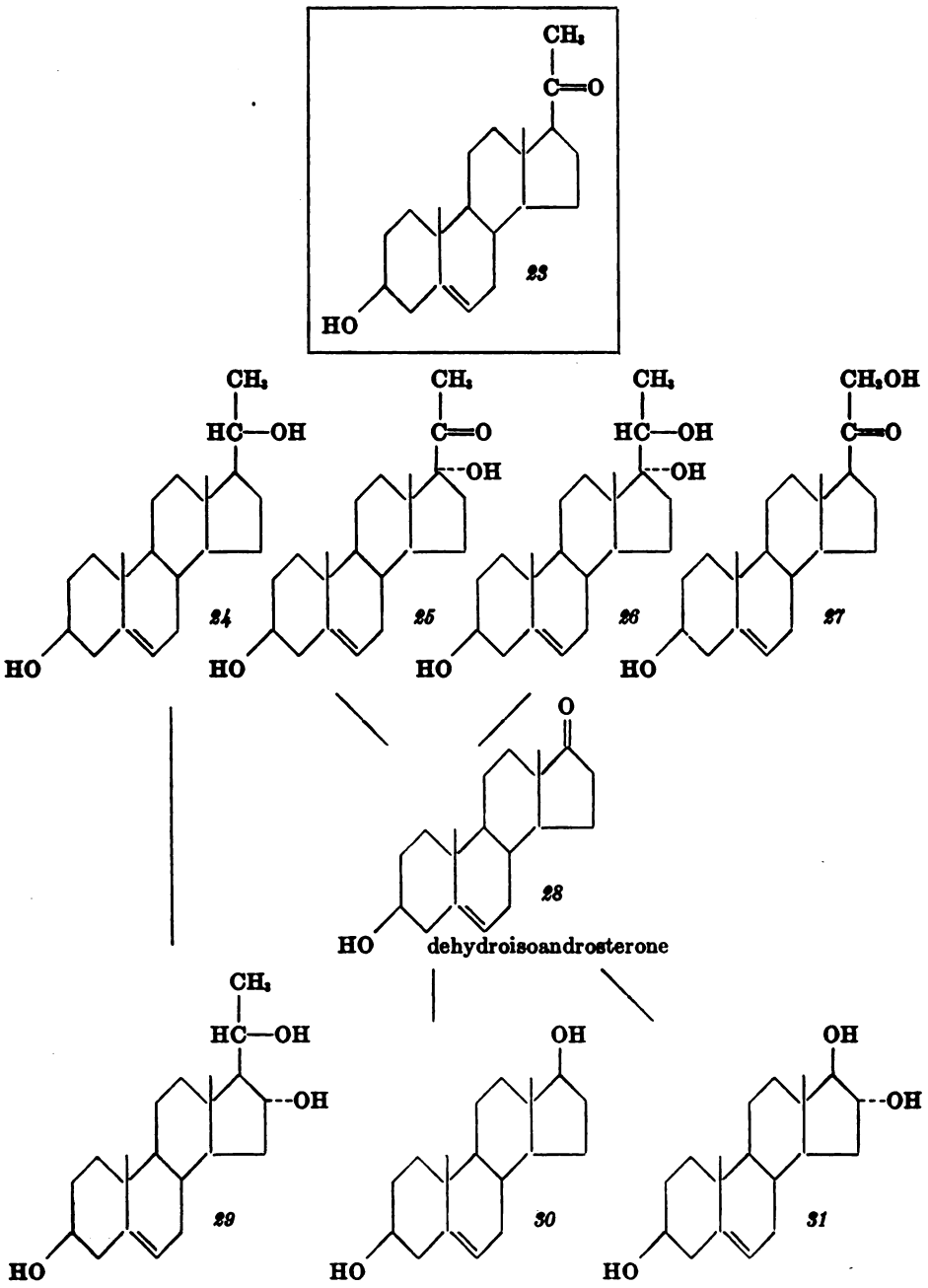


FIG. 11. 5-Pregnenolone (*23*) and the Δ^5-3β-hydroxy steroids isolated from urine.

21-Hydroxypregnenolone (*27*), probably a precursor of desoxycorticosterone, has been isolated from the urine only after the administration of ACTH (126). Since these urinary metabolites, *24*, *27* and *29*, do not possess the C_{17}-hydroxyl

group it is unlikely that they give rise to dehydroisoandrosterone, for there is no evidence that C_{21}-pregnane derivatives without a tertiary hydroxyl group at C_{17} can be transformed to 17-ketosteroids. But *24*, *27* and *29* can provide information as to the nature of the precursor of *28*, since they appear to represent urinary metabolites of *intermediates* in the biogenesis of the corticosteroids, Compounds F and B. This will become more evident by recalling that the adrenal gland has the capacity to introduce hydroxyl groups into preformed steroids. As was mentioned previously in the section concerned with the biogenesis of the adrenal hormones (Section II), Hechter *et al.* (234) isolated 11β-hydroxyprogesterone, 17α-hydroxyprogesterone, corticosterone (Compound B) and hydrocortisone (Compound F), following the perfusion of progesterone through an isolated adrenal gland. When pregnenolone (*23*) was added to the perfusate, progesterone, Compound F and Compound B were isolated. Thus, the reactions by which the adrenal gland can transform pregnenolone into Compound F involve: oxidation of a Δ^5-3β-hydroxy group to a Δ^4-3-keto group (445) and hydroxylation at C_{11}, at C_{17} and at C_{21}. It is highly unlikely that all four of these reactions occur simultaneously and therefore a variety of intermediates can be produced. To account for the urinary steroids *25*, *26* and *27*, it is merely necessary to assume that hydroxylation at C_{17} and at C_{21} can occasionally occur prior to the dehydrogenation in ring A. Thus, hydroxylation at C_{17} of a properly constituted precursor(s) possessing the Δ^5-3β-ol group would yield *25* and *26*, whereas hydroxylation at C_{21} of the same precursor would give rise to *27*. These partially hydroxylated products may be further metabolized (*e.g.*, *25* $\rightarrow$ *26* or *25* $\rightarrow$ *28*) and excreted into the urine. Therefore, it could be said that these substances as well as dehydroisoandrosterone are not the metabolites of a hormone but are the metabolites of an intermediate in the biosynthesis of the adrenal hormones.

The nature of this intermediate is strongly suggested by the occurrence in the urine of 5-pregnene-3β,20α-diol (*24*). The chemical nature of pregnenediol (*24*) as well as *25*, *26*, *27* and the 16-hydroxylated compound, *29*, points directly to some compound structurally similar to these urinary steroids, possibly pregnenolone (*23*), as an *in vivo* intermediate in the synthesis of Compounds B and F and as a precursor of urinary dehydroisoandrosterone. The fact that pregnenolone can be hydroxylated and serve as a precursor for the active hormones *in vitro* led Hechter *et al.* (234) to postulate that it was an *in vivo* precursor in corticosteroidogenesis. The Hirschmanns have already suggested that pregnenolone (*23*) is the metabolic precursor of *24*, for, despite the fact that *23* has not been isolated from adrenal extracts, progesterone and 3β-hydroxyallopregnan-20-one (*17*) have been. Pregnenolone has been isolated by Ruzicka and Prelog (440) from pigs' testes. Whether pregnenolone is indeed this precursor remains to be proved by experiment.

The hypothesis that many urinary metabolites may be formed from adrenal intermediates rather than from active hormones will be utilized as a basis to relate the urinary metabolites to their possible adrenal secretory precursors.

In the charts (Figures 12–15) that follow, the possible glandular precursors are placed above those urinary steroids which they most closely resemble. The

routes represented in these figures are all predicated upon the hypothesis that pregnenolone or some structurally similar compound is an *in vivo* precursor of the adrenal secretory products. Furthermore, assumption is made that partial or complete hydroxylation of this precursor, with or without oxidation to the Δ^4-3-keto product, may result in some, if not all, possible combinations and these, in turn, may lead to the formation of the urinary products by well established metabolic routes.

Most, but not all, of the precursors given in Figures 12–15 have been isolated from extracts of adrenal glands. As mentioned earlier, pregnenolone itself has not been isolated from adrenals, although the closely related products, progesterone (*13*) and 3β-hydroxyallopregnan-20-one (*17*), have. 17α-Hydroxypregnenolone (*25*) and 21-hydroxypregnenolone (*27*) have not been isolated from glandular extracts, but since they have been found in urine and may logically be considered to be partially hydroxylated intermediates, they have been placed among the glandular products. 11β-Hydroxyprogesterone (*38*) has been isolated after perfusing blood containing progesterone through an isolated adrenal gland (234). 11β,17α-Dihydroxyprogesterone (*43*) has not been isolated but has been included in Figure 15 because it completes the logically possible combinations and because 21-desoxytetrahydrocortisone (*49*) (126), one of its reduction products, has been isolated from urine. Five other logically possible Δ^5-3β-ol precursors have been omitted since they have been isolated from neither glandular nor urinary extracts. All the compounds listed below the hypothetical precursors have been isolated from human urine. It must be reiterated that the guide lines joining the individual urinary steroids are not to establish that the transformations indicated have necessarily been demonstrated by experiment.

Figure 12 shows at the top those adrenal secretory products which may be considered to be derived from the hypothetical Δ^5-3β-ol intermediate, pregnenolone or some structurally similar compound, before any hydroxylation has been effected. In cases of adrenal hyperactivity, such hormonal precursors may be produced in excessive amounts and may escape from the adrenals before further hydroxylation. While pregnenolone itself has not been found in the urine of such patients, its reduction product, 5-pregnenediol (*24*) (243, 346) has been isolated, as well as the chemically related 16α-hydroxylated compound (*29*) (245). The origins of allopregnane-3β,20α-diol (*32*) (333) and allopregnane-3β,16α,20β-triol (*33*) (333) are obscure. Perfusion experiments (234) as well as tissue slice experiments (445) have demonstrated that the adrenals and other tissues can convert pregnenolone to progesterone. The progesterone so formed could follow the pathways indicated in Figure 10. This could account for the presence of the trace amounts of pregnanediol found in the urine of men (219, 147). In this way, also, the excessive amounts of pregnanediol (*21*) found in the urine of patients with adrenal tumors and hyperplasia might be explained (339). Exogenously administered pregnenolone has already been shown to result in the excretion of pregnanediol in the urine (385).

In Figure 13 are found the adrenal secretory products which may result from the supposed precursor by hydroxylation at C_{17}. The presence of 17-hydroxy-

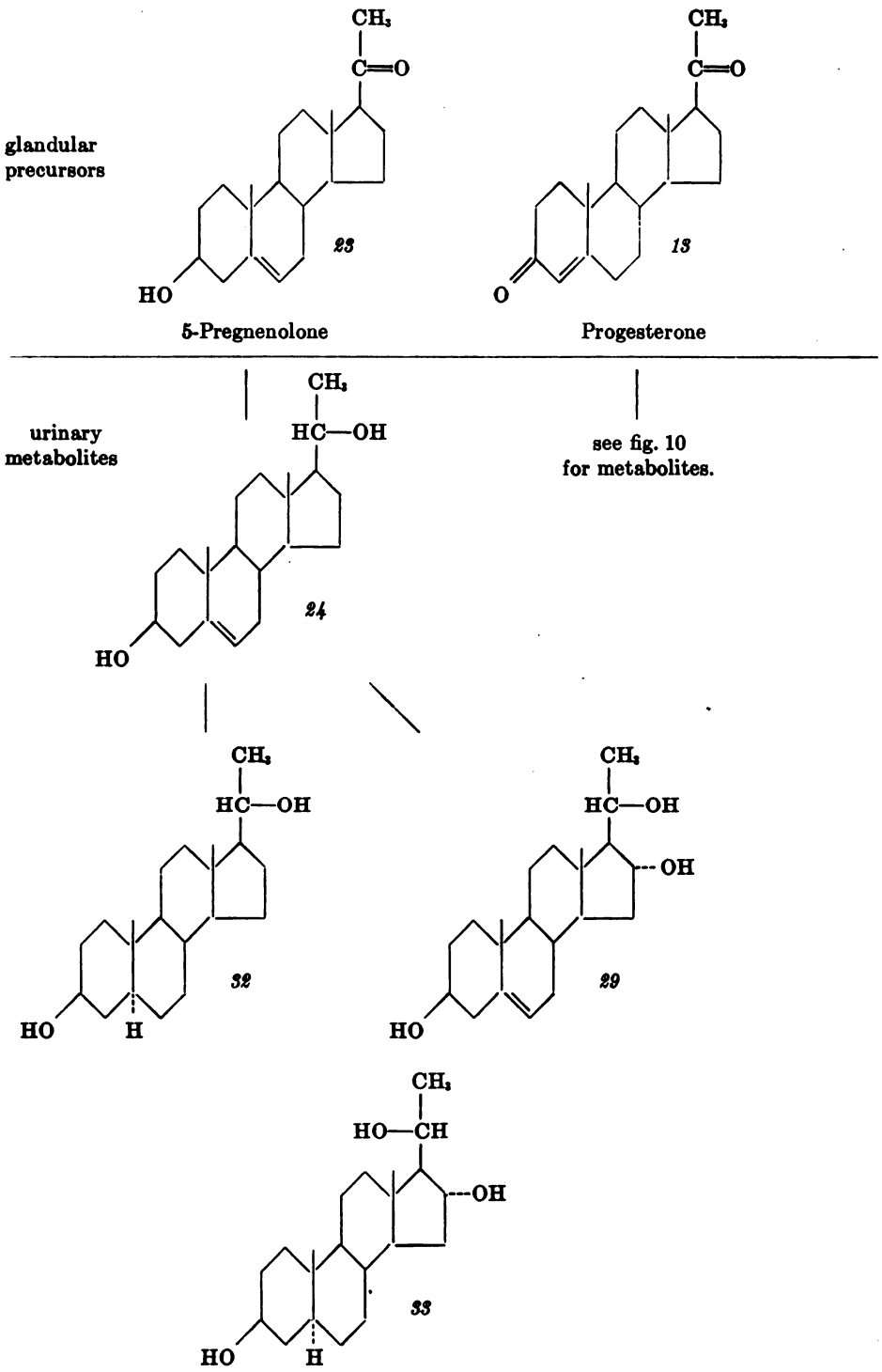


FIG. 12. Possible $C_{21}O_2$ adrenal hormonal precursors and the known urinary metabolites that can be related to them.

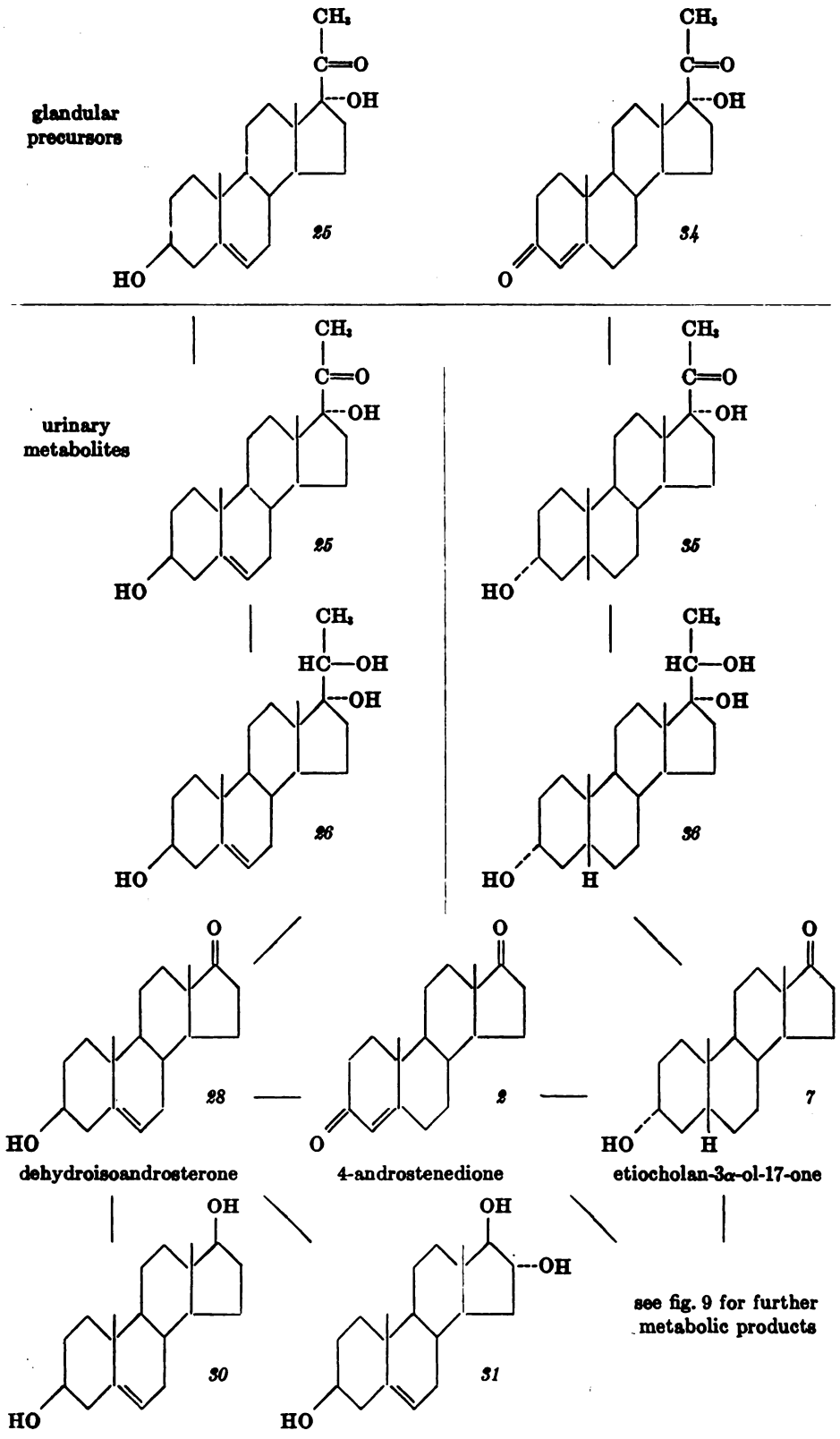


FIG. 13. Possible adrenal hormonal precursors hydroxylated at C_{17}; and the known urinary metabolites that can be related to them.

pregnenolone (*25*) (244), 17-hydroxypregnenediol (*26*) (246), dehydroisoandrosterone (*28*), and also *30* and *31* (242) in the urine of patients with adrenal cancer has previously been commented upon. Likewise, earlier mention has been made of the *in vivo* conversion of 17α-hydroxyprogesterone (*34*) into androsterone (*6*) and etiocholanolone (*7*) (168). The substances 17α-hydroxypregnanolone (*35*) (315) and 17α-hydroxypregnanediol (*36*) (339) have been isolated from the urine of patients with adrenal hyperplasia or tumors. 4-Androstenedione (*2*) has been isolated from adrenal as well as urinary extracts (315).

Figure 14 shows the glandular precursors that could be derived from pregnenolone (a) by hydroxylation at C_{21}, (b) by hydroxylation at C_{11}, and (c) by the introduction of 2 hydroxyl groups, one at C_{11} and the other at C_{21}. 21-Hydroxypregnenolone (*27*) has been isolated from the urine of patients to whom ACTH has been administered (126). It is conceivable that pregnenediol (*24*) is formed from 21-hydroxypregnenolone (*27*) by reduction of the C_{21}-carbinol to a C_{21}-methyl group, since the ability of the organism to effect such a transformation has been demonstrated by the conversion of desoxycorticosterone (*37*) to pregnanediol (*21*). 11-Ketopregnanolone (*39*) (316) could arise from corticosterone (*40*) by a similar process or could be a metabolic product of 11β-hydroxyprogesterone (*38*). 11-Ketopregnanediol (*41*) has been isolated from the urine of individuals to whom Compound A, the 11-keto analogue of corticosterone, had been administered (336) as well as from the urine of untreated subjects (316).

Figure 15 presents the remainder of the postulated adrenal precursors. Those shown are precursors that could result by the introduction of (a) two hydroxyl groups, one at C_{17} and the other at C_{21}, (b) two hydroxyl groups, one at C_{11} and the other at C_{17}, and finally (c) three hydroxyl groups, one at C_{11}, one at C_{17} and one at C_{21}. 11β,17α-Dihydroxyprogesterone (*43*), as mentioned earlier, is suppositional. Evidence already exists for the metabolic formation of 17-ketones from compounds containing a 17α-hydroxy-20-keto side chain, with or without a 21-hydroxyl group. A similar cleavage of the side chain of Compound S (*42*) would result in 4-androstenedione (*2*). The possibility further exists that dehydroisoandrosterone could likewise be formed from the hypothetical Δ^5-3β-ol precursor (not shown) of Compound S. The further metabolism of 4-androstenedione and dehydroisoandrosterone has already been discussed (Figure 9). Both Compound E (*44*) and Compound F (*45*) are found in normal urines in trace amounts. The most important urinary corticoid, quantitatively speaking, is 3α,17α,21-trihydroxypregnane-11,20-dione (tetrahydrocortisone) (*47*) (317, 460), although both 17α,21-dihydroxypregnane-3,11,20-trione (dihydrocortisone) (*46*) (317, 460) and 3α,11β,17α,21-tetrahydroxypregnan-20-one (*48*) (126) are excreted normally. 21-Desoxytetrahydrocortisone (*49*) (126) may be a further metabolite of *47*, arising by reduction of the 21-alcohol group, or it may be derived directly from the hypothetical 11β,17α-dihydroxyprogesterone (*43*). Further conversions shown in Figure 15 would account for the six known C_{11}-oxygenated C_{19}-metabolites (*50–55*) (315, 316, 339, 442a). The most important of these is 11-ketoetiocholanolone (*52*) which is excreted by normal individuals in approximately 1–2 mg. amounts per day (124, 315).

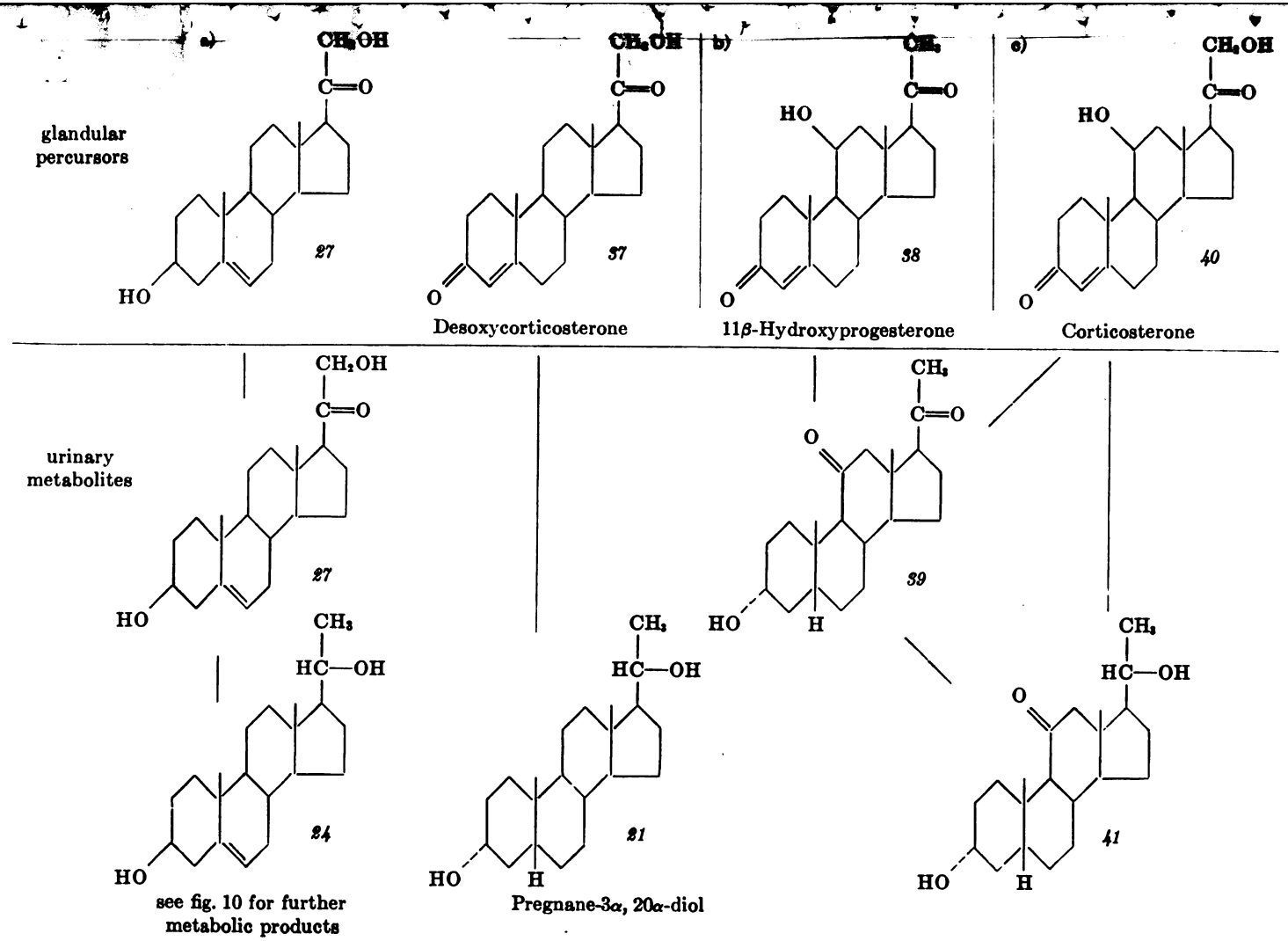
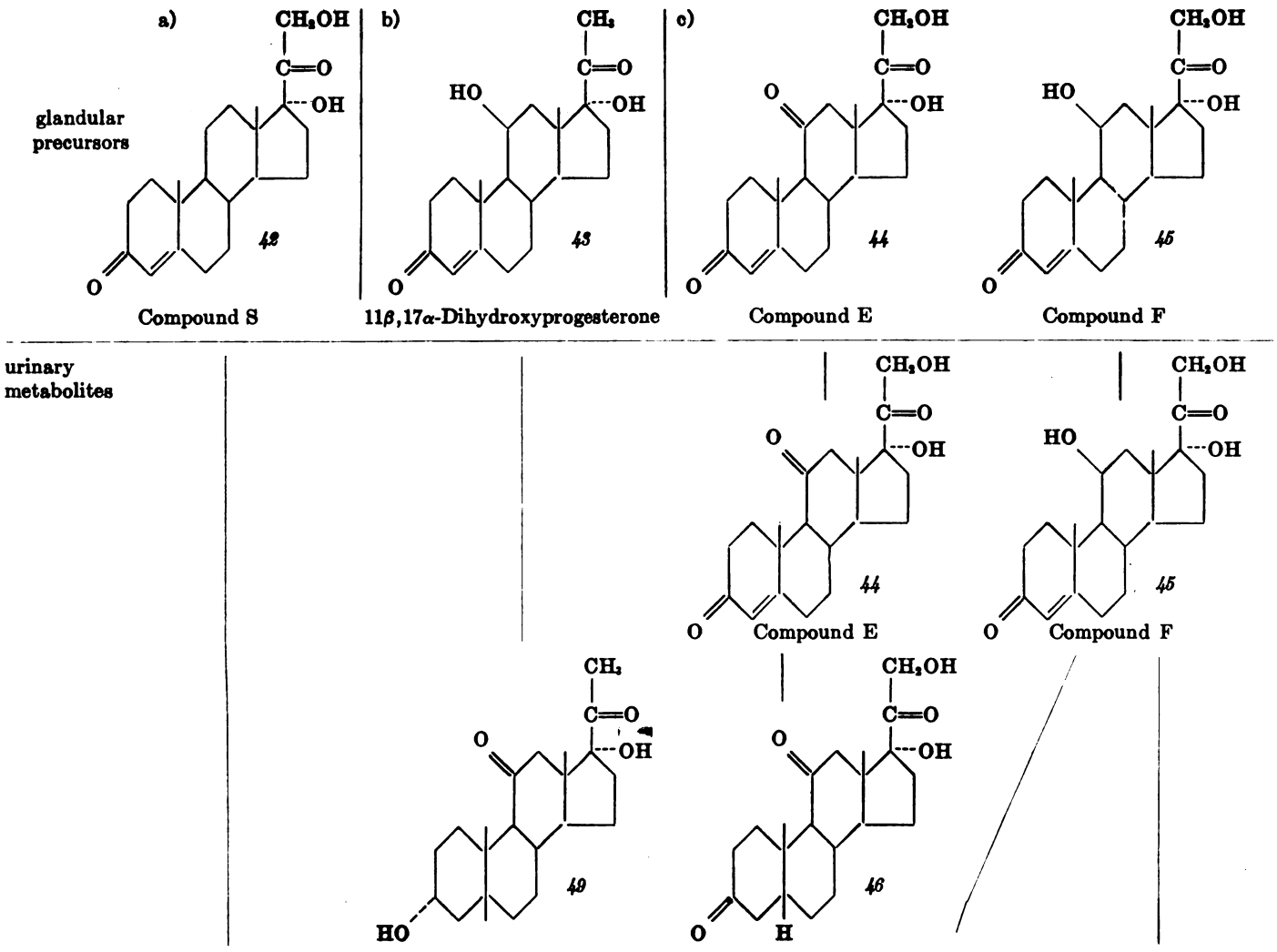


FIG. 14. Possible adrenal hormonal precursors, hydroxylated at a) C_{21}, b) C_{11}, and c) C_{11} and C_{21}; and the known urinary metabolites that can be related to them.


a)
b)
c)
glandular precursors
urinary metabolites
Compound S
11β,17α-Dihydroxyprogesterone
Compound E
Compound F
42
43
44
45
46
49

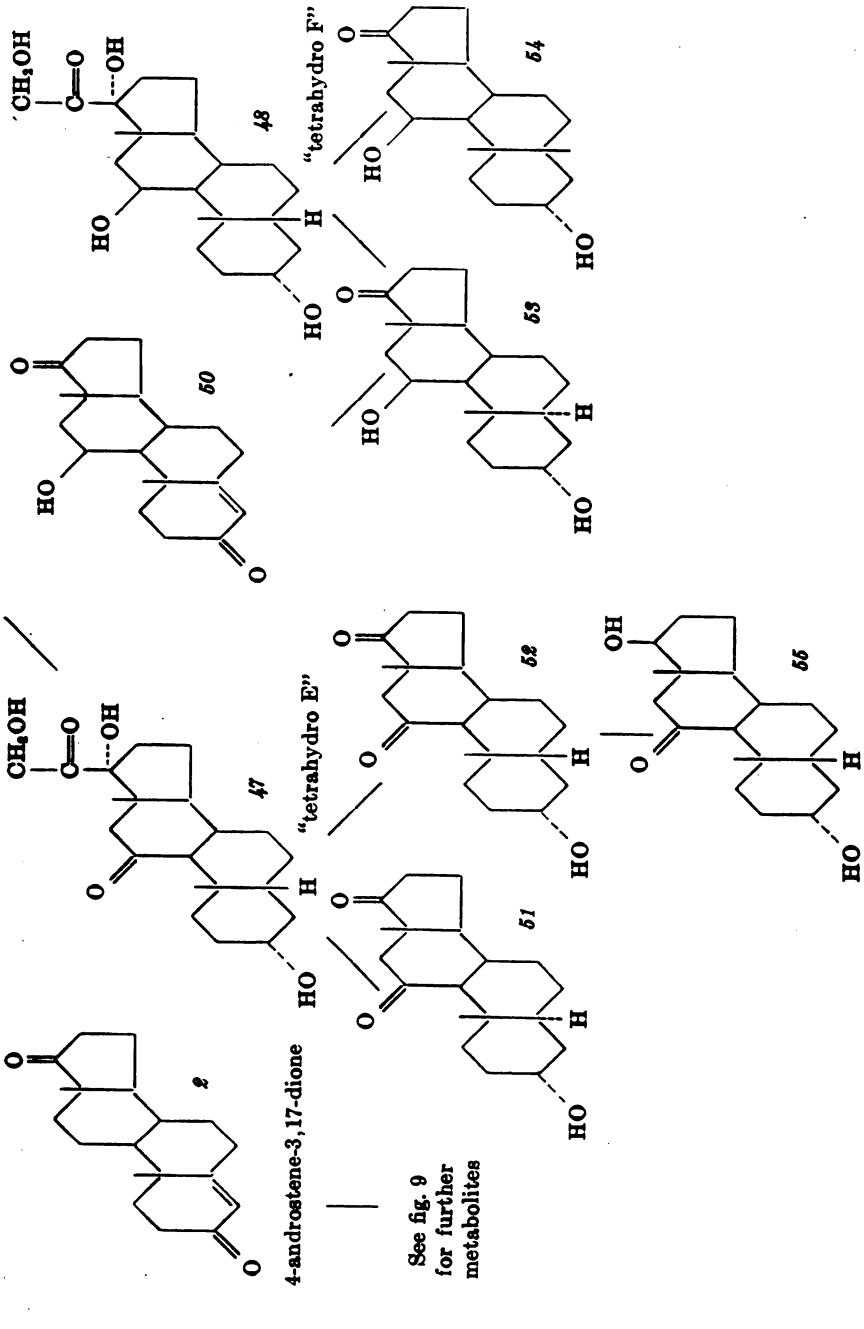


FIG. 15. Possible adrenal hormonal precursors, hydroxylated at (a) C_{17} and C_{21}, (b) C_{11} and C_{17}, and (c) C_{11}, C_{17} and C_{21}; and the known urinary metabolites that can be related to them.

It is obvious from the consideration of Figures 9–15 that almost every non-benzenoid steroid ever isolated from urine can be thought of as having been derived from adrenal cortical precursors. Only those labeled by the characteristic oxygen function at C_{11} or the tertiary hydroxyl group at C_{17} or the highly oxygenated side chain are unequivocally known to arise from these sources. Nevertheless, the compounds illustrated in the figures which do not possess these structural labels may likewise be metabolites of testosterone and progesterone, as well as of the adrenal hormones. The alterations that occur during metabolism tend to eliminate the structural differences that exist in the various hormonal progenitors, and, in some cases, lead to the formation of the same urinary end-product. Consequently, the correlation of some urinary metabolites to their true precursors frequently becomes difficult. Further consideration of Figures 9–15 will emphasize that adrenal cortical activity may be determined by an analysis of the urinary 17-ketosteroids, the "pregnanediol complex", as well as the corticoids. Since the urinary levels of each of these classes of substances depend, in part, upon different biogenetic and catabolic processes, the estimation of only one of these classes of urinary steroids may give an incomplete or unbalanced picture of adrenal function.

The contributions that investigations using isotopically labeled steroids have made to the general understanding of the catabolism of adrenal cortical hormones in man have been scant. Mention has already been made of the demonstrated conversion of deuterated 17α-hydroxyprogesterone to labeled etiocholanolone and androsterone (168). The synthesis of tritiated cortisone has been reported (172) and has been administered to humans (48), but no details on its metabolic fate have as yet been reported.

The studies in animals (mice and rats) have likewise been few. When 11-dehydrocorticosterone-21-C^{14} was administered to rats by Hsia *et al.* (252), no radioactivity was found in the expired CO_2 and most of the activity was found in the feces. Results with desoxycorticosterone-3,21-C_2^{14}-acetate injected intramuscularly into mice (307) showed that the radioactivity appearing in urine was greater than that in the feces and was present mainly in the water-soluble fraction. About 7% of the radioactivity appeared as CO_2 during the first 12 hours after injection.

Urinary estrogens. The schematic representation for the metabolism of the estrogens (given in Figure 16) proposed by Pincus and Pearlman (399) over a decade ago still describes the status of our knowledge. Estradiol-17β (*56*) is the most potent estrogen and is considered to be the true hormone. It is excreted in small amounts in the urine together with estrone (*57*) and much larger amounts of estriol (*58*). In experiments with exogenously administered estrogens, only a fraction (as determined either by isolation of crystalline materials or by biological activity) of the administered substance is recovered from the urine. This fact, together with the results obtained *in vitro* with tissue preparations, indicates that major pathways other than those shown in Figure 16 exist. As mentioned earlier, the bile and feces are of some importance as excretory routes for the estrogens in humans.

The conversion of estradiol into estrone has been demonstrated in several animals and man (219, 458). This transformation is not dependent upon the presence of ovaries or uterus, since it occurs in both males and females. The conversion of estrone to estradiol and estriol has also been observed in men and non-pregnant women (400). The ratio of the three estrogens in these experiments (as determined by partition methods involving distribution between an organic phase and solutions of different base strengths) was estriol: estradiol-17β: estrone as 5:4:1. The conversion of estradiol-17β to estriol was found to occur in 5.4% yield as determined from urinary studies in estradiol-treated men (458).

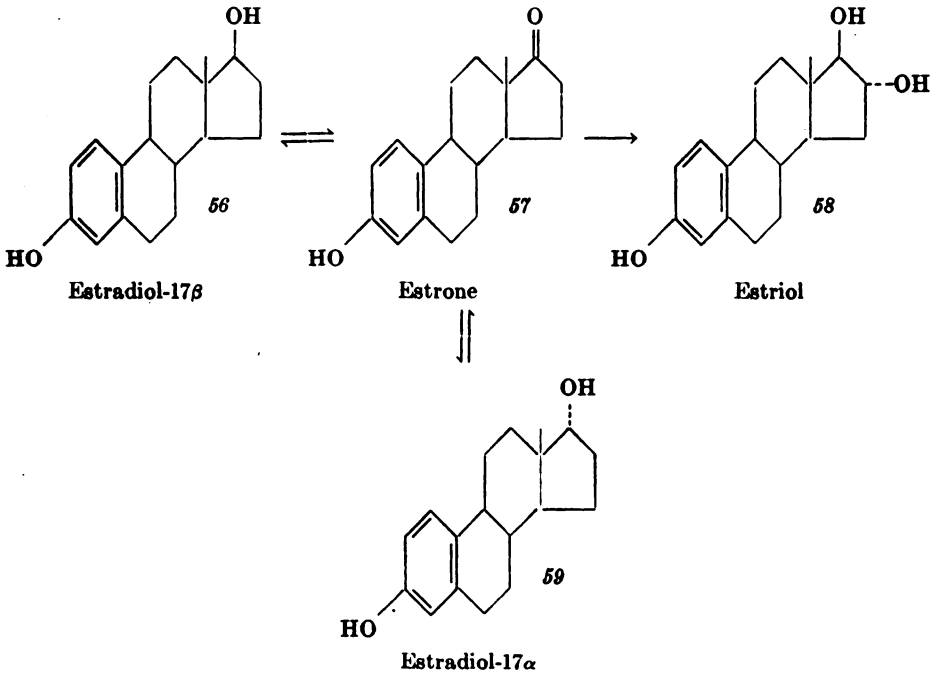


FIG. 16. Interconversions of the estrogens.

The significance of urinary estradiol-17α (*59*) is not clear for whereas 12% of the estradiol-17β administered to rabbits was recovered from the urine as estradiol-17α (218), little or none of this 17α-isomer was found in human urine after the administration of estrone (386).

The formation of estriol from estrone and estradiol-17β appears to be irreversible. After the administration of estriol to man, 50% could be recovered (by bioassay) in the urine (458); there was no increase in the excretion of estrone nor estradiol-17β. These results and others led to the formulation of the scheme as shown in Figure 16.

The excretion pattern of estrone, estradiol and estriol of 10 normal women following injection of 2.0 mg. of estrone to each was studied by Stimmel and Stealy (495) by means of a chromatographic technique. In both the pre-ovulatory

and post-ovulatory phases of the cycle, the urinary estrogen patterns were similar, leading the authors to conclude that the status of the endometrium cannot be deduced from these patterns.

Since estrone can be isolated from adrenal tissue, it has long been recognized that urinary estrogens may, in part, be derived from adrenal precursors. This idea is supported by the observed increase of urinary estrogens in patients with adrenal tumors, adrenal hyperplasia and Cushing's syndrome as has recently been reported by Migeon and Gardner (345). When cortisone was given to patients with adrenal hyperplasia, the urinary estrogens as well as the 17-ketosteroids decreased.

Isotopically labeled estrogens can aid in the elucidation of many unsolved problems. Extensive experimentation, mostly in animals, has already been carried out using estradiol-2(4)-I^{131} (2) and estrone-7,8-Br_2^{82} (515) as well as stilbesterol-C^{14} (202, 516) and sodium estrone sulfate-S^{35} (112, 313) and these have been adequately reviewed elsewhere (217, 514). Since these compounds cannot be considered to be natural hormones, the significance of the results obtained with them for estrogen metabolism is not clear. In addition, most of these experiments were conducted in animals and not in humans.

Estrone-16-C^{14} has recently become available and a few preliminary investigations of the metabolism of this compound have been reported. When labeled estrone was administered to tumor-bearing mice (307), some radioactivity was found in the bile, considerable quantities in the feces, about 20% in the urine and about 12% in the CO_2 in the first 12 hours. Of the radioactivity in the urine and feces, only 15% was extractable by ether; acid hydrolysis of the urine still left about 75% of the radioactivity water-soluble. Similarly, it has been reported that after the administration of estrone-16-C^{14} (130) or 17α-methyl-C^{14}-estradiol-17β (a compound equal in estrogenic activity to estradiol-17β) (41, 130, 362) to rats, most of the activity appeared in the feces. When bile fistula-animals were used, the major portion of the radioactivity was found in the bile (70%) (41), whereas bile-duct ligation resulted in the appearance of most of the activity in the urine.

Estrone-6,7-d_2 has been employed by Pearlman to test the thesis of Smith and Smith who believe that progesterone significantly influences the course of estrogen metabolism in human pregnancy (477). Since similar recoveries (about 4%) of estriol were obtained from the administration of estrone-6,7-d_2 (as the acetate) to women in late pregnancy (384) as had been previously obtained from (unlabeled) estrone-treated men (386), the conversion of estrone to estriol apparently did not depend upon the endogenous production of progesterone. A similar conclusion had been reached by Heard *et al.* (218) based upon experiments with estrone- or estradiol-treated rabbits. They found no estriol in the urine of these animals either with or without the simultaneous administration of progesterone.

VI. CONCLUDING REMARKS

Although only certain aspects of steroid hormone biochemistry have been considered in this essay, its length and breadth provide ample evidence of the

tremendous interest and effort that this subject has elicited. The progress which has resulted from this effort has, indeed, been striking, but in many instances it has served merely to emphasize the gaps in our knowledge and to suggest further researches. Thus, in spite of the fact that most of the active steroid hormones apparently have been isolated from glandular sources, there are indications that other active principles, as for example, the salt-retaining factor present in the "amorphous fraction" of adrenal gland extracts, remain to be isolated and identified. Spectacular advances have been made, in recent years, in the elucidation of the biogenesis of the adrenal hormones. Although the key roles of acetate and cholesterol in this biosynthesis have been indicated, the *in vivo* intermediates between acetate and cholesterol and between acetate and the adrenal hormones still remain obscure. The biosynthetic pathways leading to the formation of the estrogens and testosterone are at present all but unknown. Similarly, little information is available concerning the manner in which the steroid hormones are transported in the blood, although important progress is currently being made in the determination of the exact hormonal levels in blood. That the steroid hormones can influence every phase of metabolism is by now well established; nevertheless, the biochemical mechanisms by which these effects are mediated are completely unknown. The many studies of the metabolic effects of steroids upon a wide variety of enzyme systems, both *in vivo* and *in vitro*, have not as yet clarified the mechanism of hormone action. Despite the fact that the catabolic fate of the steroid hormones may be completely unrelated to their physiological and biochemical functions as hormones, more information concerning this aspect is available than about almost any other phase of steroid biochemistry. When considered together with the biogenesis of the steroid hormones, the catabolic fate of these biologically important substances affords significant information which can be correlated with the internal hormonal environment. Such information is essential for the proper understanding of normal physiological processes as well as many disease states.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. Wm. D. Cohen for his advice and aid in the preparation of the section on the "Metabolic effects of steroids at the enzymatic level".

The authors also gratefully acknowledge the partial support given them by grants from the National Institutes of Health, United States Public Health Service, and the Damon Runyon Memorial Fund.

REFERENCES

1. Adams, D. H.: Hormonal factors influencing catalase activity in mice. Testicular and adrenal factors. Biochem. J., 50: 496-493, 1952.
2. Albert, S., Heard, R. D. H., Leblond, C. P. and Saffran, J.: Distribution and metabolism of iodo-α-estradiol labeled with radioactive iodine. J. Biol. Chem., 177: 247-266, 1949.
3. Aldman, B., Dicefalusy, E., Högberg, B. and Rosenberg, T.: Inhibition of alkaline phosphatase by estradiol phosphates. Biochem. J., 49: 218-222, 1951.
4. Alfin-Slater, R. B., Deuel, H. J. Jr., Schots, M. C. and Shimoda, F. K.: Study of cholesterol turnover rate in using deuterium as a tracer element. Federation Proc., 9: 144-145, 1950.
5. Allen, W. M., Hayward, S. J. and Pinto, A.: A color test for dehydroisoandrosterone and closely related steroids, of use in the diagnosis of adreno-cortical tumors. J. Clin. Endocrinol., 10: 54-70, 1950.

6. ANDERSON, A. J. AND WARREN, F. L.: Extraction of conjugated androgens from normal human urine. J. Endocrinol., 7: lxv, 1951.
7. ANNER, G. AND MIESCHER, K.: Die Totalsynthese des natürlichen Östrons. Experientia, 4: 25–26, 1948.
8. ASHMORE, J. AND DOISY, E. A.: Metabolic studies of radiotestosterone. Federation Proc., 11: 182, 1952.
9. AXELROD, L. R.: Paper partition chromatography of progesterone and related compounds. Federation Proc., 11: 182–183, 1952.
10. AXELROD, L. R.: The quantitative separation of estrogens by paper partition chromatography. J. Biol. Chem., 201: 59–69, 1953.
11. BACHMANN, W. E., KUSHNER, S. AND STEVENSON, A. C.: The total synthesis of a stereoisomer of the sex hormone estrone. J. Am. Chem. Soc., 64: 974–981, 1942.
12. BAGGETT, B., GLICK, J. H. JR. AND KINSELLA, R. A. JR.: Urinary excretion of tetrahydrocortisone. J. Clin. Investigation, 31: 615–616, 1952.
13. BALMAIN, J. H., FOLLEY, S. J. AND GLASCOCK, R. F.: Inhibition of fatty acid synthesis in rat mammary gland slices by cortisone *in vitro* and its antagonism by insulin. Nature, 169: 447–449, 1952.
14. BARGONI, N. AND DIBELLA, S.: Dell'Azione di steroidi sulla citochromossidasi. Boll. Soc. ital. di biol. sper., 26: 1095–1096, 1950.
15. BARRY, M. C., EIDINOFF, M. L., DOBRINER, K. AND GALLAGHER, T. F.: The fate of C^{14}-testosterone and C^{14}-progesterone in mice and rats. Endocrinology, 50: 587–599, 1952.
16. BARTLETT, G. R. AND MACKAY, E. M.: Insulin stimulation of glycogen formation in rat abdominal muscle. Proc. Soc. Exper. Biol. & Med., 71: 493–495, 1949.
17. BARTLETT, G. R., WICK, A. N. AND MACKAY, E. M.: The influence of insulin and adrenal cortical compounds on the metabolism of radioactive C^{14}-glucose in the isolated rat diaphragm. J. Biol. Chem., 178: 1003–1004, 1949.
18. BATES, R. W. AND COHEN, H.: Experimental basis for selecting the optimal conditions for quantitative fluorimetry of natural estrogens. Endocrinology, 47: 166–181, 1950.
19. BATES, R. W. AND COHEN, H.: Fluorescence spectra of natural estrogens and their application to biological extracts. Endocrinology, 47: 182–192, 1950.
20. BAULD, W. S.: Estimation of urinary estrogens. 2nd Internat. Cong. Biochem., Abstr. of Communications, p. 120, Paris, July 1952.
21. BAYLISS, R. I. S.: Some observations on the hydrolysis and extraction of formaldehydogenic corticosteroids. Biochem. J., 52: 63–70, 1952.
22. BEER, C. T., DOBRINER, K., EIDINOFF, M. L. AND GALLAGHER, T. F.: Radioactive estrogens in humans. Federation Proc., 12: 176, 1953.
23. BERNSTEIN, S. AND MCGILVERY, R. W.: The enzymatic conjugation of m-aminophenol. J. Biol. Chem., 198: 195–203, 1952.
24. BILLETER, J. R. AND MIESCHER, K.: Über Steroide. 106. Mitteilung zur Totalsynthese der Steroide. Helv. Chim. Acta, 34: 2053–2073, 1951.
25. BISCHOFF, F. AND PILHORN, H. R.: The state and distribution of steroid hormones in biologic systems. III. Solubilities of testosterone, progesterone, and α-estradiol in aqueous salt and protein solution and in serum. J. Biol. Chem., 174: 663–682, 1948.
26. BISCHOFF, F. AND KATHERMAN, R. E.: Solubility of estrone in biologic systems. Federation Proc., 11: 188, 1952.
27. BISCHOFF, F., KATHERMAN, R. E., YEE, Y. S. AND MORAN, J. J.: Solubilities of testosterone and estradiol esters in biologic systems. Federation Proc., 11: 189, 1952.
28. BITMAN, J. AND COHEN, S. L.: Comparison of various techniques for hydrolysis of conjugated 17-ketosteroids in urine. Federation Proc., 9: 152, 1950.
29. BITMAN, J. AND COHEN, S. L.: Hydrolysis of urinary conjugated 17-ketosteroids by acetate buffer. J. Biol. Chem., 191: 351–363, 1951.
30. BLOCH, K.: Some aspects of the metabolism of leucine and valine. J. Biol. Chem., 155: 255–263, 1944.
31. BLOCH, K.: The biological conversion of cholesterol to pregnanediol. J. Biol. Chem., 157: 661-666, 1945.
32. BLOCH, K.: Biosynthesis of steroids, in A Symposium on Steroid Hormones, p. 33–45, The Univ. of Wisconsin Press, 1950.
33. BLOCH, K.: The biological synthesis of cholesterol, in Recent Progress in Hormone Research, 6: 111–129, New York, N. Y., Academic Press Inc., 1951.
34. BLOCH, K., BERG, B. N. AND RITTENBERG, D.: The biological conversion of cholesterol to cholic acid. J. Biol. Chem., 149: 511–517, 1943.
35. BLOCH, K., BOREK, E. AND RITTENBERG, D.: Synthesis of cholesterol in surviving liver. J. Biol. Chem., 162: 441–449, 1945.
36. BLOCH, K. AND RITTENBERG, D.: The biological formation of cholesterol from acetic acid. J. Biol. Chem., 143: 297–298, 1942.
37. BLOCH, K. AND RITTENBERG, D.: On the utilization of acetic acid for cholesterol formation. J. Biol. Chem., 145: 625–636, 1942.
38. BLOCH, K. AND RITTENBERG, D.: The preparation of deuteriocholesterol. J. Biol. Chem., 149: 505-509, 1943.
39. BLOCH, K. AND RITTENBERG, D.: An estimation of acetic acid formation in the rat. J. Biol. Chem., 159: 45–58, 1945.
41. BOCKLAGE, B. C., DOISY, E. A. JR., ELLIOTT, W. H., THAYER, S. A., NICHOLAS, H. J. AND DOISY, E. A.: Excretion of radioactive 17-methylestradiol. Federation Proc., 10: 164, 1951.
42. BONGIOVANNI, A. M. AND EISENMENGER, W. J.: Adrenal cortical metabolism in chronic liver disease. J. Clin. Endocrinol., 11: 152–172, 1951.

43. Bonner, J. and Arreguin, B.: The biochemistry of rubber formation in the guayule. I. Rubber formation in seedlings. Arch. Biochem., 21: 109–124, 1949.
44. Borek, E. and Rittenberg, D.: The metabolism of acetone by surviving rat liver. J. Biol. Chem., 179: 843–845, 1949.
45. Borth, R.: The chromatographic method for the determination of urinary pregnanediol, in Ciba Foundation Colloquia on Endocrinology, 2: 45–57, Philadelphia-N. Y., The Blakiston Co., 1952.
46. Boscott, R. J.: Paper chromatographic separation of urinary phenols and estrogens. Biochem. J., 48: xlvii–xlviii, 1951.
47. Boscott, R. J.: Paper chromatography of urinary phenolic acids, polyphenols and estrogens. Biochem. J., 51: xlv, 1952.
48. Bradlow, H. L., Kritchevsky, T. H., Katzenellenbogen, E. R., Dobriner, K. and Gallagher, T. F.: Hydrolysis of the metabolites of cortisone in humans. Federation Proc., 12: 181, 1953.
49. Brady, R. O.: Biosynthesis of radioactive testosterone *in vitro*. J. Biol. Chem., 193: 145–148, 1951.
50. Brady, R. O.: A rapid method for the qualitative and quantitative estimation of the physiological activity of the adrenal cortex. Endocrinology, 52: 49–53, 1953.
51. Brady, R. O. and Gurin, S.: The biosynthesis of radioactive fatty acids and cholesterol. J. Biol. Chem., 186: 461–469, 1950.
52. Brady, R. O. and Gurin, S.: The synthesis of radioactive cholesterol and fatty acids *in vitro*. J. Biol. Chem., 189: 371–377, 1951.
53. Braunsberg, H.: The fluorimetric determination of estrogens. J. Endocrinol., 8: 11–17, 1952.
54. Brookesbank, B. W. L. and Haslewood, G. A. D.: The nature of pregnanediol-like glucuronide. Biochem. J., 47: 36–43, 1950.
55. Brookesbank, B. W. L. and Haslewood, G. A. D.: The isolation of androst-16-en-3α-ol from women's urine. Biochem. J., 51: 286–288, 1952.
56. Bucher, N. L. R.: The formation of radioactive cholesterol and fatty acids from C^{14}-labeled acetate by rat liver homogenates. J. Am. Chem. Soc., 75: 498, 1953.
57. Bucher, N. L. R., McGovern, N. H., Kingston, R. and Kennedy, M. H.: Factors affecting biosynthesis of cholesterol from acetate by rat liver homogenates. Federation Proc., 12: 184, 1953.
58. Buehler, H. J., Katzman, P. A. and Doisy, E. A.: Hydrolysis of conjugates of neutral ketosteroids. Proc. Soc. Exper. Biol. & Med., 78: 3–8, 1951.
59. Burstein, S., Savard, K. and Dorfman, R. I.: *In vivo* metabolism of hydrocortisone and 21-desoxycortisone. Federation Proc., 12: 185, 1953.
60. Burton, R. B., Zaffaroni, A. and Keutmann, E. H.: Corticosteroids in urine of normal persons determined by paper chromatography. J. Biol. Chem., 193: 769–779, 1951.
61. Bush, I. E.: Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues. Biochem. J., 50: 370–378, 1952.
62. Bush, I. E.: Species differences in adrenocortical secretion. J. Endocrinol., 9: 95–100, 1953.
63. Bush, I. E.: Hormones in the adrenal venous effluent. J. Physiol., 112: 10P, 1951.
64. Bush, I. E., de Courcy, C. and Gray, C. H.: Adrenal cortical steroids in urine. J. Endocrinol., 8: xii, 1952.
65. Butt, H. R., Comfort, M. W., Power, M. H. and Mason, H. L.: Observations on the effect of cortisone acetate on two patients with hepatic disease. J. Lab. & Clin. Med., 37: 870–884, 1951.
66. Butt, W. R., Morris, P. and Morris, C. J. O. R.: Determination of Δ^4-3-ketosteroids in blood. 1st Internat. Cong. Biochem., Abstr. of Communications, p. 405–406, Cambridge, 1949.
67. Butt, W. R., Morris, P., Morris, C. J. O. R. and Williams, D. C.: Polarographic estimation of steroid hormones. 5. Determination of progesterone in blood. Biochem. J., 49: 434–438, 1951.
68. Cantarow, A., Paschkis, K. E., Williams, T. L. and Havens, W. P. Jr.: Urinary 17-ketosteroids in chronic liver disease. Federation Proc., 10: 23–24, 1951.
69. Cantarow, A., Paschkis, K. E., Williams, T. L. and Havens, W. P. Jr.: Studies on *in vitro* inactivation of estradiol. Federation Proc., 11: 22, 1952.
70. Cantarow, A., Rakoff, A. E., Paschkis, K. E. and Hansen, L. P.: Excretion of estrogen in bile. Proc. Soc. Exper. Biol. & Med., 49: 707–710, 1942.
71. Cantarow, A., Rakoff, A. E., Paschkis, K. E., Hansen, L. P. and Walkling, A. A.: Excretion of estrogen in bile. Endocrinology, 31: 515–519, 1942.
72. Cantarow, A., Rakoff, A. E., Paschkis, K. E., Hansen, L. P. and Walkling, A. A.: Excretion of exogenous and endogenous estrogen in bile of dogs and humans. Proc. Soc. Exper. Biol. & Med., 52: 256–257, 1943.
73. Cardwell, H. M. E., Cornforth, J. W., Duff, S. R., Holtermann, H. and Robinson, R.: Total synthesis of androgenic hormones. Chemistry & Industry, p. 389–390, 1951.
74. Carol, J., Haenni, E. O. and Baneo, D.: The preparation of β-dihydroequilin. J. Biol. Chem., 185: 267–270, 1950.
75. Carroll, K. K., McAlpine, H. T. and Noble, R. L.: Studies on the excretion of cortisone and compound F using the Porter-Silber method. Canad. M. A. J., 65: 363–367, 1951.
76. Case, E. M. and Dickens, F.: Inhibition of rat-liver succinoxidase by synthetic estrogens and related substances. Biochem. J., 42: 1, 1948.
77. Chaikoff, I. L., Siperstein, M. D., Dauben, W. G., Bradlow, H. L., Eastham, J. F., Tomkins, G. M. Meier, J. R., Chen, R. W., Hotta, S. and Sreeb, P. A.: C^{14}-cholesterol. II. Oxidation of carbons 4 and 26 to carbon dioxide by the intact rat. J. Biol. Chem., 194: 413, 1952.
78. Chaney, A. L., McKee, W. E., Fischer, R. H. and McColgan, S. P.: A new and simplified procedure for the

determination of free pregnanediol in urine, and its evaluation. J. Clin. Endocrinol. & Metab., 12: 735–743, 1952.

79. Channon, H. J.: The biological significance of the unsaponifiable matter of oils. I. Experiments with the unsaturated hydrocarbon, squalene (spinacene). Biochem. J., 20: 400–408, 1926.
80. Chen, C. and Tewell, H. E. Jr.: Corticosteroid determination with blue tetrazolium BT. Federation Proc., 10: 377–378, 1951.
81. Chiu, C. Y.: The effect of adrenal cortical preparations added *in vitro* upon the carbohydrate metabolism of liver slices. 2. The effect of some pure steroids upon carbohydrate synthesis, oxygen uptake and non-protein nitrogen. Biochem. J., 46: 120–124, 1950.
82. Chiu, C. Y. and Needham, D. M.: The effect of adrenal cortical preparations added *in vitro* upon the carbohydrate metabolism of liver slices. 1. The effect of adrenal cortical extract (eschatin) upon synthesis of glycogen and of total carbohydrate. Biochem. J., 46: 114–120, 1950.
83. Clark, D. H.: Cited in ref. 448.
84. Clark, I.: The effect of cortisone upon protein synthesis. J. Biol. Chem., 200: 69–76, 1953.
85. Clark, L. C. Jr. and Kochakian, C. D.: The *in vitro* metabolism of testosterone to Δ^4-androstenedione-3,17, cis-testosterone, and other steroids by rabbit liver slices. J. Biol. Chem., 170: 23–33, 1947.
86. Clark, L. C. Jr., Kochakian, C. D. and Fox, R. P.: Differential inhibition between normal and tumor (crown gall) tissue in beet roots. Science, 98: 89, 1943.
87. Cohen, H. and Bates, R. W.: Hydrolysis of conjugated sulfates of estrogens by commercial enzyme preparation of aspergillus oryzal. Endocrinology, 44: 317–324, 1949.
88. Cohen, H. and Bates, R. W.: Occurrence and determination of conjugated sulfates of estrogens in urine from pregnant women. Endocrinology, 45: 86–90, 1949.
89. Cohen, S. L.: The hydrolysis of steroid glucuronides with calf-spleen glucuronidase. J. Biol. Chem., 192: 147–160, 1951.
90. Cohen, S. L. and Goldfine, M. M.: The use of spleen glucuronidase as a hydrolytic agent for pregnanediol assays. J. Clin. Endocrinol. & Metab., 12: 916, 1952.
91. Cohen, S. L. and Oneson, I. B.: Dioxane-acid hydrolysis of ketosteroid conjugates. Federation Proc., 12: 191, 1953.
92. Cohn, C. and Kolinsky, M.: Influence of the previous diet on glucose utilization by isolated diaphragm of adrenalectomised rats. Am. J. Physiol., 171: 714, 1952.
93. Cohn, E. J.: Interactions of proteins with alkaline earths, with steroids, with blood cells and with specific polysaccharides, in Blood Cells & Plasma Proteins, their State in Nature, p. 43–60, New York, N. Y., Academic Press, Inc., 1953.
94. Colingsworth, D. R., Brunner, M. P. and Haines, W. J.: A partial microbiological synthesis of adrenal cortex hormones. J. Am. Chem. Soc., 74: 2381–2382, 1952.
95. Conroy, H.: Picrotoxin. II. The skeleton of picrotoxin. The total synthesis of dl-picrotoxadiene. J. Am. Chem. Soc., 74: 3046–3051, 1952.
96. Conway, E. J. and Hingerty, D.: The influence of adrenalectomy on muscle constituents. Biochem. J., 40: 561–568, 1946.
97. Coon, M. J.: The metabolic fate of the isopropyl group of leucine. J. Biol. Chem., 187: 71–82, 1950.
98. Coon, M. J. and Gurin, S.: Studies on the conversion of radioactive leucine to acetoacetate. J. Biol. Chem., 190: 1159–1167, 1949.
99. Cope, C. L. and Bain, M.: Urinary reducing steroids and the adrenal. Clin. Sc., 10: 161–176, 1951.
100. Cope, C. L. and Hurlock, B.: The chemical estimation of cortisone-like hormones in urine. Brit. M. J., 4792: 1020–1023, 1952.
101. Corcoran, A. C., Page, I. H. and Dustan, H. P.: Urinary formaldehydogenic corticoids, normal values and observations in hypertension. J. Lab. & Clin. Med., 36: 297–301, 1950.
102. Corcoran, A. C. and Page, I. H.: Methods for the chemical determination of corticosteroids in urine and plasma. J. Lab. & Clin. Med., 33: 1326–1333, 1948.
103. Cornforth, J. W., Hunter, G. D. and Popják, G.: The biosynthesis of cholesterol from acetate. Arch. Biochem. & Biophys., 42: 481–482, 1953.
104. Cox, R. I.: A method for the quantitative determination in urinary extracts of C_{21} 17:20-dihydroxy-20-methylsteroids. Biochem. J., 52: 339–345, 1952.
105. Csapó, Á.: Actomyosin content of the uterus. Nature, 162: 218–219, 1948.
106. Curran, G. L.: Utilization of acetoacetic acid in cholesterol synthesis by surviving rat liver. J. Biol. Chem., 191: 775–782, 1951.
107. Curran, G. L. and Rittenberg, D.: The role of ethyl alcohol in the biological synthesis of cholesterol. J. Biol. Chem., 190: 17–20, 1951.
108. Dauben, W. G. and Bradlow, H. L.: The synthesis of C^{14}-labeled "squalene" J.. Am. Chem. Soc., 74: 5204–5206, 1952.
109. Dauben, W. G., Bradlow, H. L., Freeman, W. K., Kritchevsky, D. and Kirk, M.: The regeneration of squalene from its solid hexahydrochloride. J. Am. Chem. Soc., 74: 4321–4323, 1952.
110. Daughaday, W. H., Jaffe, H., and Williams, R. H.: Chemical assay for "cortin". Determination of formaldehyde liberated on oxidation with periodic acid. J. Clin. Endocrinol., 8: 166–174, 1948.
111. Davis, J. S., Meyer, R. K. and McShan, W. H.: Effect of androgen and estrogen on succinic dehydrogenase and cytochrome oxidase of rat prostate and seminal vesicle. Endocrinology, 44: 1–7, 1949.
112. Davis, M. E., Kelsey, F. E., Fugo, N. W., Loucks, J. E., Horner, E. N. and Voskuil, P.: Metabolism and

excretion of estrone sulfate labeled with radioactive sulfur (S^{35}). Proc. Soc. Exper. Biol. & Med., 74: 501-505, 1950.

113. De Meio, R. H. and Tkacz, L.: Conjugation of phenol by rat liver slices and homogenates. J. Biol. Chem., 195: 175-184, 1952.
114. Devis, R.: Méthodes chimiques et applications cliniques du fractionnement chromatographique des stéroides urinaires. Ann. d'endocrinol. 12: 451-480, 1951.
115. de Watteville, H.: Le prégnandiol et son dosage dans les urines. Gynéc. et obst., 49: 155-166, 1950.
116. de Watteville, H., de Borth, R. and Gsell, M.: Effect of dl-α-tocopherol acetate on progesterone metabolism. J. Clin. Endocrinol., 8: 982-992, 1948.
117. Dicefalusy, E.: Progesterone in human placental tissue. Acta Endocrinol., 10: 373-389, 1952.
118. Dicefalusy, E.: A qualitative study on the estrogens in the human placenta. 2nd Internat. Cong. Biochem., Abstr. of Communications, p. 125, Paris, July 1952.
119. Dimter, A.: Untersuchungen über das Unverseifbare. I. Miteilung über das Unverseifbare des Ovarialdermoidcystenfettes. Ztschr. f. physiol. Chem., 270: 247-265, 1941.
120. Dingemanse, E., Huis in't Veld, L. G. and de Laat, B. M.: Clinical method for the chromatographic-colorimetric determination of urinary 17-ketosteroids. J. Clin. Endocrinol., 6: 535-548, 1946.
121. Dingemanse, E., Huis in't Veld, L. G. and Hartogh-Katz, S. L.: Clinical method for the chromatographic-colorimetric determination of urinary 17-ketosteroids. II. Normal adults. J. Clin. Endocrinol. & Metab., 12: 66-85, 1952.
122. Dirscherl, W., Bergmeyer, H. and Breuer, H.: Wirkung von Testosteronpropionat auf Atmung sowie aerobe und anaerobe Glykolyse der Vesiculardrüsen junger und alter Mäuse *in vitro*. Biochem. Ztschr., 322: 245-262 ff., 1952.
123. Dirscherl, W. and Hauptmann, K. H.: Analyse der Wirkung von Androgen und Estrogen sowie Cancergenen auf den Kohlehydratstoffwechsel der Leber. Biochem. Ztschr., 320: 199-227, 1949/50.
124. Dobriner, K. and Lieberman, S.: The metabolism of steroid hormones in humans, in A Symposium on Steroid Hormones, p. 46-88, Madison, The Univ. of Wisconsin Press, 1950.
125. Dobriner, K., Lieberman, S., Rhoads, C. P. and Taylor, H. C. Jr.: Urinary excretion of ketosteroids in pregnancy, in The Normal and Pathological Physiology of Pregnancy, p. 75-77, Baltimore, The Williams & Wilkins Co., 1948.
126. Dobriner, K., Lieberman, S., Wilson, H., Ekman, B. and Rhoads, C. P.: Steroid excretion and adrenal function in neoplastic disease, in Pituitary-Adrenal Function, p. 158, Washington, D. C., Am. Assoc. Advancement Sc., 1950.
127. Dobriner, K., Rhoads, C. P., Lieberman, S., Hill, B. R. and Fieser, L. F.: Abnormal α-ketosteroid excretion in patients with neoplastic disease. Science, 99: 494-496, 1944.
128. Dostech, R.: Die Glykogen-Phosphorylierung durch Muskel und Leber bei normalen und adrenalektomierten Tieren. Helv. Chim. Acta, 28: 31-41, 1945.
129. Dohan, F. C., Richardson, E. M., Bluemle, L. W. Jr. and Gyorgy, P.: Hormone excretion in liver disease. J. Clin. Investigation, 31: 481-498, 1952.
130. Doisy, E. A. Jr., Bocklage, B. C., Shen, N. H. and Valcourt, A. F.: A comparison of the metabolism of some female sex hormones, Science, 116: 520, 1952.
131. Done, A. K., Ely, R. S., Raile, R. B. and Kelley, V. C.: Species differences in circulating 17-hydroxycorticosteroid concentrations. Proc. Soc. Exper. Biol. & Med., 81: 667-669, 1952.
132. Dorfman, R. I.: Steroids and tissue oxidation, Vitamins & Hormones, 10: 331-370, New York, N. Y., Academic Press, Inc., 1952.
133. Dorfman, R. I., Ungar, F. and Savard, K.: Conversion of C^{14} acetate to labeled steroids. 2nd Internat. Cong. Biochem., Abstr. of Communications, p. 126, Paris, July 1952.
134. Dorfman, R. I., Wise, J. E. and Shipley, R. A.: Metabolism of the steroid hormones. Endocrinology, 46: 127-131, 1950.
135. Drekter, I. J., Heisler, A., Scism, G. R., Stern, S., Pearson, S. and McGavack, T. H.: The determination of urinary steroids. I. The preparation of pigment-free extracts and a simplified procedure for the estimation of total 17-ketosteroids. J. Clin. Endocrinol. & Metab., 12: 55-65, 1952.
136. Dustan, H. P., Mason, H. L. and Corcoran, A. C.: Urinary formaldehydogenic corticoid as determined after enzymatic hyrolysis in normal subjects and in patients with adrenal and hypertensive disease. J. Clin. Investigation, 32: 60-67, 1953.
137. Edgar, D. G.: Progesterone in body fluids. Nature, 170: 543-544, 1952.
138. Eidinoff, M. L. and Knoll, J. E.: The measurement of radioactive hydrogen in solid samples. Comparison without gas counting. Science, 112: 250-251, 1950.
139. Eisenberg, E., Gordan, G. S. and Elliott, H. W.: The effect of castration and of testosterone upon the respiration of rat brain. Science, 109: 337-338, 1949.
140. Eisenstein, A. B.: Steroid compounds resulting from incubation of cortisone with surviving liver slices. Science, 116: 520-521, 1952.
141. Elliott, W. H., Ashmore, J. and Hyde, P. M.: The metabolism of androgens. Science, 116: 520, 1952.
142. Elliott, W. H., Doisy, E. A. Jr., Bocklage, B. C., Shen, F. N. S., Thayer, S. A., Hyde, P. M. and Doisy, E. A.: Role of the liver in the metabolism of steroids. 12th Internat. Cong. of Pure and Applied Chemistry, Abstr. of Communications, p. 105-106, New York, N. Y., September 1951.
143. Engel, L. L.: The chemical estimation of steroid hormone metabolites, in Recent Progress Hormone Research, 5: 335-379, New York, N. Y., Academic Press, Inc., 1950.

144. ENGEL, L. L., SLAUNWHITE, W. R. JR., CARTER, P. AND OLMSTED, P. C.: A relation between phase composition and partition coefficients for estrogens. J. Biol. Chem., 191: 621–625, 1951.
145. ENGEL, L. L. AND RYAN, K. J.: The *in vitro* metabolism of the natural estrogens by normal and neoplastic tissue. 2nd Internat. Cong. Biochem., Abstr. of Communications, p. 469, Paris, July 1952.
146. ENGEL, L. L., SLAUNWHITE, W. R. Jr., CARTER, P., OLMSTEAD, P. C. AND NATHANSON, I. T.: The application of counter-current distribution to the separation and characterization of urinary estrogens, in Ciba Foundation Colloquia on Endocrinology, 2: 104–116, Philadelphia-New York, The Blakiston Co., 1952.
147. ENGEL, L. L., THORN, G. W. AND LEWIS, R. A.: The urinary excretion of steroid compounds. I. Normal male subjects. J. Biol. Chem., 137: 205–215, 1941.
148. ENGSTROM, W. W., MUNSON, P. L., COOK, L. AND COSTA, P. J.: Studies on the conversion of testosterone to urinary 17-ketosteroids in normal human males and males with hepatic and other diseases. J. Clin. Endocrinol., 11: 416–426, 1951.
149. EPPSTEIN, S. H., MEISTER, P. D., PETERSON, D. H., MURRAY, H. C., LEIGH, H. M., LYTTLE, D. A., REINEKE, L. M. AND WEINTRAUB, A.: Microbiological transformations of steroids. III. Preparation of 11-epi-corticosterone and of 6β-hydroxy-11-desoxycorticosterone. J. Am. Chem. Soc., 75: 408–412, 1953.
150. EPPSTEIN, S. H., PETERSON, D. H., LEIGH, H. M., MURRAY, H. C., WEINTRAUB, A., REINEKE, L. M. AND MEISTER, P. D.: Microbiological transformations of steroids. VII. Preparation of 11α-hydroxy-pregnane-3,20-dione and 11α-hydroxyallopregnane-3,20-dione. J. Am. Chem. Soc., 75: 421, 1953.
151. EVANS, J. M., YOUNG, J. P., HERTZ, R., TULLNER, W. W., WILMER, G. and WOOD, O.: The ability of the human liver to reduce the biologic activity of injected estrogens. J. Clin. Endocrinol. & Metab., 12: 495–501, 1952.
152. EVERETT, J. W. AND SAWYER, C. H.: Effects of castration and treatment with sex steroids on the synthesis of serum cholinesterase in the rat. Endocrinology, 39: 323–343, 1946.
153. FIESER, L. F. AND FIESER, M.: Natural Products Related to Phenanthrene, New York, N. Y., Rheingold Publishing Corp., 1949.
154. FINKELSTEIN, M. AND GOLDENBERG, C.: Inactivation of estrone by liver homogenates determined by fluorimetry. Bull. Research Council Israel, 1: 154–156, 1951.
155. FINKELSTEIN, M., HESTRIN, S. AND KOCH, W.: Estimation of steroid estrogens by fluorimetry. Proc. Soc. Exper. Biol. & Med., 64: 64–71, 1947.
156. FISCHER, R. H., MCCOLGAN, S. P. AND CHANEY, A. L.: Progesterone metabolism. I. Pregnanediol excretion in the menstrual cycle. Am. J. Obst. & Gynec., 63: 613–619, 1952.
157. FISH, C. A., HAYANO, M. AND PINCUS, G.: Conversion of cortisone to 17-hydroxycorticosterone by liver homogenates. Arch. Biochem. & Biophys., 42: 480–481, 1953.
158. FISHMAN, W. H.: The Enzymes, 1: 648, New York, N. Y., Academic Press, Inc., 1950.
159. FISHMAN, W. H.: Relationship between estrogens and enzyme activity. Vitamins & Hormones, 9: 213–236, 1951.
160. FISHMAN, W. H., ODELL, L. D., GILL, J. E. AND CHRISTENSEN, R. A.: II. The influence of stilbestrol on serum β-glucuronidase in women following pasturition. Am. J. Obst. & Gynec., 59: 414–418, 1950.
161. FOLCH-PI, J.: Linkages between proteins and lipids, in Blood Cells and Plasma Proteins, Their State in Nature, p. 378–381, New York, N. Y., Academic Press, Inc., 1953.
162. FORBES, T. R.: Systemic levels of plasma progesterone during pregnancy in women. Endocrinology, 49: 218–224, 1951.
163. FRAENKEL-CONRAT, H., SIMPSON, M. E. AND EVANS, H. M.: Influence of adrenalectomy and of adrenocortical steroids on liver arginase. J. Biol. Chem., 147: 99–108, 1943.
164. FRIED, J., THOMA, R. W., GERKE, J. R., HERR, J. E., DONIN, M. N. AND PERLMAN, D.: Oxidation of steroids by micro-organisms. II. Hydroxylation in position 11 and synthesis of cortisone from Reichstein's compound S. J. Am. Chem. Soc., 74: 3963–3963, 1952.
165. FRIEDEN, E. H., STEELE, A. C. AND TELFER, M. A.: Hormonal effects on the incorporation of labeled amino acids in tissues of the reproductive tract. Federation Proc., 11: 215–216, 1952.
166. FRIEDMAN, M., SANDFORD, O. AND MICHAELIS, F.: Production and excretion of cholesterol in mammals. Am. J. Physiol., 164: 789–791, 1951.
167. FUJIMOTO, G. I.: Labeling of steroids in the 4-position. J. Am. Chem. Soc., 73: 1856, 1951.
168. FUKUSHIMA, D. K., DOBRINER, K. AND GALLAGHER, T. F.: Metabolic studies with deuterium steroid hormones. Federation Proc., 10: 185, 1951.
169. FUKUSHIMA, D. K., DOBRINER, K. AND GALLAGHER, T. F.: Testosterone production in a normal man. Federation Proc., 11: 217, 1952.
170. FUKUSHIMA, D. K. AND GALLAGHER, T. F.: Isotopic distribution in cholesterol after platinum-catalysed hydrogen-deuterium exchange. J. Biol. Chem., 196: 861–869, 1952.
171. FUKUSHIMA, D. K. AND GALLAGHER, T. F.: Studies on the platinum-catalysed exchange of hydrogen isotopes with steroids. J. Biol. Chem., 196: 871–884, 1952.
172. FUKUSHIMA, D. K., KRITCHEVSKY, T. H., EIDINOFF, M. L. AND GALLAGHER, T. F.: Synthesis of radioactive cortisone. J. Am. Chem. Soc., 74: 487–490, 1952.
173. FUKUSHIMA, D. K., STOKEM, M. B., BRADLOW, H. L., KRITCHEVSKY, T. H., HELLMAN, L., DOBRINER, K. AND GALLAGHER, T. F.: Urinary steroids after testosterone and progesterone in humans. Federation Proc., 12: 206–207, 1953.
174. GALLAGHER, T. F.: Studies with deuterium steroids, in Isotopes in Biochemistry, p. 28–40, Philadelphia, Pa., The Blakiston Co., 1951.

175. Gallagher, T. F., Fukushima, D. K., Barry, M. C. and Dobriner, K.: Studies with isotopic steroid hormones, in Recent Progress in Hormone Research, 6: 131–152, New York, N. Y., Academic Press, Inc., 1951.
176. Garst, J. B. and Friedgood, H. B.: On the nature of the so-called background material in estrogen fractions of extracts prepared from hydrolyzed urine. Science, 116: 65–66, 1952.
177. Gilder, H. and Hoagland, C. L.: Urinary excretion of estrogens and 17-ketosteroids in young, adult males with infectious hepatitis. Proc. Soc. Exper. Biol. & Med., 61: 62–65, 1946.
178. Glass, S. J.: The influence of the liver on sex endocrine functions. Progress in Clinical Endocrinology, p. 489–503, New York, N. Y., Grune & Stratton, Inc., 1950.
179. Goldzieher, J. W., Bodenchuk, J. M. and Nolan, P.: The fluorescence reactions of steroids. I. Estrogens. J. Biol. Chem., 199: 621–629, 1952.
180. Goldzieher, J. W. and Roberts, I. S.: Identification of estrogen in the human testis. J. Clin. Endocrinol. & Metab., 12: 143–150, 1952.
181. Gordan, G. S. and Elliott, W. H.: The action of diethylstilbestrol and some steroids on the respiration of rat brain homogenates. Endocrinology, 41: 517–581, 1947.
182. Gornall, A. G. and Macdonald, M. P.: A quantitative colorimetric procedure for corticosteroids. Rev. Can. Biol., 11: 62, 1952.
183. Gould, R. G., Campbell, D. J., Taylor, C. B., Kelly, F. B. Jr., Warner, I. and Davis, C. B. Jr.: Origin of plasma cholesterol using carbon-14. Federation Proc., 10: 191, 1951.
184. Grady, H. J., Elliott, W. H., Doisy, E. A. Jr., Bocklage, B. C. and Doisy, E. A.: Synthesis and biological studies of progesterone-21-C^{14}. Abstr., Am. Chem. Soc. 118th Meeting, 28C, Chicago, Ill., September, 1950.
185. Grady, H. J., Elliott, W. H., Doisy, E. A. Jr., Bocklage, B. C. and Doisy, E. A.: Synthesis and metabolic studies of progesterone-21-C^{14}. J. Biol. Chem., 195: 755–762, 1952.
186. Granata, L.: Biossigenazione del desossicorticosterone mediante sezioni ore omogenati di corteccia surrenale, sua conversione in prodotti ad attivita glicogenetica. Boll. Soc. ital. di biol. sper., 27: 228–230, 1951.
187. Grant, G. A. and Beall, D.: Studies on estrogen conjugates, in Recent Progress Hormone Research, 5: 307–334, 1950.
188. Grant, J. K.: Studies on the metabolism of progesterone and related steroids *in vitro*. 2. Factors influencing the metabolism of pregnane-3α, 20α-diol by rat-liver homogenates and investigation of products of metabolism. Biochem. J., 51: 358–362, 1952.
189. Grant, J. K. and Marrian, G. F.: Studies on the metabolism of progesterone and related steroids *in vitro*. 1. The metabolism of pregnane-3α, 20α-diol by liver preparations. Biochem. J., 47: 497–502, 1950.
190. Grant, J. K. and Taylor, W.: The influence of steroids on citrate metabolism. Biochem. J., 52: xxiv, 1952.
191. Graubard, M. and Pincus, G.: Steroid metabolism, estrogens and phenolases. Endocrinology, 30: 285–289, 1942.
192. Guidry, M. A., Segaloff, A. and Altschul, A. M.: Effect of α-estradiol, some of its esters and estrone on the respiration of tissue homogenates. Endocrinology, 50: 29–36, 1952.
193. Gurin, S. and Brady, R. O.: The biosynthesis of radioactive cholestrol by surviving liver slices, in Isotopes in Biochemistry, p. 17–27, Philadelphia, Pa., The Blakiston Co., 1951.
194. Hagerman, D. D. and Villee, C. A.: Effects of estradiol on human endometrium and placenta *in vitro*. Federation Proc., 11: 235, 1952.
195. Hagerman, D. D. and Villee, C. A.: Effects of estradiol in metabolism of human endometrium *in vitro*. Arch. Biochem. & Biophys., 40: 481–483, 1952.
196. Haines, W. J.: Studies on the biosynthesis of adrenal cortex hormones, in Recent Progress in Hormone Research, 7: 255–305, New York, N. Y., Academic Press, Inc., 1952.
197. Haines, W. J., Nelson, E. D., Drake, N. A. and Woods, O. R.: Biosynthesis of 17-α-hydroxycorticosterone from acetate, Arch. Biochem. & Biophys., 32: 218–220, 1951.
198. Hamburger, C.: A new and simple method of preparing alcoholic potassium hydroxide for 17-ketosteroid determinations. Acta Endocrinol., 9: 129–134, 1952.
199. Hamilton, J. G. Jr. and Holman, R. T.: Displacement analysis of lipids. VIII. Preliminary studies with steroids. Arch. Biochem. & Biophys., 36: 456–461, 1952.
200. Hanahan, D. J. and Wakil, S. J.: The origin of some of the carbon atoms of the side chain of C^{14}-ergosterol. J. Am. Chem. Soc., 75: 273–275, 1953.
201. Hanahan, D. J. and Wakil, S. J.: The biosynthesis of ergosterol from isotopic acetate. Arch. Biochem. & Biophys., 37: 167–171, 1952.
202. Hanahan, D. J., Daskalakis, E. G., Edwards, T., Dauben, J. H. Jr. and Meikle, R. W.: Observations on metabolism of C^{14}-diethylstilbestrol. Arch. Biochem. & Biophys., 33: 342–343, 1951.
203. Harris, R. S. and Cohen, S. L.: The influence of ovarian hormones on the enzymic activities of tissues. Endocrinology, 48: 264–272, 1951.
204. Haskins, A. L. Jr.: Assay of circulating progesterone by ultraviolet spectroscopy. Proc. Soc. Exper. Biol. & Med., 73: 439–443, 1950.
205. Haslam, R. M. and Klyne, W.: Allopregnane-3β,20β-diol as a standard for determination of urinary pregnanediol. Lancet, I: 399, 1952.
206. Hayano, M. and Dorfman, R. I.: Studies on the inhibition of various enzymes by steroids. Ann. N. Y. Acad. Sc., 54: 608–618, 1951.
207. Hayano, M. and Dorfman, R. I.: The action of adrenal homogenates on progesterone, 17-hydroxyprogesterone and 21-desoxycortisone. Arch. Biochem. & Biophys., 36: 237–239, 1952.
208. Hayano, M. and Dorfman, R. I.: The enzymatic C-11β-hydroxylation of steroids. J. Biol. Chem., 201: 175–188, 1953.

209. HAYANO, M., DORFMAN, R. I. AND PRINS, D. A.: Metabolism of steroid hormones. Conversion of desoxycorticosterone to glycogenic material *in vitro*. Proc. Soc. Exper. Biol. & Med., 72: 700–701, 1949.
210. HAYANO, M., DORFMAN, R. I. AND YAMADA, E. Y.: The inhibition of D-amino acid oxidase by desoxycorticosterone. J. Biol. Chem., 186: 603–614, 1950.
211. HAYANO, M., DORFMAN, R. I. AND YAMADA, E. Y.: The conversion of desoxycorticosterone to glycogenic material by adrenal homogenates. J. Biol. Chem., 193: 175–181, 1951.
212. HAYANO, M., SCHILLER, S. AND DORFMAN, R. I.: Influence of various steroids on the oxidative function of rat tissue preparations. Endocrinology, 46: 387–391, 1950.
213. HAYANO, M., WIENER, M. AND LINDBERG, M. C.: Steroid hydroxylases of adrenal and corpus luteum. Federation Proc., 12: 216, 1953.
214. HAYNES, R., SAVARD, K. AND DORFMAN, R. I.: An *in vitro* effect of ACTH. J. Clin. Endocrinol. & Metab., 12: 972, 1952.
215. HAYNES, R., SAVARD, K. AND DORFMAN, R. I.: An action of ACTH on adrenal slices. Science, 116: 690–691, 1952.
216. HAYNES, R., SAVARD, K. AND DORFMAN, R. I.: Effect of ACTH on corticoid production of adrenal cortical tissue slices. Federation Proc., 12: 216, 1953.
217. HEARD, R. D. H.: The metabolism of the estrogens, Part I, in Recent Progress in Hormone Research, 4: 25–37, New York, N. Y., Academic Press, Inc., 1949.
218. HEARD, R. D. H., BAULD, W. S. AND HOFFMAN, M. M.: Steroids. V. α-estradiol and progesterone metabolism. J. Biol. Chem., 141: 709–710, 1941.
219. HEARD, R. D. H. AND HOFFMAN, M. M.: Steroids. IV. The fate in man of injected α-estradiol. J. Biol. Chem., 141: 329–342, 1941.
220. HEARD, R. D. H. AND SAFFRAN, J. C.: The metabolism of the estrogens, Part II, in Recent Progress in Hormone Research, 4: 43–63, New York, N. Y., Academic Press, Inc., 1949.
221. HEARD, R. D. H., SAFFRAN, J. C. AND THOMPSON, L.: Cited in Leblond, C. P., Metabolism of C^{14}-labeled steroids, in Isotopes in Biochemistry, p. 61, Philadelphia, Pa., The Blakiston Co., 1951.
222. HEARD, R. D. H. AND SOBEL, H.: Steroids, VIII. A colorimetric method for the estimation of reducing steroids. J. Biol. Chem., 165: 687–698, 1946.
223. HEARD, R. D. H. AND ZIEGLER, P.: The preparation of radioactive progesterone and desoxycorticosterone labeled in ring A. J. Am. Chem. Soc., 72: 4328, 1950.
224. HEARD, R. D. H. AND ZIEGLER, P.: Steroids. IX. An improved method of preparation of cholestenone-4-C^{14}. J. Am. Chem. Soc., 73: 4036–4038, 1951.
225. HECHTER, O.: Lymphocyte discharge from the isolated rabbit spleen by adrenal cortical extract. Endocrinology 42: 295–306, 1948.
226. HECHTER, O.: Corticosteroid release from an isolated adrenal gland. Federation Proc., 8: 70–71, 1949.
227. HECHTER, O.: Characterization of corticosteroids released from perfused cow adrenals. Federation Proc., 9: 58–59, 1950.
228. HECHTER, O.: Comments, in Recent Progress in Hormone Research, 7: 301–304, New York, N. Y., Academic Press, Inc., 1951.
229. HECHTER, O., JACOBSEN, R. P., JEANLOZ, R. W., LEVY, H., MARSHALL, C. W., PINCUS, G. AND SCHENKER, V.: The bio-oxygenation of 11-desoxycorticosterone at C_{11}. J. Am. Chem. Soc., 71: 3261–3262, 1949.
230. HECHTER, O., JACOBSEN, R. P., JEANLOZ, R. W., LEVY, H., MARSHALL, C. W., PINCUS, G. and SCHENKER, V.: Bio-oxygenation of steroids at C_{11}. Arch. Biochem., 25: 457–459, 1950.
231. HECHTER, O., JACOBSEN, R. P., JEANLOZ, R. W., LEVY, H., PINCUS, G. AND SCHENKER, V.: Pathways of corticosteroid synthesis. J. Clin. Endocrinol., 10: 827–828, 1950.
232. HECHTER, O., JACOBSEN, R. P., JEANLOZ, R. W., LEVY, H., PINCUS, G. AND SCHENKER, V.: Pathways of corticosteroid synthesis. Proc. Am. Diabetes Assoc., 10: 39–45, 1950.
233. HECHTER, O., SOLOMON, M. M., MACCHI, I. A., CASPI, E. AND FEINSTEIN, M.: Corticosteroid metabolism in isolated perfused rat livers. J. Clin. Endocrinol. & Metab., 12: 935–936, 1952.
234. HECHTER, O., ZAFFARONI, A., JACOBSEN, R. P., LEVY, H., JEANLOZ, R. W., SCHENKER, V. AND PINCUS, G.: The nature and the biogenesis of the adrenal secretory product, in Recent Progress in Hormone Research, 6: 215–246, New York, N. Y., Academic Press, Inc., 1951.
235. HEFTMANN, E.: Identification of estrogens by paper chromatography. Science, 3: 571–572, 1950.
236. HELLER, C.: Metabolism of the estrogens. The effect of liver and uterus upon estrone, estradiol, and estriol. Endocrinology, 26: 619–630, 1940.
237. HELLMAN, L., ROSENFELD, R. R., FUKUSHIMA, D. K., GALLAGHER, T. F. AND DOBRINER, K.: Utilization of C^{14}-acetate for cholesterol and steroid hormone synthesis in the human. J. Clin. Endocrinol. & Metab., 12: 934–935, 1952.
238. HELMREICH, M. L., LASATER, M. B. AND SAMUELS, L. T.: Influence of chorionic gonadotrophin on activity of dehydrogenase in testis which converts 3-hydroxysteroids to 3-ketones. Federation Proc., 11: 228, 1952.
239. HENLY, A. A.: Estimation of corticosteroids. Nature, 169: 877, 1952.
240. HENLY, A. A. AND POTTER, M.: The estimation of formaldehydogenic steroids. Lancet, II: 697–698, 1952.
241. HERTZ, R., TULLNER, W. W., WESTFALL, B. B., MORROW, A. G. AND EMGE, M. K.: Intravenous administration of massive dosages of estrogen to the human subject. Blood levels attained. Proc. Soc. Exper. Biol. & Med., 72: 187–191, 1949.
242. HIRSCHMANN, H.: Steroid excretion in a case of adrenocortical carcinoma. I. The isolation of a Δ^4-androstenetriol-3(β),16,17. J. Biol. Chem., 150: 363–379, 1943.

243. HIRSCHMANN, H. AND HIRSCHMANN, F. B.: Steroid excretion in a case of adrenocortical carcinoma. II. An examination of the non-ketonic fraction precipitable with digitonin. J. Biol. Chem., 157: 601–612, 1945.
244. HIRSCHMANN, H. AND HIRSCHMANN, F. B.: Steroid excretion in a case of adrenocortical carcinoma. III. The isolation of Δ^5-pregnenediol-3(β),17(β)-one-20 and of 17a-methyl-Δ^5-D-homoandrostenediol-3(β),17a(α)-one-17. J. Biol. Chem., 167: 7–25, 1947.
245. HIRSCHMANN, H. AND HIRSCHMANN, F. B.: Steroid excretion in a case of adrenocortical carcinoma. IV. Δ^5-pregnenetriol-3β,16α,20α. J. Biol. Chem., 184: 259–282, 1950.
246. HIRSCHMANN, H. AND HIRSCHMANN, F. B.: Steroid excretion in a case of adrenocortical carcinoma. V. Δ^5-pregnenetriol-3β,17α,20α. J. Biol. Chem., 187: 137–146, 1950.
247. HOCHSTER, R. M. AND QUASTEL, J. H.: Effects of steroids and diethylstilbestrol on dehydrogenase systems. Ann. N. Y. Acad. Sc., 54: 626–635, 1951.
248. HOFMANN, H. AND STAUDINGER, HJ.: Zur Biosynthese der Nebennierenrinden-Hormone. Arzneimittel-Forsch., 1: 416–417, 1951.
249. HOFMANN, H. AND STAUDINGER, HJ.: Papierchromatographische Trennung und Bestimmung der Nebennierenrinden-Hormone. Biochem. Ztschr., 322: 230–236, 951.
250. HOLMAN, H. R., WHITE, A. AND FRUTON, J. S.: Relation of adrenal cortex to serum peptidase activity. Proc. Soc. Exper. Biol. & Med., 65: 196–199, 1947.
251. HOOKER, C. W. AND FORBES, T. R.: The transport of progesterone in blood. Endocrinology, 44: 61–66, 1949.
252. HSIA, R. S., ELLIOTT, W. H., DOISY, E. A. JR. AND THAYER, S. A.: Synthesis and metabolic studies of 11-dehydrocorticosterone-21-C^{14} and its acetate. Federation Proc., 11: 232–233, 1952.
253. HUGGINS, C. AND HODGES, C. V.: Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injection on serum phosphotases in metastatic carcinoma of the prostate. Cancer Research, 1: 293–297, 1941.
254. HUGGINS, C., SCOTT, W. W. AND HODGES, C. V.: Studies on prostatic cancer. III. The effects of fever, of desoxycorticosterone and of estrogen on clinical patients with metastatic carcinoma of the prostate. J. Urol., 46: 997–1006, 1941.
255. HYDE, P. M., ELLIOTT, W. H. AND DOISY, E. A.: Metabolic studies of 17-methyl-C^{14}-testosterone. Federation Proc., 12: 223, 1953.
256. IZZO, J. I. AND GABIGA, A. M.: Colorimetric determination of corticosteroid-like substances in urine with blue tetrasolium. Federation Proc., 12: 224, 1953.
257. JAYLE, M. F.: Exploration biochimique de la cortico-surrénale. Ann. d'Endocrinol., 12: 404–450, 1951.
258. JAYLE, M. F.: Exploration de la fonction endocrinienne de la gestation. Gynéc. & Obst., 49: 336–356, 1950.
259. JAYLE, M. F. AND CRÉPY, O.: The excretion and measurement of urinary phenolsteroids. Endocrinology, 2: 84–103, Philadelphia, Pa.-New York, N. Y., The Blakiston Co., 1952.
260. JEANLOZ, R. W., LEVY, H., HECHTER, O., SCHENKER, V., JACOBSEN, R. P. AND PINCUS, G.: Chemical transformation of Δ^4-androstene-3,17-dione by adrenal perfusion. Abstr., 118th Meeting Am. Chem. Soc. 20C, September 1950.
261. JEANLOZ, R. W., LEVY, H., JACOBSEN, R. P., HECHTER, O., SCHENKER, V. AND PINCUS, G.: The chemical transformation of progesterone by adrenal perfusion. Abstr., 118th Meeting Am. Chem. Soc. 29C, September 1950.
262. JENSEN, H. AND GRAY, J. L.: The influence of hormones on the amino acid dehydrogenase systems of the liver and kidney. Ann. N. Y. Acad. Sc., 54: 619–625, 1951.
263. JENSEN, C. C.: Quantitative determination of dehydroisoandrosterone. I. Determination in pure solutions. Acta Endocrinol., 4: 140–152, 1950.
264. JENSEN, C. C.: Quantitative determination of dehydroisoandrosterone. II. Determination in urinary extracts. Acta Endocrinol., 4: 374–400, 1950.
265. JENSEN, C. C. AND TÖTTERMAN, L. E.: Hydrolysis of urinary neutral 17-ketosteroid conjugates. Acta Endocrinol., 10: 221–232, 1952.
266. JENSEN, C. C. AND TÖTTERMAN, L. E.: Hydrolysis of urinary neutral 17-ketosteroid conjugates. II. A fractional hydrolytic procedure. Acta Endocrinol., 11: 33–48, 1952.
267. JOHANSEN, A. AND THYGESEN, J. E.: The effect of pituitary adrenocorticotropic hormone on human serum peptidase activity. Scand. J. Clin. Lab. Invest., 3: 66–79, 1951.
268. JOHNSON, W. S., BANNISTER, B., BLOOM, B. M., KEMP, A. D., PAPPO, R., ROGIER, E. R. AND SZMUSZKOVICZ, S.: Total synthesis of epiandrosterone. J. Am. Chem. Soc., 75: 2275–2276, 1953.
269. JOHNSON, W. S. AND CHRISTIANSEN, R. G.: A new total synthesis of estrone. J. Am. Chem. Soc., 73: 5511–5512, 1951.
270. JONES, R. N. AND DOBRINER, K.: Infrared spectrometry applied to steroid structure and metabolism, in Vitamins and Hormones, 7: 293–363, New York, N. Y., Academic Press, Inc., 1949.
271. KAHNT, F. W. AND WETTSTEIN, A.: Die 11-Oxydation von Desoxycorticosteron und Reichsteins Substanz S mit Hilfe tierischer Organhomogenate. Bildung von Corticosteron und 17-Oxy-corticosteron. Helv. Chim. Acta, 34: 1790, 1951.
272. KALMAN, S. M.: The effect of androgens on the succinoxidase system of rat liver. Endocrinology, 50: 361–365, 1952.
273. KAPELLER-ADLER, R.: Effects of steroid hormones and of gonadotrophine on histaminase. Biochem. J., 48: xxl, 1951.
274. KARNOFSKY, D. A., HAMRE, P. J. AND HYSOM, G.: Toxicity of progesterone in the new-born mouse, Proc. Soc. Exper. Biol. & Med., 79: 641–643, 1952.
275. KATZMAN, P. A., KINSELLA, R. A. JR. AND DOYLE, M. L.: The production of bacterial β-glucuronidase and its use for the hydrolysis of steroid glucuronides. Science, 116: 524, 1952.

276. KEMP, T. AND PEDERSEN-BJERGAARD, K.: Uber die Aufnahme-und Ausscheidungs-verhaltnisse des Follikulins beim Menschen. Endokrinologie, 13: 156–167, 1933.
277. KERR, L. M. H., CAMPBELL, J. G. AND LEVVY, G. A.: β-glucuronidase as an index of growth in the uterus and other organs. Biochem. J., 44: 487–494, 1949.
278. KERR, L. M. H., CAMPBELL, J. G. AND LEVVY, G. A.: Further observations on changes in β-glucuronidase activity in the mouse. Biochem. J., 46: 278–284, 1950.
279. KINSELLA, R. A. JR., DOISY, R. J. AND GLICK, J. H. JR.: Enzymatic hydrolysis of urinary reducing lipids. Federation Proc., 9: 190, 1950.
280. KIRGIS, H. AND ROTHSCHILD, I.: *In vivo* inactivation of estradiol by the human liver. Endocrinology, 50: 269–270, 1952.
281. KLYNE, W. AND PATERSON, J. Y. F.: Further observations on the steroids of pregnant mare's urine. Biochem. J., 42: i–ii, 1948.
282. KNOBIL, E.: The relation of some steroid hormones to β-glucuronidase activity. Endocrinology, 50: 16–28, 1952.
283. KOCH, F. C.: The chemistry and biology of male sex hormones. Harvey Society Lectures, Series 33, p. 205–236, New York, N. Y., 1937.
284. KOCHAKIAN, C. D.: The effect of dose and nutritive state on kidney arginase after steroid stimulation. J. Biol. Chem., 161: 115–125, 1945.
285. KOCHAKIAN, C. D.: The role of hydrolytic enzymes in some of the metabolic activities of steroid hormones, in Recent Progress in Hormone Research, 1: 177–216, 1947.
286. KOCHAKIAN, C. D.: Recent studies on the *in vivo* and *in vitro* effect of hormones on enzymes. Ann. N. Y. Acad. Sc., 54: 534–547, 1951.
287. KOCHAKIAN, C. D. AND APOSHIAN, H. V.: The *in vitro* metabolism of 3α,17β-androstanediol by liver and kidney. Arch. Biochem., 37: 442–448, 1952.
288. KOCHAKIAN, C. D., GARBER, E. E. AND BARTLETT, M. N.: Effect of estrogen alone and in combination with testosterone on the body and organ weights and the arginase and phosphotases of the organs of the mouse. Am. J. Physiol., 155: 265–271, 1948.
289. KOCHAKIAN, C. D., PARENTE, N. AND APOSHIAN, H. V.: Metabolism of testosterone and androstanediol-3α,17α by liver and kidney homogenates. Federation Proc., 8: 214, 1949.
290. KOCHAKIAN, C. D. AND ROBERTSON, E.: The effect of androgens and hypophysectomy on arginase and phosphotases of the kidneys and liver of the rat. Arch. Biochem., 29: 114–123, 1950.
291. KOCHAKIAN, C. D. AND STIDWORTHY, G.: Paper chromatography of C_{19}-steroids. J. Biol. Chem., 199: 607–612, 1952.
292. KOECHLIN, B. A., KRITCHEVSKY, T. H. AND GALLAGHER, T. F.: Preparation of deuterium steroid hormones from bile acids and the formation of α,β-unsaturated ketones from 3-ketocholane derivatives. J. Biol. Chem., 184: 393–404, 1950.
293. KRITCHEVSKY, T. H.: Reversed phase paper chromatography of steroids. Federation Proc., 11: 243, 1952.
294. KRITCHEVSKY, T. H. AND GALLAGHER, T. F.: Partial synthesis of compounds related to adrenal cortical hormones. XII. Preparation of 17α-hydroxyprogesterone and other 17α-hydroxy-20-ketosteroids. J. Biol. Chem. 179: 507–508, 1949.
295. KRITCHEVSKY, D. AND GRAY, I.: Biosynthesis of cholesterol from isobutyrate. Experientia, 7: 183, 1951.
296. KRITCHEVSKY, D. AND KIRK, M. R.: Paper chromatography of steroids. J. Am. Chem. Soc., 74: 4484–4486, 1952.
297. KRITCHEVSKY, D. AND KIRK, M. R.: Detection of steroids in paper chromatography. Arch. Biochem. & Biophys., 35: 346–351, 1952.
298. KRITCHEVSKY, T. K. AND TISELIUS, A.: Reversed phase partition chromatography of steroids on silicone-treated paper. Science, 114: 299–300, 1951.
299. KRUPP, M. A., ENGLEMAN, E. P., WELSH, J. E. AND WRENN, H. T.: Measurement of the excretion of cortisone and compound F, using the Porter-Silber method. J. Clin. Endocrinol. & Metab., 12: 1163–1171, 1952.
300. LAKSHMANAN, T. K., AND LIEBERMAN, S.: Application of principle of gradient elution to analysis of urinary ketosteroids. Federation Proc., 12: 235, 1953.
301. LANDAU, R. L., KNOWLTON, K., LUGIBIHL, K. AND KENYON, A. T.: The effect of adrenal stimulation with adrenocorticotrophic hormone on the excretion of dehydroisoandrosterone in man. Endocrinology, 48: 489–505, 1951.
302. LANDAU, R. L., KNOWLTON, K., LUGIBIHL, K. AND MUNSON, P. L.: The determination of urinary dehydroisoandrosterone in normal men and women and in adrenal disease. Endocrinology, 48: 506–517, 1951.
303. LANGDON, R. G. AND BLOCH, K.: Biosynthesis of squalene and cholesterol. J. Am. Chem. Soc., 74: 1869–1870, 1952.
304. LANGDON, R. G. AND BLOCH, K.: The biosynthesis of squalene. J. Biol. Chem., 200: 129–134, 1953.
305. LANGDON, R. G. AND BLOCH, K.: The utilization of squalene in the biosynthesis of cholesterol. J. Biol. Chem., 200: 135–144, 1953.
306. LANGDON, R. G. AND BLOCH, K.: Accumulation of Δ^7-cholestenol after squalene feeding. Federation Proc., 12: 235–236, 1953.
307. LEBLOND, C. P.: Metabolism of C^{14}-labeled steroids, Ciba Foundation Conference on Isotopes in Biochemistry, p. 4–13, Philadelphia, Pa., The Blakiston Co., 1951.
308. LEDOGAR, J. A. AND JONES, H. W. JR.: The enzymatic dehydrogenation of estradiol to estrone. Science, 112: 536–537, 1950.
309. LEHNINGER, A. L. AND SCOTT, W. W.: The action of human prostate slices on α-estradiol and estrone. Endocrinology, 40: 9–13, 1947.

310. Leslie, I.: Cortisone, growth hormone, and insulin action on embryonic tissue growing *in vitro*. Biochem. J., 52: xxi, 1952.
311. Levitz, M.: Personal communication.
312. Levy, H.: On the inactivation of estradiol by rat liver *in vitro*. Arch. Biochem., 14: 325–334, 1947.
313. Lewison, E. F., Levi, J. E., Jones, G. S., Jones, H. W. Jr. and Silberstein, H. E.: Tracer studies of radioactive sodium estrone sulfate (S^{35}) in cases of advanced breast cancer. Cancer, 4: 537–548, 1951.
314. Lieberman, S. and Dobriner, K.: Steroid excretion in health and disease. I. Chemistry. Recent Progress in Hormone Research, 6: 71–101, New York, N. Y., Academic Press, Inc., 1948.
315. Lieberman, S., Dobriner, K., Hill, B. R., Fieser, L. F. and Rhoads, C. P.: Studies in steroid metabolism. II. Identification and characterization of ketosteroids isolated from urine of healthy and diseased persons. J. Biol. Chem., 172: 263–295, 1948.
316. Lieberman, S., Fukushima, D. K. and Dobriner, K.: Studies in steroid metabolism. VII. Identification and characterization of additional ketosteroids isolated from urine of healthy and diseased persons. J. Biol. Chem., 182: 299–316, 1950.
317. Lieberman, S., Hariton, L. B. and Dobriner, K.: Alterations in steroids excretion after administration of ACTH in man. Federation Proc., 9: 196–197, 1950.
318. Lieberman, S., Praetz, B., Humphries, P. B. and Dobriner, K.: The isolation of two new urinary steroid triols from human urine. Abstr., 117th Meeting Am. Chem. Soc. 19C, 1950.
319. Lieberman, S., Tagnon, H. J. and Schulman, P.: *In vitro* metabolism of estrogens by the liver studied by means of a colorimetric method. J. Clin. Investigation, 31: 341–345, 1952.
320. Lipsett, M. N. and Moore, F. J.: Effects of adrenalectomy and cortisone treatment on fatty acid oxidation by mouse liver homogenates. J. Biol. Chem., 197: 303–308, 1952.
321. Little, H. N. and Bloch, K.: Studies on the utilization of acetic acid for the biological synthesis of cholesterol. J. Biol. Chem., 183: 33–46, 1950.
322. London, I. M. and Rittenberg, D.: Deuterium studies in normal man. I. The rate of synthesis of serum cholesterol. J. Biol. Chem., 184: 687–691, 1950.
323. Long, C. N. H.: The conditions associated with the secretion of the adrenal cortex. Federation Proc., 6: 461–471, 1947.
324. Long, C. N. H.: The relation of cholesterol and ascorbic acid to the secretion of the adrenal cortex, in Recent Progress in Hormone Research, 1: 99, 1947.
325. Louchart, J. and Jailer, J. W.: Inactivation of 11-dehydro-17-hydroxycorticosterone by tissue slices. Proc. Soc. Exper. Biol. & Med., 79: 393–396, 1952.
326. Lumry, R., Samuels, L. T., Helmreich, M. L. and Lasater, M. B., Cited in Samuels, L. T. and West, C. D.: Vitamins & Hormones, 10: 274, 1952.
327. MacKenna, R. M. B., Wheatley, V. R. and Wormall, A.: The composition of the surface skin fat (sebum) from the human forearm. J. Invest. Dermat., 15: 33–47, 1950.
328. MacPhillamy, H. B. and Scholz, C. R.: The synthesis of 17-(methyl-C^{14})-testosterone and 21-C^{14}-progesterone. J. Biol. Chem., 178: 37–40, 1949.
329. McCullagh, E. P., McCullagh, D. R. and Hicken, N. F.: Diagnosis and treatment of hypogonadism in the male. Endocrinology, 17: 49–63, 1933.
330. McGinty, D. A., Smith, G. N., Wilson, M. L. and Worrel, C. S.: The biosynthesis of 17-hydroxycorticosterone from 11-desoxy-17-hydroxy-corticosterone. Science, 112: 506, 1950.
331. Mader, W. J. and Buck, R. R.: Colorimetric determination of cortisone and related ketol steroids. Anal. Chem., 24: 666–667, 1952.
332. Mancera, O., Zaffaroni, A., Rubin, B. A., Sondheimer, F., Rosenkranz, G. and Djerassi, C.: Steroids. XXXVII. A ten step conversion of progesterone to cortisone. J. Am. Chem. Soc., 74: 3711–3712, 1952.
333. Marker, R. E. and Rohrmann, E.: Pregnenediol in mares' pregnancy urine and its conversion into progesterone. J. Am. Chem. Soc., 60: 1565–1567, 1938.
334. Marrian, G. F.: The quantitative determination of the urinary adrenocortical steroids. J. Endocrinol., 7: lxix–lxxiii, 1951.
335. Marrian, G. F. and Bauld, W. S.: The hydrolysis of the conjugated estrogens in human urine. Acta Endocrinol., 7: 240–256, 1951.
336. Mason, H. L.: Metabolites of 11-dehydrocorticosterone: pregnane-3(α),20-diol-11-one. J. Biol. Chem., 172: 783–787, 1948.
337. Mason, H. L.: Urinary steroids in adrenal disease and the metabolism of adrenal hormones, in Recent Progress in Hormone Research, 3: 103–123, New York, N. Y., Academic Press, Inc., 1948.
338. Mason, H. L.: Isolation of adrenal cortical hormones from urine. 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone. J. Biol. Chem., 182: 131–149, 1950.
339. Mason, H. L. and Kepler, E. J.: Isolation of steroids from the urine of patients with adrenal cortical tumors and adrenal cortical hyperplasia. A new 17-ketosteroid, androstane-3(α),11-diol-17-one. J. Biol. Chem., 161: 235–257, 1945.
340. Mason, H. L. and Strickler, H. S.: Identification of pregnane-3(α),17-diol-20-one as the steroid moiety of a new glucuronide isolated from human urine. J. Biol. Chem., 171: 543–549, 1947.
341. Meister, P. D., Peterson, D. H., Murray, H. C., Eppstein, S. H., Reineke, L. M., Weintraub, A. and Leigh, H. M.: Microbiological transformations of steroids. II. The preparation of 11α-hydroxy-17α-progesterone. J. Am. Chem. Soc., 75: 55–57, 1953.
342. Meister, P. D., Petersen, D. H., Murray, H. C., Spero, G. B., Eppstein, S. H., Weintraub, A., Reineke,

L. M. AND LEIGH, H. M.: Microbiological transformations of steroids. V. The oxygenation of 17α-hydroxyprogesterone to 6β,17α-dihydroxyprogesterone and 11α,17α-dihydroxyprogesterone. J. Am. Chem. Soc., 75: 416, 1953.

343. MEYER, R. K. AND MCSHAN, W. H.: Hormone enzyme relationships, in Recent Progress in Hormone Research, 5: 464–515, 1950.

344. MIESCHER, K. AND WIELAND, P.: Über Steroide. Zur Biosynthese der Steroide. Helv. Chim. Acta, 33: 1847–1864, 1950.

345. MIGEON, C. J. AND GARDNER, L. I.: Urinary estrogens (measured fluorimetrically and biologically) in hyperadrenocorticism. Influence of cortisone, compound F, compound B and ACTH. J. Clin. Endocrinol. & Metab., 12: 1513–1531, 1952.

346. MILLER, A. M. AND DORFMAN, R. I.: Metabolism of the steroid hormones. Isolation of 13 steroid metabolites from a patient with (probable) adrenal hyperplasia. Endocrinology, 46: 514–525, 1950.

347. MILLER, A. M., DORFMAN, R. I. AND MILLER, M.: Metabolism of the steroid hormones. Metabolism of dehydroisoandrosterone. Endocrinology, 46: 105–110, 1950.

348. MITCHELL, F. L.: Chromatographic isolation and estimation of the natural estrogens from tissue. Nature, 170: 621–622, 1952.

349. MONTIGEL, C.: Die Wirkung von Steroidhormonen auf die Glykogen-Phosphorylierung im Muskelbrei. Helv. Chim. Acta, 28: 42–50, 1945.

350. MONTIGEL, C. AND VERZAR, F.: Untersuchungen über den Kohlehydratstoffwechsel nach Adrenalektomie. I. Mitteilung. Abnahme der Glykogen-Phosphorylierung bei adrenalektomierten Katsen und Hunden und Wiederherstellung durh Desoxycorticosteron und andere Steroidhormone. Helv. Physiol. Acta, 1: 115–141, 1943.

351. MUELLER, G. C. AND YANAGI, G. N.: Activating influence of α-estradiol on the *in vitro* incorporation of glycine-2-C^{14} into surviving rat uteri. Federation Proc., 11: 262, 1952.

352. MÜHLBOCK, O.: Über die Estrogenenstoffe und den Cholesteringehalt im Serum trächtiger Stuten. Hoppe-Seyler's Ztschr. f. physiol. Chem., 250: 139–146, 1937.

353. MUNSON, P. L., GALLAGHER, T. F. AND KOCH, F. C.: Isolation of dehydroisoandrosterone sulfate from norma male urine. J. Biol. Chem., 152: 67–77, 1944.

354. MUNSON, P. L., JONES, M. E., MCCALL, P. J. AND GALLAGHER, T. F.: A colorimetric method for estimation of dehydroisoandrosterone and its application to urine extracts. J. Biol. Chem., 176: 73–82, 1948.

355. MUNSON, P. L., LABHART, A. AND FORSHAM, P. H.: Cortisone and hydrocortisone as precursors of urinary dehydroepiandrosterone. J. Clin. Endocrinol. & Metab., 12: 969, 1952.

356. NEHER, R. AND WETTSTEIN, A.: Papierchromatographie von schwach polaren Steroiden. Helv. Chim. Acta, 35: 276–283, 1952.

357. NELSON, D. H., REICH, H. AND SAMUELS, L. T.: Isolation of a steroid hormone from the adrenal-vein blood of dogs. Science, 111: 578–579, 1950.

358. NELSON, D. H. AND SAMUELS, L. T.: A method for the determination of 17-hydrocycorticoids in blood. 17-hydroxycorticosterone in the peripheral circulation. J. Clin. Endocrinol. & Metab., 12: 519–526, 1952.

359. NELSON, D. H., SAMUELS, L. T. AND REICH, H.: The cortical steroid in mammalian blood after ACTH stimulation, Proc. in the Second Clinical ACTH Conference, 1: 49–53, New York, N. Y., The Blakiston Co., 1951.

360. NELSON, D. H., SAMUELS, L. T., WILLARDSON, D. G. AND TYLER, F. H.: The levels of 17-hydroxycorticosteroids in peripheral blood of human subjects. J. Clin. Endocrinol., 11: 1021–1029, 1951.

361. NELSON, D. H., SANDBERG, A. A., PALMER, J. G. AND TYLER, F. H.: Blood levels of 17-hydroxycorticosteroids following the administration of adrenal steroids and their relation to levels of circulating leukocytes. J. Clin. Investigation, 31: 843–849, 1952.

362. NICHOLAS, H. J., THAYER, S. A., DOISY, E. A. JR., ELLIOTT, W. H., BOCKLAGE, B. AND DOISY, E. A.: Preparation, bioassay and metabolism of radioactive 17-methylestradiol. Federation Proc., 9: 209, 1950.

363. NIELSEN, E. D., DRAKE, N. A. AND HAINES, W. J.: Incorporation of radioacetate into 17-hydroxycorticosterone by adrenal cortex slices. Federation Proc., 10: 228–229, 1951.

364. NISSIM, J. A. Studies on the metabolism of adrenal steroid hormones. J. Endocrinol., 8: 257–264, 1952.

365. NISSIM, J. A. AND ROBSON, J. M.: The conversion of pregnenolone to a more active progestational substance by incubation with corpus luteum, (adrenal) or placenta. J. Physiol., 114: 12P–13P, 1951.

366. NORYMBERSKI, J. K.: Determination of urinary corticosteroids. Nature, 170: 1074–1075, 1952.

367. NYC, J. F., MABON, D. M., GARST, J. B. AND FRIEDGOOD, H. B.: Chromatographic separation of estrone, estradiol and estriol. Proc. Soc. Exper. Biol. & Med., 77: 466–469, 1951.

368. ONCLEY, J. L. AND GURD, F. R. N.: The lipoproteins of human plasma, in Blood Cells & Plasma Proteins, their State in Nature, p. 337–357, New York, N. Y., Academic Press, Inc., 1953.

369. ONESON, I. B. AND COHEN, S. L.: The nature of the conjugated estrone in human pregnancy urine. Endocrinology, 51: 173–182, 1952.

370. OPSAHL, J.: Hyaluronidase and the adrenal cortical hormones. Conference on the Adrenal Cortex, 2: 115–163, 1950.

371. OTTKE, R. C., TATUM, E. L., ZABIN, I. AND BLOCH, K.: Ergosterol synthesis in neurospora. Federation Proc., 9: 212, 1950.

372. PASCHKIS, K. E., CANTAROW, A. AND HAVENS, W. P. JR.: Studies on progesterone metabolism in cases of liver disease. Federation Proc., 10: 101, 1951.

373. PASCHKIS, K. E., CANTAROW, A. AND BOYLE, D.: *In vitro* inactivation of cortisone by tissues of normal and stressed adrenalectomized rats. Federation Proc., 10: 110, 1951.

374. PASCHKIS, K. E., CANTAROW, A., RAKOFF, A. E., HANSEN, L. P. AND WALKLING, A. A.: Excretion in the dog of

androgens and estrogens in the bile following injections of androgens. Proc. Soc. Exper. Biol. & Med., 55: 127–130, 1944.

375. Paschkis, K. E., Cantarow, A., Walkling, A. A. and Boyle, D.: Adrenal cortical hormone levels in adrenal vein and peripheral blood. Endocrinol., 47: 338–346, 1950.
376. Paterson, J. Y. F., Cox, R. I. and Marrian, G. F.: Some observations on the adreno-cortical steroids in human urine. Biochem. J., 46: xxix-xxx, 1950.
377. Paterson, J. Y. F. and Marrian, G. F.: Some observations on the chloroform-soluble conjugated adrenocortical steroids in human urine. Biochem. J., 48: xxxiii, 1951.
378. Pearlman, W. H.: The identification of compound B, a substance occurring in ox bile, as allopregnanediol-3(β),-20(β). J. Biol. Chem., 166: 473–476, 1946.
379. Pearlman, W. H. and Cerceo, E.: The isolation of pregnanol-3(α)-one-20, pregnanediol-3(α),20(β), and etiocholanediol-3(α),17(β) from the bile of pregnant cows. J. Biol. Chem., 176: 847–856, 1948.
380. Pearlman, W. H. and Cerceo, E.: The isolation of C21-steroids from human placenta. J. Biol. Chem., 194: 807–814, 1952.
381. Pearlman, W. H. and Cerceo, E.: The isolation of progesterone from human placenta. J. Biol. Chem., 198: 79–83, 1952.
382. Pearlman, W. H., Paschkis, K. E., Rakoff, A. E., Cantarow, A., Walkling, A. A. and Hansen, L. E.: A note on the biliary excretion of exogenous estrogen. Endocrinology, 36: 284–286, 1945.
383. Pearlman, W. H. and Pearlman, M. R. J.: Preparation of 6,7-d2-estrone acetate. J. Am. Chem. Soc., 72: 5781, 1950.
384. Pearlman, W. H., Pearlman, M. R. J. and Rakoff, A. E.: Estrogen metabolism in human pregnancy. A study with the aid of deuterium. Federation Proc., 11: 268, 1952.
385. Pearlman, W. H. and Pincus, G.: The metabolism of pregnenolone. Federation Proc., 5: 79–80, 1946.
386. Pearlman, W. H. and Pincus, G.: The metabolism of estrone in men. J. Biol. Chem., 147: 379–387, 1943.
387. Pearlman, W. H. and Rakoff, A. E.: A note on the estrogens in the bile of pregnant women. Endocrinology, 44: 199–201, 1949.
388. Pearlman, W. H., Rakoff, A. E., Cantarow, A. and Paschkis, K. E.: The isolation of estrone from the bile of pregnant cows. J. Biol. Chem., 170: 173–179, 1947.
389. Pearlman, W. H., Rakoff, A. E., Paschkis, K. E., Cantarow, A. and Walkling, A. A.: The metabolic fate of estrone in bile fistula dogs. J. Biol. Chem., 173: 175–183, 1948.
390. Perlman, D., Titus, E. and Fried, J.: Microbiological hydroxylation of progesterone. J. Am. Chem. Soc., 74: 2126, 1952.
391. Peterson, D. H., Murray, H. C., Eppstein, S. H., Reineke, L. M., Weintraub, A., Meister, P. D. and Leigh, H. M.: Microbiological transformation of steroids. I. Introduction of oxygen at C11 of progesterone. J. Am. Chem. Soc., 74: 5933–5936, 1952.
392. Peterson, D. H., Eppstein, S. H., Meister, P. D., Magerlein, B. J., Murray, H. C., Leigh, H. M., Weintraub, A. and Reineke, L. M.: Microbiological transformations of steroids. IV. The 11-epimer of compound F and other new oxygenated derivatives of Reichstein's compound S. A new route to cortisone. J. Am. Chem. Soc., 75: 412–415, 1953.
393. Peterson, D. H. and Murray, H. C.: Microbiological oxygenation of steroids at C11. J. Am. Chem. Soc., 74: 1871–1872, 1952.
394. Peterson, D. H., Nathan, A. H., Meister, P. D., Eppstein, S. H., Murray, H. C., Weintraub, A., Reineke, L. M. and Leigh, H. M.: Microbiological transformations of steroids. VI. Preparation of 11α-hydroxy-6-dehydroprogesterone. J. Am. Chem. Soc., 75: 419–421, 1953.
395. Picha, G. M., Saunders, F. J. and Green, D. M.: An oxidative metabolite of desoxycorticosterone. Science, 115: 704–705, 1952.
396. Pihl, A., Bloch, K. and Anker, H. S.: The rates of synthesis of fatty acids and cholesterol in the adult rat studied with the aid of labeled acetic acid. J. Biol. Chem., 183: 441–450, 1950.
397. Pincus, G., Hechter, O. and Hopkins, T.: The inactivation of ACTH in mammalian blood. J. Clin. Endocrinol. & Metab., 12: 920, 1952.
398. Pincus, G., Hechter, O. and Zaffaroni, A.: The effect of ACTH upon steroidogenesis by the isolated perfused adrenal gland. Proc. of the Second Clinical ACTH Conference, 1: 40–48, New York, N. Y., The Blakiston Co., 1951.
399. Pincus, G. and Pearlman, W. H.: The intermediate metabolism of sex hormones, Vitamins & Hormones, 1: 293–343, New York, N. Y., Academic Press, Inc., 1943.
400. Pincus, G. and Pearlman, W. H.: Metabolism of estrone in men and non-pregnant women. Endocrinology, 31: 507–514, 1942.
401. Plager, J. E. and Samuels, L. T.: Enzyme system involved in the oxidation of carbon-21 in steroids. Federation Proc., 11: 383, 1952.
402. Plager, J. E. and Samuels, L. T.: Factors affecting activity of the enzymes oxidising carbons 17 and 21 of the pregnene nucleus. Federation Proc., 12: 357, 1953.
403. Plager, J. E. and Samuels, L. T. Synthesis of C14-17-hydroxy-11-desoxycorticosterone and 17-hydroxycorticosterone by fractionated extracts of adrenal homogenates. Arch. Biochem. & Biophys., 42: 477–478, 1953.
404. Plant, G. W. E. and Lardy, H. A.: Enzymatic incorporation of C14-bicarbonate into acetoacetate in the presence of various substrates. J. Biol. Chem., 192: 435–445, 1951.
405. Pond, M. H.: A microchromatographic method for the separation of certain fractions of 17-ketosteroids in human urine. Lancet, II: 906–909, 1951.

406. POND, M. H.: A microchromatographic method for the separation of certain fractions of 17-ketosteroids in human urine. J. Endocrinol., 8: xii, 1952.
407. POPJÁK, G. AND BEECKMANS, M. L.: Synthesis of cholesterol and fatty acids in foetuses and in mammary glands of pregnant rabbits. Biochem. J., 46: 547–561, 1950.
408. POPJÁK, G. AND BEECKMANS, M. L.: Extrahepatic lipid synthesis. Biochem. J., 47: 233–238, 1950.
409. PORTER, C. C. AND SILBER, R. H.: A quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids. J. Biol. Chem., 185: 201–207, 1950.
410. PRICE, T. D. AND RITTENBERG, D.: The metabolism of acetone. I. Gross aspects of catabolism and excretion. J. Biol. Chem., 185: 449–459, 1950.
411. Proposed standard method of 17-ketosteroid determination. M. R. C. Committee on Clinical Endocrinology. Lancet, II: 585–586, 1951.
412. RABINOVITCH, J., DECOMBE, J. AND FREEDMAN, A. Simplified method of estimating formaldehydogenic corticosteroids in urine. Lancet, II: 1201–1202, 1951.
413. RABINOVITCH, M. AND GREENBERG, D. M.: Incorporation of radioacetate into the cholesterol of fetal rat liver homogenates. Arch. Biochem., 40: 472–474, 1952.
414. RABINOWITCH, J. L. AND GURIN, S.: The biosynthesis of radioactive cholesterol by particle-free extracts of rat liver. Biochem. & Biophys. Acta, 10: 345–346, 1953.
415. RAKOFF, A. E. AND CANTAROW, A.: Estrogen content of blood of ovarian vein of the dog and spermatic vein of the stallion. Federation Proc., 9: 103, 1950.
416. RAKOFF, A. E., PASCHKIS, K. E. AND CANTAROW, A.: Conjugated estrogens in human pregnancy serum. Am. J. Obst. & Gynec., 46: 856–860, 1943.
417. REDDY, W. J., FORSHAM, P. H. AND JENKINS, D.: Estimation of conjugated corticosteroids in urine. J. Clin. Endocrinol. & Metab., 12: 972–973, 1952.
418. REDDY, W. J., JENKINS, D. AND THORN, G. W.: Estimation of 17-hydroxycorticoids in urine. Metabolism, 1: 511–527, 1952.
419. REICH, H., NELSON, D. H. AND ZAFFARONI, A.: Isolation of 17-hydroxycorticosterone from blood obtained from adrenal veins of dogs. J. Biol. Chem., 187: 411–417, 1950.
420. RICHARDSON, D. E., BLUEMLE, L. JR. AND GYORGY, P.: Hormone excretion in diffuse liver disease. Federation Proc., 11: 35, 1952.
421. RIEGEL, B., HARTOP, W. L. JR. AND KITTINGER, G. W.: Studies on the metabolism of radioprogesterone in mice and rats. Endocrinology, 47: 311–319, 1950.
422. RIEGEL, B. AND LIU, Y. C.: The synthesis of 17α-ethynyltestosterone-20,21-C^{14}. J. Org. Chem., 16: 1610–1614, 1951.
423. RIEGEL, B. AND PROUT, F. S.: The synthesis of progesterone-21-C^{14}. J. Org. Chem., 13: 933–936, 1948.
424. RIEGEL, I. L. AND MEYER, R. K.: Intracellular distribution of estrogen inactivating mechanism in rat liver and other tissues. Proc. Soc. Exper. Biol. & Med., 80: 617–622, 1952.
425. RITTENBERG, D. AND PRICE, T. D.: Stable isotopes in biochemical research. Ann. Rev. Nuclear Sc., 1: 509–596, 1952.
426. RITTENBERG, D. AND SCHOENHEIMER, R.: Deuterium as an indicator in the study of intermediate metabolism. X. Further studies on the biological uptake of deuterium into organic substances with special reference to fat and cholesterol formation. J. Biol. Chem., 121: 235–253, 1937.
427. ROBERTS, S. AND SZEGO, C. M.: The nature of circulating estrogen. Lipoprotein-bound estrogen in human plasma. Endocrinology, 39: 183–187, 1946.
428. ROBERTS, S. AND SZEGO, C. M.: The influence of steroids on uterine respiration and glycolysis. J. Biol. Chem., 201: 21–30, 1953.
429. ROBINSON, A. M. AND GOULDEN, F.: A qualitative study of urinary 17-ketosteroids in normal males and in men with prostatic disease. Brit. J. Cancer, 3: 62–71, 1949.
430. ROBINSON, R.: Structure of cholesterol. J. Soc. Chem. Ind. (London), 53: 1062–1063, 1934.
431. ROGERS, J. AND MCLELLAN, F.: Isolation of pregnanediol from human bile after oral administration of progesterone. J. Clin. Endocrinol., 11: 246–250, 1951.
432. ROMANOFF, L. P., PLAGER, J. AND PINCUS, G.: The determination of adrenocortical steroids in human urine. Endocrinology, 45: 10–20, 1949.
433. ROMANOFF, E. B., UNGAR, F., DORFMAN, R. I. AND PINCUS, G.: Perfusion of the isolated human adrenal gland. J. Clin. Endocrinol. & Metab., 12: 968, 1952.
434. ROMANOFF, L. P., WOLF, R. S. AND PINCUS, G. Extraction and fractionation of urinary corticoids. Federation Proc., 11: 131, 1952.
435. RONZONI, E. The excretion of dehydroisoandrosterone during adrenal stimulation with adrenocorticotropic hormone. J. Clin. Endocrinol. & Metab., 12: 527–540, 1952.
436. ROTHCHILD, I.: A simple method for the preparation of aqueous solutions of steroid hormones for intravenous administration. Endocrinology, 50: 583–585, 1952.
437. RUBIN, B. L., DORFMAN, R. I. AND MILLER, M.: Androgen excretion in human bile. Endocrinology, 51: 463–468, 1952.
438. RUBIN, B. L. AND ROSENKRANTZ, H.: Separation of ketosteroids in a 24-hour urine sample. Federation Proc., 11: 133, 1952.
439. RUBIN, B. L., ROSENKRANTZ, H., DORFMAN, R. I. AND PINCUS, G.: Separation of 17-ketosteroids in a 24-hour urine sample. J. Clin. Endocrinol. & Metab., 13: 568–580, 1953.
440. RUZICKA, L. AND PRELOG, V.: Zur Kenntnis der Lipoide aus Schweinetestes. Helv. Chim. Acta, 26: 975–994, 1943.

441. Saffran, M., Grad, B. and Baylis, M. J.: Stimulation by corticotropin of formation of corticoids by isolated rat adrenals. Federation Proc., 11: 135, 1952.
442. Saffran, M., Grad, B. and Baylis, M. J.: Production of corticoids by rat adrenals *in vitro*. Endocrinology, 50: 639–643, 1952.
442a. Salamon, I. I. and Dobriner, K.: The isolation of a potential adrenal androgen from human urine after administration of ACTH. J. Clin. Endocrinol. & Metab., 12: 967, 1952.
443. Salhanick, H. A., Noall, M. W., Zarrow, M. X. and Samuels, L. T.: The isolation of progesterone from human placentae. Science, 115: 708–709, 1952.
444. Samuels, L. T.: Enzyme systems involved in the metabolism of androgens in the liver. A Symposium on Steroid Hormones, p. 241–258, Madison, Wis., The Univ. of Wisconsin Press, 1950.
445. Samuels, L. T., Helmreich, M. L., Lasater, M. B. and Reich, H.: An enzyme in endocrine tissues which oxidizes Δ^5-3-hydroxy steroids to α,β unsaturated ketones. Science, 113: 490–491, 1951.
446. Samuels, L. T., McCauley, C. and Sellers, D. M.: Metabolism of steroids by tissues. II. The enzymic destruction of testosterone by liver tissue. J. Biol. Chem., 168: 477–483, 1947.
447. Samuels, L. T., Sweat, M. L., Levedahl, B. H., Pottner, M. M. and Helmreich, M. L.: Metabolism of testosterone by livers of different species of animals. J. Biol. Chem., 183: 231–239, 1950.
448. Samuels, L. T. and West, C. D.: The intermediary metabolism of the non-benzenoid steroid hormones. Vitamins & Hormones, 10: 251–295, New York, N. Y., Academic Press, Inc., 1952.
449. Sarett, L. H., Arth, G. E., Lukes, R. M., Beyler, R. E., Poos, G. I., Johns, W. F. and Constantin, J. M.: Stereospecific total synthesis of cortisone. J. Am. Chem. Soc., 74: 4974, 1952.
450. Savard, K.: Paper partition chromatography of C_{19}-and C_{21}-ketosteroids. J. Biol. Chem., 202: 457–477, 1953.
451. Savard, K., Dorfman, R. I. and Poutasse, E.: Biogenesis of androgens in human testis. J. Clin. Endocrinol. & Metab., 12: 935, 1952.
452. Savard, K., Green, A. A. and Lewis, L. A.: Oxidation of 11-desoxycorticosteroids in the adrenal tissue homogenates. Endocrinology, 47: 418–428, 1950.
453. Savard, K., Kolff, W. J. and Corcoran, A. C.: Corticosteroids of peripheral blood. Endocrinology, 50: 366–373, 1952.
454. Savard, K., Lewis, L. A. and Green, A. A.: Effect of adrenal mince and cell-free filtrate on desoxycorticosterone. Federation Proc., 9: 223, 1950.
455. Schacter, B. and Marrian, G. F.: The isolation of estrone sulfate from the urine of pregnant mares. J. Biol. Chem., 126: 663–669, 1938.
456. Schellman, J., Lumry, R., Samuels, L. T., Eik-Nes, K., Helmreich, M. and Lasater, M.: Interaction of steroids with bovine serum albumin. 122nd Meeting Am. Chem. Soc., Abstract of Communications, 17C, Atlantic City, 1952.
457. Schiller, J.: Metabolic changes in estrogen induced by rat organs. Endocrinology, 36: 7–15, 1945.
458. Schiller, J. and Pincus, G.: The fate of α-estradiol and of estriol injected into a human male subject. Arch. Biochem., 2: 317–322, 1943.
459. Schiller, J. and Pincus, G.: Perfusion of rat livers with eterogen *in vitro*. Science, 98: 410–412, 1945.
460. Schneider, J. J.: Studies on the excretion of adrenocortical compounds. I. Isolation of 17-hydroxy-11-dehydrocorticosterone and other compounds from the urine of normal males. J. Biol. Chem. 183: 365–376, 1950.
461. Schneider, J. J.: Further isolation of adrenocortical compounds from male urine. J. Biol. Chem., 194: 337–348, 1952.
462. Schneider, J. J.: Conversion of desoxycorticosterone to four allopregnane metabolites by rat liver *in vitro*. J. Biol. Chem., 199: 235–244, 1952.
463. Schneider, J. J. and Horstmann, P. M.: Effects of incubating DOC with various rat tissues. J. Biol. Chem., 191: 327–338, 1951.
464. Schneider, J. J. and Horstmann, P. M.: Effects of incubating Compound E and related steroids with various surviving rat tissues. J. Biol. Chem., 196: 629–639, 1952.
465. Schneider, J. J. and Mason, H. L.: Studies on intermediary steroid metabolism. I. Isolation of Δ^5-androstene-3(β), 17(α)-diol and Δ^5-androstene-3(β), 16(β), 17(α)-triol following the incubation of dehydroisoandrosterone with surviving rabbit liver slices. J. Biol. Chem., 172: 771–782, 1948.
466. Schneider, J. J. and Mason, H. L.: Studies on intermediary steroid metabolism. II. Compounds isolated following the incubation of androsterone and etiocholan-3(α)-ol-17-one with surviving rabbit liver slices. J. Biol. Chem., 175: 231–240, 1948.
467. Schwartz, T. B. and Engel, F. L.: The adrenal cortex and serum peptidase activity. J. Biol. Chem., 180: 1047–1052, 1949.
468. Schwenk, E. and Werthessen, N. T.: Studies on the biosynthesis of cholesterol. III. Purification of C^{14}-cholesterol from perfusion of livers and other organs. Arch. Biochem., 40: 334–341, 1952.
469. Schwenk, E. and Werthessen, N. T.: Studies on the biosynthesis of cholesterol. IV. Higher counting substances accompanying C^{14}-cholesterol in the intact rat. Arch. Biochem., 42: 91–93, 1953.
470. Seckel, H. P. G.: The influence of various physiological substances on the glycogenolysis of surviving rat liver. Endocrinology, 26: 97–101, 1940.
471. Seneca, H., Ellenbogen, E., Henderson, E., Collins, A. and Rockenbach, J.: The *in vitro* production of cortisone by mammalian cells. Science, 112: 524–525, 1950.
472. Shull, G. M., Sardinas, J. L. and Nubel, R. C.: Paper chromatography of steroid compounds. Arch. Biochem., 37: 186–198, 1952.

473. SINEX, F. M.: Effect of the adrenal cortical hormones and insulin on alanine metabolism. Federation Proc., 10: 247, 1951.
474. SIPERSTEIN, M. D., CHAIKOFF, I. L. AND CHERNICK, S. S.: Significance of endogenous cholesterol in arteriosclerosis: Synthesis in arterial tissue. Science, 113: 747-749, 1951.
475. SIPERSTEIN, M. D., JAYKO, M. E., CHAIKOFF, I. L. AND DAUBEN, W. G.: Nature of the metabolic products of C^{14}-cholesterol excreted in bile and feces. Proc. Soc. Exper. Biol. & Med., 81: 720, 1952.
476. SLAUNWHITE, W. R. JR., ENGEL, L. L., Olmsted, P. C. AND CARTER, P.: The fluorescence and extinction and partition coefficients of estrogens. J. Biol. Chem., 191: 627-631, 1951.
477. SMITH, G. V. S. AND SMITH, O. W.: Internal secretions and toxemia of late pregnancy. Physiol. Rev., 28: 1-22, 1948.
478. SMITH, O. W. AND BARKER, C.: Factors influencing the composition of the sodium "pregnanediol" glucuronide complex in human pregnancy, with especial reference to diethylstilbestrol therapy. J. Clin. Endocrinol. & Metab., 12: 151-168, 1952.
479. SOMMERVILLE, I. F. AND BIGLER, R. cited in SAMUELS, L. T. AND WEST, C. D., Vitamins & Hormones, 10: p. 279, New York, N. Y., Academic Press, Inc., 1952.
480. SOMMERVILLE, I. F., GOUGH, N. AND MARRIAN, G. F.: The quantitative determination of small amounts of pregnanediol in human urine. J. Endocrinol., 5: 247-257, 1948.
481. SONDERHOFF, R. AND THOMAS, H.: Die enzymatische Dehydrierung der Trideutero-Essigsäure. Ann. Chem., 530: 195-213, 1937.
482. SOURKES, T. L. AND HENEAGE, P.: Some studies on the oxidative metabolism of the adrenal gland. Endocrinology, 50: 73-82, 1952.
483. SPRAGUE R. G., POWER, M. H., MASON, H. L. AND CLUXTON, H. E.: Metabolic effects of synthetic Compound E (17-hydroxy-11-dehydrocorticosterone) in 2 patients with Addison's disease and in 1 with coexisting Addison's disease and diabetes mellitus. J. Clin. Investigation, 28: 812, 1949.
484. SREBE, P. A., CHAIKOFF, I. L. AND DAUBEN, W. G.: The *in vitro* synthesis of cholesterol from acetate by surviving adrenal cortical tissue. J. Biol. Chem., 176: 829-833, 1948.
485. SREBE, P. A., CHAIKOFF, I. L., TREITMAN, S. S. AND BURSTEIN, L. S.: The extrahepatic synthesis of cholesterol. J. Biol. Chem., 182: 629-634, 1950.
486. STÄHELIN, D. AND VÖGTLI, W.: Glykogen-Phosphorylierung bei adrenalektomierten Katsen und Ratten durch Muskel und Leber. Helv. Physiol. Acta, 6: 317-330, 1948.
487. STAUDINGER, H. AND SCHMEISSER, M.: Bestimmung der Nebennierenrindenhormone im Harn. Biochem. Ztschr., 321: 83-92, 1950.
488. STEROID METABOLISM AND ESTIMATION.: Vol. II, Endocrinology, New York, N. Y., The Blakiston Co., 1952.
489. STEROID NOMENCLATURE.: Chemistry and Industry, SN1-SN11, 1951.
490. STEVENSON, M. F. AND MARRIAN, G. F.: The determination of estrogens in human pregnancy urine. A new method of correcting for the brown color developed in the Kober reaction by non-estrogenic substances. Biochem. J., 41: 507-511, 1947.
491. STIMMEL, B. F.: The fractionation and photometric estimation of the estrogens in human pregnancy urine. J. Biol. Chem., 162: 99-109, 1946.
492. STIMMEL, B. F.: Simultaneous biliary and urinary exogenous estrogen excretion studies in man. Federation Proc., 10: 254, 1951.
493. STIMMEL, B. F.: Spectrophotometric estimation of fecal estrogens in pregnant women. Federation Proc., 11: 294, 1952.
494. STIMMEL, B. F., RANDOLPH, J. D. AND CONN, W. M.: A procedure for concomitant photometric estimation of pregnanediol and estrogens in human urine. J. Clin. Endocrinol. & Metab., 12: 371-384, 1952.
495. STIMMEL, B. F. AND STEALY, C. L.: Metabolism of estrone in phases of menstrual cycles. J. Clin. Endocrinol. & Metab., 12: 489, 1952.
496. STONE, D., GOBIEL, E., FEINSTEIN, M. AND HECHTER, O.: Influence of ACTH on progesterone transformation to corticosteroid, in perfused bovine adrenals. Federation Proc., 12: 139, 1953.
497. SUTHERLAND, E. S. AND MARRIAN, G. F.: The separation of non-ketonic and ketonic sodium glucuronidates from the 'sodium pregnanediol glucuronidate' of human pregnancy urine: the isolation of pure sodium pregnane-3(α), 20(α)-diol glucuronidate. Biochem. J., 41: 193-196, 1947.
498. SWEAT, M. L.: Enzymatic synthesis of 17-hydroxy-corticosterone. J. Am. Chem. Soc., 73: 4056, 1951.
499. SWEAT, M. L., ABBOTT, W. E., JEFFERIES, W. MCK. AND BLISS, E. L.: Adrenocortical steroids in human peripheral and adrenal venous blood as determined by fluorescence. Federation Proc., 12: 141, 1953.
500. SWEAT, M. L. AND FARRELL, G. L.: Resolution and quantitative analysis of steroids in adrenal-vein blood. J. Clin. Endocrinol. & Metab., 12: 968, 1952.
501. SWEAT, M. L., SAMUELS, L. T. AND LUMRY, R.: Preparation and characterisation of the enzyme which converts testosterone to androstenedione. J. Biol. Chem., 185: 75-84, 1950.
502. SWYER, G. I. M. AND BRAUNSBERG, H.: The separation and estimation of urinary estrone and estradiol. J. Endocrinol., 7: lx-lxi, 1951.
503. SZEGO, C. M. AND ROBERTS, S.: Nature of circulating estrogen. Proc. Soc. Exper. Biol. & Med., 61: 161-164, 1946.
504. SZEGO, C. M. AND ROBERTS, S.: In Recent Progress in Hormone Research, Vol. VIII, in press.
505. TAGNON, H. J., LIEBERMAN, S., SCHULMAN, P. AND BRUNSCHWIG, A.: The metabolism of estrogens by the human liver studied *in vitro*. J. Clin. Investigation, 31: 346-350, 1952.
506. TALBOT, N. B., RYAN, J. AND WOLFE, J. K.: Enzymatic hydrolysis of urinary sodium pregnanediol glucuronidate to free pregnanediol. J. Biol. Chem., 151: 607-614, 1943.

507. TALBOT, N. B., SALTMAN, A. H., WIXOM, R. L. AND WOLFE, J. K.: The colorimetric assay of urinary corticosteroid-like substances. J. Biol. Chem., 160: 535-546, 1945.
508. TEICH, S., ROGERS, J., LIEBERMAN, S., ENGEL, L. L. AND DAVIS, J. W.: The origin of 3,5-cycloandrostan-6β-ol-17-one (i-androsten-6β-ol-17-one) in urinary extracts. J. Am. Chem. Soc., 75: 2523-2525, 1953.
509. TIPTON, S. R., LEATH, M. J., TIPTON, I. H. AND NIXON, W. L.: The effects of feeding thyroid substance and of adrenalectomy on the activities of succinoxidase and cytochrome oxidase in the liver tissue of rats. Am. J. Physiol., 145: 693-698, 1946.
510. TOMKINS, G. M., CHAIKOFF, I. L., DAUBEN, W. G., BRADLOW, H. L. AND SREBR, P. A.: Synthetic C^{14}-squalene: concerning its incorporation with cholesterol by liver. J. Am. Chem. Soc., 74: 6145-6146, 1952.
511. TORDA, C. AND WOLFF, H. G.: Effects of hypophysectomy and adrenocorticotrophic hormone on neuromuscular function and acetylcholine synthesis. Am. J. Physiol., 161: 534-539, 1950.
512. TSCHESCHE, R. AND KORTE, F.: Zum biochemischen Syntheseweg der Steroide. Angewandte Chemie, 64: 633-638, 1952.
513. TURNER, R. B : Steroids labeled with isotopic carbon: cholestenone and testosterone. J. Am. Chem. Soc., 72: 579-585, 1950.
514. TWOMBLY, G. H.: The synthesis and metabolism of radioactively-labeled steroids. Vitamins & Hormones, 9 237-261, New York, N. Y. Academic Press, Inc., 1951.
515. TWOMBLY, G. H. AND SCHOENEWALDT, E. F.: The metabolism of radioactive dibromoestrone in man. Cancer, 3: 601-607, 1950.
516. TWOMBLY, G. H. AND SCHOENEWALDT, E. F.: Tissue localization and excretion routes of radioactive diethylstilbestrol. Cancer, 4: 296-302, 1951.
517. UMBREIT, W. W.: The influence of adrenalectomy and cortisone treatment on enzymatic reactions in rat tissues. Ann. N. Y. Acad. Sc., 54: 569-574, 1951.
518. UMBREIT, W. W. AND TONHAZY, N. E.: Metabolic effects of cortisone. I. Oxidation of proline. J. Biol. Chem., 191: 249-256, 1951.
519. UMBREIT, W. W. AND TONHAZY, N. E.: Metabolic effects of cortisone. II. The adenylate system. J. Biol. Chem., 191: 257-261, 1951.
520. UNGAR, F. AND DORFMAN, R. I.: Metabolism of Δ^4-androstene-3,17-dione. Science, 115: 115-116, 1952.
521. UNGAR, F., DORFMAN, R. I. AND PRINS, D. A.: Metabolism of cistestosterone, androstanediol-3β,17β, and etiocholanol-17β-one-3 *in vivo*. J. Biol. Chem., 189: 11-17, 1951.
522. VENNING, E. H.: Further studies on the estimation of small amounts of sodium pregnanediol glucuronidate in urine. J. Biol. Chem., 126: 595-602, 1938.
523. VENNING, E. H.: Section III. Assay of Hormone secretions in Methods in Medical Research, 2: 245-343, Chicago, Ill., The Year Book Publishers, Inc., 1950.
524. VENNING, E. H.: Conjugated corticoids. Federation Proc., 11: 302, 1952.
525. VENNING, E. H. AND BROWNE, J. S. L.: Excretion of glycogenic corticoids and of 17-ketosteroids in various endocrine and other disorders. J. Clin. Endocrinol., 7: 79-101, 1947.
526. VENNING, E. H., HOFFMAN, M. M. AND BROWNE, J. S. L.: The extraction of cortin-like substances from human post-operative urine. Endocrinology, 35: 49-62, 1944.
527. VENNING, E. H., HOFFMAN, M. M. AND BROWNE, J. S. L.: Isolation of androsterone sulfate. J. Biol. Chem., 146: 369-379, 1942.
528. VERZÁR, F.: *In vitro* influences of corticosteroids on phosphorylating enzymes. Ann. N. Y. Acad. Sc., 54: 716-727, 1951.
529. VERZÁR, F.: The influence of corticoids on enzymes of carbohydrate metabolism. Vitamins & Hormones, 10: 297-330, New York, N. Y., Academic Press, Inc., 1952.
530. VERZÁR, F. AND WENNER, V.: The influence *in vitro* of deoxycorticosterone on glycogen formation in muscle. Biochem. J., 42: 35-41, 1948.
531. VERZÁR, F. AND WENNER, V.: The action of steroids on glycogen breakdown in surviving muscle. Biochem. J., 42: 48-51, 1948.
532. VESTLING, C. S. AND LATA, G. F.: Steroid changes in incubating adrenal homogenates. Science, 113: 582-583, 1951.
533. VOGT, M.: The output of cortical hormone by the mammalian suprarenal. J. Physiol., 102: 341-356, 1943.
534. WATTENYL, H., BESSINGER, A., MARITZ, A. AND ZELLER, E. A.: Cholin-Esterase und Geschlechtsfunktion beim Meerschweinchen. Helv. Chim. Acta, 26: 2063-2070, 1943.
535. WEICHSELBAUM, T. E., MARGRAF, H. W. AND ELMAN, R.: Quantitative determination of 17-hydroxycorticosteroids and 21-hydroxy-20 ketosteroids in the peripheral blood of humans before and after operative trauma. Federation Proc., 12: 287, 1953.
536. WELT, I. D., STETTEN, D. JR., INGLE, D. J. AND MORLEY, E. H.: Effect of cortisone upon rates of glucose production and oxidation in the rat. J. Biol. Chem., 197: 57-66, 1952.
537. WERTHESSEN, N. T., BAKER, C. F. AND BORCI, B.: The presence of an alcoholic, ketonic derivative of estrone in human and rabbit blood. Science, 107: 64-65, 1948.
538. WERTHESSEN, N. T., BAKER, C. F. AND FIELD, N. S.: Conversion of estrone on incubation in blood. J. Biol. Chem., 184: 145-154, 1950.
539. WERTHESSEN, N. T., BAKER, C. F. AND FIELD, N. S.: Conversion of estrone by organs *in vitro*. Am. J. Physiol. 167: 166-170, 1951.
540. WEST, C. D.: The metabolism of intravenous testosterone by experimental animals. Endocrinol., 49: 467-473, 1951.
541. WEST, C. D., HOLLANDER, V. P., KRITCHEVSKY, T. H. AND DOBRINER, K.: The isolation and identification of

testosterone, Δ^4-androstenedione-3,17, and 7-ketocholesterol from spermatic vein blood. J. Clin. Endocrinol. & Metab., 12: 915–916, 1952.

542. West, C. D., Reich, H. and Samuels, L. T.: Urinary metabolites after intravenous injection of human subjects with testosterone. J. Biol. Chem., 193: 219–226, 1951.
543. West, C. D. and Samuels, L. T.: The metabolism of testosterone and related steroids by kidney tissue. J. Biol. Chem., 190: 827–835, 1951.
544. West, C. D., Tyler, F. H. and Brown, H.: The effect of intravenous testosterone on nitrogen and electrolyte metabolism. J. Clin. Endocrinol., 11: 833–842, 1951.
545. West, C. D., Tyler, F. H., Brown, H. and Samuels, L. T.: The role of the liver and kidneys in the metabolism of intravenous testosterone by human subjects. J. Clin. Endocrinol., 11: 897–912, 1951.
546. Wilson, H.: Chromogenic values of ketosteroids in the Zimmermann reaction. Federation Proc., 9: 246, 1950.
547. Wilson, H.: Fractionation of formaldehydogenic substances in urine extracts. Federation Proc., 11: 311–312, 1952.
548. Wiswell, J. G.: Studies on the metabolism of progesterone. J. Clin. Endocrinol., 11: 765, 1951.
549. Wiswell, J. G. and Samuels, L. T.: The metabolism of progesterone by liver tissue *in vitro*. J. Biol. Chem., 201: 155–160, 1953.
550. Wolfson, W. Q., Eya, M. and Robinson, W. D.: Corticosterone, the principal precursor of urinary neutral Pettenkofer chromogens. (Dehydroepiandrosterone and derivatives). J. Clin. Endocrinol. & Metab., 12: 970, 1952.
551. Wollman, S. H. and Scow, R. O.: Effect of hypophysectomy and adrenalectomy on succinoxidase activity in the livers of alloxan diabetic rats. Endocrinology, 49: 105–109, 1951.
552. Woodward, R. B. and Bloch, K.: The cyclization of squalene in cholesterol synthesis. J. Am. Chem. Soc., 75: 2023–2024, 1953.
553. Woodward, R. B., Sondheimer, F., Taub, D., Hensler, K. and McLamore, W. M.: The total synthesis of a steroid. J. Am. Chem. Soc., 73: 2403–2404, 1951.
554. Woodward, R. B., Sondheimer, F., Taub, D., Hensler, K. and McLamore, W. M.: The total synthesis of steroids. J. Am. Chem. Soc., 74: 4223–4251, 1952.
555. Wüerch, J., Huang, R. L. and Bloch, K.: The origin of the isooctyl side chain of cholesterol. J. Biol. Chem., 195: 439–446, 1952.
556. Zabin, I. and Bloch, K.: The formation of ketone bodies from isovaleric acid. J. Biol. Chem., 185: 117–129, 1950.
557. Zabin, I. and Bloch, K.: The utilization of isovaleric acid for the synthesis of cholesterol. J. Biol. Chem., 185: 131–138, 1950.
558. Zabin, I. and Bloch, K.: The utilization of butyric acid for synthesis of cholesterol and fatty acids. J. Biol. Chem., 192: 261–266, 1951.
559. Zabin, I. and Bloch, K.: Studies on the utilization of isovaleric acid in cholesterol synthesis. J. Biol. Chem., 192: 267–273, 1952.
560. Zaffaroni, A.: Absorption spectra of sulfuric acid chromogens obtained from adrenal steroids and related compounds. J. Am. Chem. Soc., 72: 3838, 1950.
561. Zaffaroni, A. and Burton, R. B.: Corticosteroids present in adrenal vein blood of dogs. Arch. Biochem. & Biophys., 42: 1–6, 1953.
562. Zaffaroni, A., Burton, R. B. and Keutmann, E.: The application of paper partition chromatography to steroid analysis. J. Biol. Chem., 177: 109–116, 1949.
563. Zaffaroni, A., Hechter, O. and Pincus, G.: Conversion of C^{14} labeled acetate and cholesterol to adrenocortical hormones by perfused adrenal glands. Federation Proc., 10: 150, 1951.
564. Zaffaroni, A., Hechter, O. and Pincus, G.: Adrenal conversion of C^{14} labeled cholesterol and acetate to adrenal cortical hormones. J. Am. Chem. Soc., 73: 1390–1391, 1951.
565. Zaffaroni, A. and Hendricks, J.: Biochemical synthesis of the 11α epimers of the adrenal cortical hormones. Abstr., 122nd Meeting, Am. Chem. Soc. 9C–10C, Atlantic City, September 1952.
566. Zimmermann, W.: Zur Methodik der Bestimm ung der 17-Ketosteroide von Zimmermann, W. Chem. Abstr., 46: 4597, 1952.
567. Zimmermann, W., Anton, H.-U. und Pontius, D.: Die Eliminierung störender Chromogene bei der Bestimmung der 17-Ketosteroide mit m-Dinitrobenzol Hoppe-Seyler's Ztschr. f. physiol. Chem., 289: 91–102, 1951–1952.
568. Zondek, B.: Über das Schicksal des Follikelhormons (Follikulin) im Organismus. Skand. Arch. Physiol., 70: 133–167, 1934.
569. Zygmuntowicz, A. S., Wood, M., Christo, E. and Talbot, N. B.: Studies of urinary 17-ketosteroid excretion by means of a new microchromatographic fractionation procedure. J. Clin. Endocrinol. 11: 578–596, 1951.