[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. XL. The Origin and Interrelationships of the Steroidal Hormones

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In the thirty-nine papers which have been reported from this Laboratory, we have described the progress made in our investigations of the steroid content of urines. Heretofore, our results have not been discussed from the broader standpoint of the origin and interrelationships of the steroidal hormones, but now sufficient information is at hand to make such a correlation desirable.

The isolation and synthesis of sex hormones, and more recent work on the isolation of steroids from the adrenal cortex, have led biochemists to the increasing realization of the important role played by steroids in the animal. It appears to be generally assumed that these various steroids arise from cholesterol.[1] This hypothesis has many more or less obvious difficulties. It is hard to understand how oxygen atoms such as occur at C-11 in cortical derivatives, or at C-12 in bile acids, could be introduced into the cholesterol molecule. Attempts to show that cholesterol may be converted by biochemical methods into sex hormones have been inconclusive.[2] Moreover, the assumption that sex hormones are derived from cholesterol through the intermediate formation of Δ^5-pregnenol-3β-one-20 and dehydroisoandrosterone leads to contradictions when the relative physiological activities of the hormones are considered. Thus it is not apparent, if progesterone is derived from Δ^5-pregnenol-3β-one-20, why the latter should show no progestational activity. The inactivity of this unsaturated hydroxy ketone suggests that it is a product of the utilization of progesterone and not a precursor. Moreover, the drastic conditions which must be employed in the laboratory to oxidize the cholesterol side chain to yield carbonyl and acetyl groups at C-17, and the fact that phytosterols are not utilized in the animal organism,[1] throw grave doubt on the possibility of a biochemical oxidation of the cholesterol side chain. Although Shimizu[3] has suggested that the isolation of trihydroxybufosterocholenic acid indicates a genetic relationship between sterols and bile acids, experiments by Schoenheimer and co-workers[4] on the deuterium content of bile acids of dogs injected with an emulsion of coprostanone-4,5d_2 show that the conversion of cholesterol to bile acids is extremely unlikely.

In the course of comprehensive studies in this Laboratory of the numerous steroids present in urine from various animals, a number of substances have been isolated which, we believe, cannot be accounted for satisfactorily on the assumption of an oxidative degradation of cholesterol in the animal. It now appears that sex hormones and the cortical substances may be derived from another precursor.

Before considering the nature of this precursor it seems desirable to attempt a correlation between the various urinary steroids that have been isolated. The isolation from urines of numerous reduction products of progesterone such as pregnanediol-3α,20α,[5,6] *allo*-pregnanediol-3α,20α,[6,7] *allo*-pregnanol-3α-one-20,[8] pregnanol-3α-one-20,[9] *allo*-pregnanedione.[10] pregnenediol-3β,20-α,[11] pregnanetriol-3α,4-β,20α,[12] pregnanedione,[10] *allo*-pregnanol-3β-one-20,[10] and *allo*-pregnanediol-3β-20α,[11] suggests that these are all formed by the biochemical reduction of progesterone, possibly according to the scheme of Fig. 1.

According to this hypothesis, most of the substances isolated are formed from progesterone either by initial reduction of the $\Delta^{4,5}$-double bond (Type I reduction), or by reduction of the carbonyl group at C-3 to a 3-β-OH with simultaneous migration of the double bond to the 5,6-position (Type II reduction). It is supposed that in the initial biochemical reduction of the double bond of progesterone according to Type I, both regular and *allo* series compounds are formed, in complete agreement with the course of reduction

(1) Fieser, "Chemistry of Natural Products Related to Phenanthrene," 2nd ed., Reinhold Publishing Corp., New York, N. Y., pp. 251 ff., 1937.

(2) Rondoni, Carminati and Corbellini, *Z. physiol. Chem.*, **241**, 71 (1936); Rondoni, *ibid.*, **245**, 78 (1936); Voss and Rabald, *ibid.*, **245**, 76 (1936).

(3) Shimizu, "Chemie und Physiologie der Gallensäure."

(4) Schoenheimer, Rittenberg, Berg and Rousselot, *J. Biol. Chem.*, **115**, 635 (1936).

(5) Marrian, *Biochem. J.*, **23**, 1090 (1929); Butenandt, *Ber.*, **63**, 659 (1930).

(6) Marker, Kamm, Crooks, Oakwood, Lawson and Wittle, THIS JOURNAL, **59**, 2297 (1937).

(7) Hartmann and Locher, *Helv. Chim. Acta*, **18**, 160 (1935).

(8) Marker, Kamm and McGrew, THIS JOURNAL, **59**, 616 (1937).

(9) Marker and Kamm, *ibid.*, **59**, 1373 (1937).

(10) Marker, Lawson, Wittle and Crooks, *ibid.*, **60**, 1559 (1938).

(11) Marker and Rohrmann, *ibid.*, **60**, 1565 (1938).

(12) Haslewood, Marrian and Smith, *Biochem. J.*, **28**, 1316 (1934).

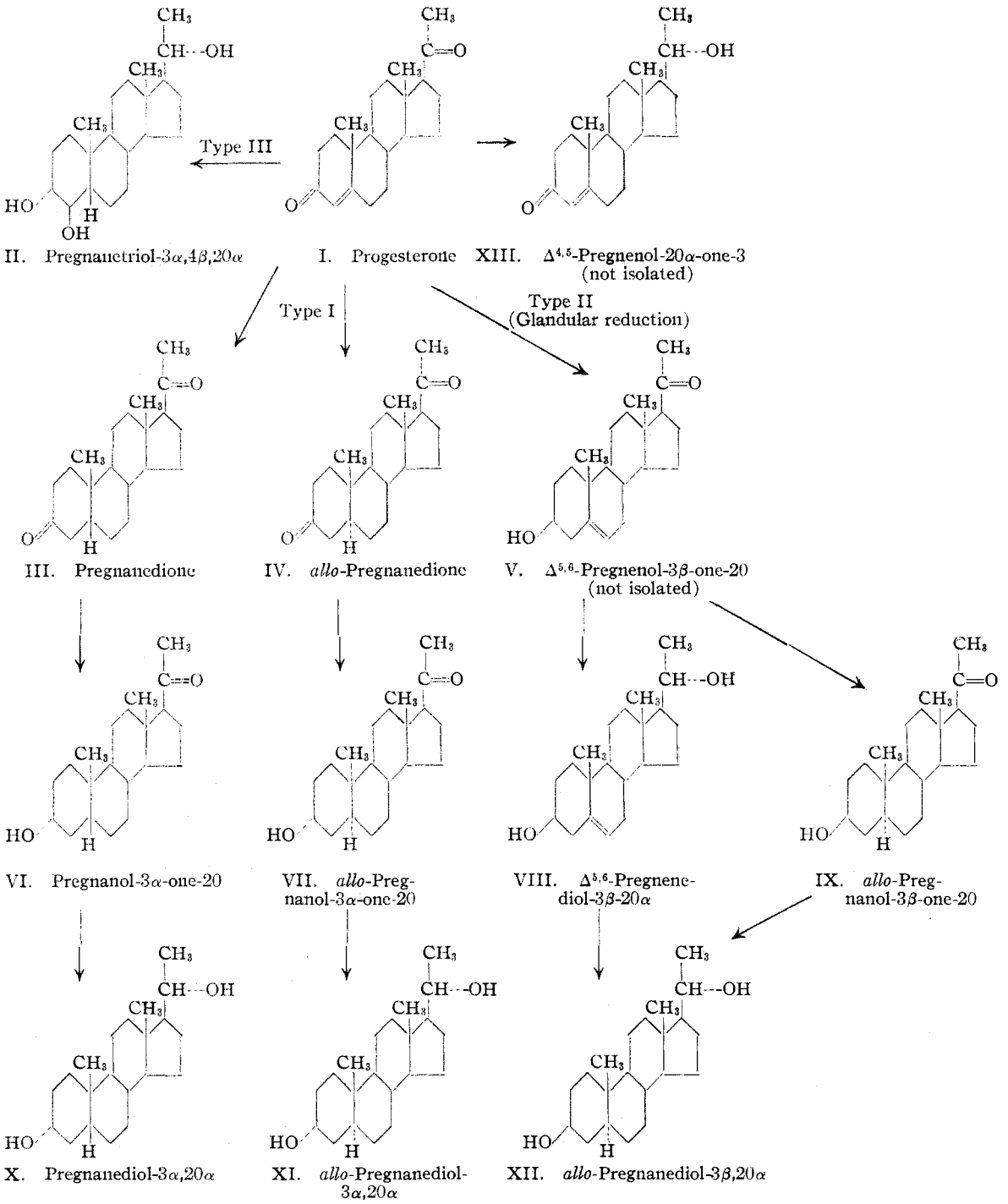

FIG. 1.—THE POSSIBLE COURSE OF REDUCTION OF PROGESTERONE

of $\Delta^{4,5}$-double bonds *in vitro*. It is interesting, in this connection, to note that Ercoli and Mamoli[13] have obtained aetiocholanedione-3,17, XVI, in 65% yield from Δ^4-androstenedione-3,17 by biochemical hydrogenation with an enzyme obtained from stallions' testes. The pregnanedione and *allo*-pregnanedione thus formed then probably suffer further reduction, first at the 3-carbonyl group, and then at the 20-carbonyl group. While the order of attack of these ketonic groups parallels the corresponding reactions under laboratory

(13) Ercoli and Mamoli, *Ber.*, **71**, 156 (1938).

conditions,[14] the stereochemical relationships involved are entirely different *in vivo* from those *in vitro*. The steric arrangement of the 3-OH formed by *in vitro* reduction of steroids is determined by the Skita rule,[15] so that under like conditions of acidity or alkalinity pregnanedione and *allo*-pregnanedione yield 3-OH groups which have opposite configurations. The configuration of 20-OH groups formed on *in vitro* catalytic hydrogenation of 20-carbonyl groups under the most varied conditions is always found to be of the β-type.[16] In contrast to these rules, *in vivo* reduction of 3- and 20-keto-steroids appears always to give α-OH groups.

In the Type II reduction of progesterone steroids of the cholesterol type (3-βOH, $\Delta^{5,6}$) are assumed to be first formed. This reaction has not yet been performed in the laboratory. The suggestion that the reduction of cholestenone to cholesterol is unlikely since it has not yet been achieved in the laboratory, loses its force because, as Schoenheimer, Rittenberg and Graff[17] have suggested, cholestenone and cholesterol probably constitute a reversible biochemical oxidation–reduction system. These workers showed that the nature of the intestinal flora, which can be controlled by a suitable choice of diet, determines the nature of the sterols excreted in the feces of test dogs. When cholestenone was fed to a dog the sterol output in its feces was increased, and depending on whether the dog's basal diet was meat or dog biscuit, the major constituent of the excreted sterols was coprostanol or cholesterol, respectively. These results strongly suggest that cholestenone may be reduced biochemically to cholesterol.

The second stage of the Type II reduction may result in the formation of either VIII or IX. Judging from the products isolated, reduction of the double bond in the 5,6-position to form IX yields only substances of the *allo* series, in complete agreement with the results of the corresponding reaction in the laboratory; for example, the hydrogenation of cholesterol yields only β-cholestanol. (A careful search for carbinols of the 3-βOH type of the regular series from mares' pregnancy urine has revealed the presence of only uranediol-3β,11-β.[86]) Finally, the further reduction of either VIII or IX gives XII, *allo*-pregnanediol-3β,20α.

At least two other modes of reduction of progesterone seem possible. Pregnanetriol-3α,4β,20α (II), which was first isolated by Marrian,[12] and later studied by Marker,[18] may arise either by hydration of the double bond of progesterone followed by subsequent reduction of the pregnanedione-3,20-ol-4 thus formed, or by the hydration of the 3-enol form of pregnanedione. The latter mechanism, which is analogous to the mechanism by which oestriol is supposed to arise from oestrone, would suggest that *allo*-pregnanetriol-2,3,20 might also be found in mares' pregnancy urine. No other compounds which might throw light on this type of reduction have yet been found. By analogy with the formation of testosterone from androstenedione in the testes, we may expect the formation of XIII, $\Delta^{4,5}$-pregnenol-20α-one-3 from progesterone in the corpus luteum. This substance, however, probably can be found only in corpus luteum extracts, just as testosterone is found only in testicular extracts. The prolonged alkaline treatment which is necessary to hydrolyze urines probably destroys all α,β-unsaturated ketones so that, even if present originally, substances of this type cannot be isolated from urines. Further reduction of $\Delta^{4,5}$-pregnenol-20α-one-3, which has been prepared by Butenandt and Schmidt,[19] can proceed according to Type I or II, giving rise to pregnanediol-3α,-20α(X), *allo*-pregnanediol-3α,20α (XI), $\Delta^{5,6}$-pregnanediol-3β,20α, (VIII), and *allo*-pregnanediol-3β,20α (XII).

In contrast to the large number of reduction products of progesterone found in human and mares' pregnancy urine, relatively few reduction products of the corresponding parent male hormone, androstenedione (XIV), have been found, probably because of the very low steroid content of male urines. As would be expected, stallions' urine apparently contains no reduction products of progesterone,[20] and it is likely that none are present in human male urine. According to the present hypothesis the course of reduction of androstenedione (Fig. 2) is supposed to be similar to that of progesterone, so that most of the ex-

(14) Marker, Kamm and Wittle, THIS JOURNAL, **59**, 1841 (1937).

(15) Ruzicka, Brungger, Eichenberger and J. Meyer, *Helv. Chim. Acta*, **17**, 1407 (1934).

(16) Marker, Kamm, Wittle, Oakwood, Lawson and Laucius, THIS JOURNAL, **59**, 2291 (1937).

(17) Schoenheimer, Rittenberg and Graff, *J. Biol. Chem.*, **111**, 183 (1935).

(18) Marker, Kamm, Wittle, Oakwood and Lawson, THIS JOURNAL, **60**, 1067 (1938).

(19) Butenandt and Schmidt, *Ber.*, **67**, 2092 (1934).

(20) Marker, Lawson, Rohrmann and Wittle, THIS JOURNAL, **60**, 1555 (1938).

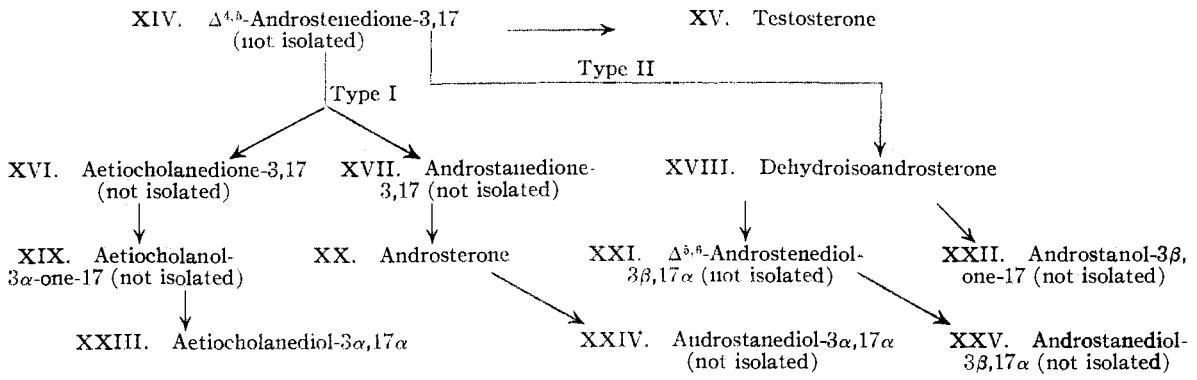

FIG. 2.—THE POSSIBLE COURSE OF REDUCTION OF ANDROSTENEDIONE

pected products would arise by Type I or Type II reduction, following the scheme indicated in Fig. 2. We have used the conventions of Ruzicka in regard to the stereochemistry of the 17-OH groups, except that we designate the configuration of the 17-OH group found naturally as α rather than *trans*. This is in agreement with the customary usage in regard to the configuration of hydroxyl groups at C-3 or C-20. Of the products listed in Fig. 2, only a few have been isolated. However, it should be noted that no C_{19} compounds have been isolated which are not predicted by the theory under discussion. The compounds isolated are androsterone,[21] dehydroisoandrosterone,[22] and aetiocholanediol-3α,17α[23] from human male urine, and testosterone[24] from testicular extracts.

In the foregoing discussion of the course of the reduction of progesterone and androstenedione to yield various steroids, most of the steroids mentioned have been found only in urines, although a few have been found only in the extracts from glands. Although our studies of the steroid content of various urines have not yet been completed, the large number of the possible reduction products of progesterone which have already been isolated suggests to us that all the reduction products possible according to the mechanism discussed in this paper occur in urine or glandular extracts.

At this point it may be profitable to compare briefly the types of compound which one may expect to isolate from urines and glandular extracts. It may be assumed that the parent hormones such as androstenedione and progesterone will exist as such in glandular extracts, but perhaps not in urines. However, it should be borne in mind that these parent substances are α,β-unsaturated ketones, and, even if present, may not survive the rigorous hydrolytic treatment to which the urines must be subjected, a treatment which is usually unnecessary to apply to glandular extracts. Besides the parent hormone such as androstenedione, progesterone, or cortin, the glandular extracts contain compounds which appear to be of only two types, corresponding in Rings A and B to cholestenone and β-cholestanol. Thus *allo*-pregnanol-3β-one-20 accompanies progesterone in corpus luteum extracts[25] and testastalone (*allo*-pregnanol-3β-one-20-al-21) accompanies testosterone in testicular extracts,[26] while numerous substances all of either the β-cholestanol or cholestenone type[27] have been found to accompany cortin in cortical extracts. The fact that only compounds of these two kinds are found in glandular extracts may indicate that glandular reduction occurs exclusively according to Type II. Although steroids of the cholesterol type have not yet been found in glandular extracts, they may be present; on the other hand, it may be that glandular reduction is so complete that no unsaturated sterols of the cholesterol type survive. The generalization that only substances of the cholestenone, β-cholestanol, and possibly of the cholesterol type, appear in glandular extracts, although it seems to be sound in view of the information now available, should of course be verified by a deliberate search

(21) Butenandt and Tscherning, *Z. angew. Chem.*, **44**, 905 (1931); *Z. physiol. Chem.*, **229**, 167 (1934).

(22) Butenandt and Dannenbaum, *ibid.*, **229**, 192 (1934).

(23) Butenandt, Tscherning and Dannenberg, *ibid.*, **248**, 206 (1937).

(24) David, Dingemanse, Freud and Laqueur, *ibid.*, **233**, 218 (1935).

(25) Butenandt, Westphal and Hohlweg, *ibid.*, **227**, 84 (1934); Slotta, Ruschig and Fels, *Ber.*, **67**, 1270 (1934); Allen and Wintersteiner, *J. Biol. Chem.*, **107**, 321 (1934).

(26) Hirano, *J. Pharm. Soc. Japan*, **56**, 717 (1936).

(27) See Reichstein, "Chemie des Cortin und seiner Begleitstoffe (pp. 347ff.) (in Ruzicka and Stepp, "Ergebnisse der Vitamin und Hormonforschung," Akademische Verlagsgesellschaft, Leipzig, 1938) for a review of the isolation and properties of the cortical steroids.

for compounds of other types. It is perhaps to be expected that glandular extracts will contain few types of steroids, for primarily the glands are the sources of the hormones, which are then passed to other localities for utilization, with the formation of reduction products. These reduction products of varied types all will be excreted ultimately in the urine so that the latter must be expected to be a source of a much wider variety of steroid types than can be found in glandular extracts. Thus the reduction products of progesterone, androstenedione, and cortin which may be expected to occur in urine will conform, in the structure of rings A and B, to all of the types indicated in Fig. 1.

From these considerations it was suspected that cortical derivatives which we regard as reduction products of the as yet unknown cortical hormone, would be found to occur in urines, and, furthermore, it was anticipated that these reduced cortin derivatives would be found present in both male and female urines. During the last year the isolation from urines of a number of steroids containing oxygen atoms at C-11 has been reported from this Laboratory, and the presence of other such substances has been indicated. These new steroids proved to be derived not from pregnane or *allo*-pregnane, but from a new hydrocarbon, urane (9β-pregnane). While the inversion of the asymmetric center at C-9 might be expected to occur in the isolation of a pregnanone-11 derivative, no such inversion could be expected to have occurred with a pregnanol-11 derivative. Therefore the uranetriol-3,11,20 isolated from mares' pregnancy urine[28] must have been present originally as such in the urine. This occurrence of steroids inverted at C-9 (β-type), of which numerous other examples have been found since, would lead one to suspect that the cortical compounds isolated in the laboratories of Reichstein, Kendall, and Wintersteiner and Pfiffner may not possess the skeleton of *allo*-pregnane, as they assumed, but the skeleton of the corresponding hydrocarbon *allo*-urane, in which the hydrogen at C-9 is inverted (β-type).[28a]

The assignment of a cholestane configuration to the cortical steroids is due mainly to the splendid work of Reichstein, who obtained androstane and *allo*-pregnane by the reduction of cortical derivatives. Since experimental details of his conversion of corticosterone to *allo*-pregnane are not yet available,[28a] it is not possible to comment on this work, but it appears that his conversion of the saturated triketone, $C_{19}H_{26}O_3$, obtained from his compounds A, C, and D, to androstane may not give conclusive information in regard to the configuration at C-9 in the cortical steroids. When this triketone was reduced by the Clemmensen method, the hydrocarbon fraction obtained was highly unsaturated. Since the unsaturation would probably be at C-11, his subsequent hydrogenation may have yielded androstane even though the original triketone had a 9β-configuration. Such a conversion from a 9β to a 9α-configuration has been observed by Tschesche and Bohle[29] in the course of their investigations of the structure of sarmentogenin and digoxigenin (Fig. 3).

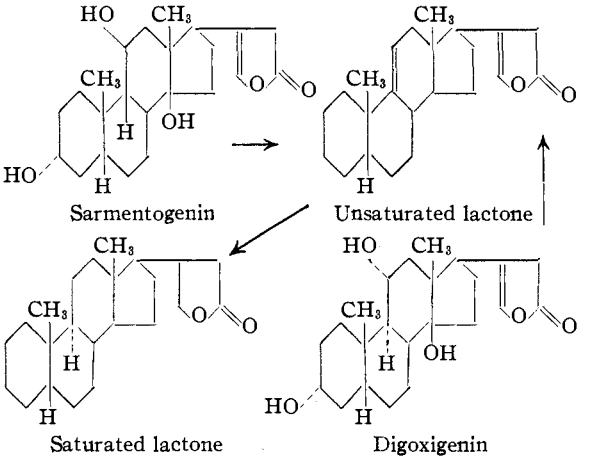

FIG. 3.—THE CORRELATION OF SARMENTOGENIN AND DIGOXIGENIN

Recent work in this Laboratory seems to support the idea that cortical steroids may be *allo*-urane derivatives. When uranetrione was reduced by the Clemmensen method in acetic acid and hydrochloric acid, the saturated hydrocarbon produced was urane. Yet when uranetrione was reduced by other means[30] with elimination of the carbonyl group at C-11 or C-20, pregnandione and uranedione were obtained. In the former case there was an inversion at C-9.

Another indication that cortical steroids may be of the *allo*-urane rather than of the *allo*-pregnane type is the apparently anomalous course of

(28) Marker, Kamm, Crooks, Oakwood, Wittle and Lawson, THIS JOURNAL, **60**, 210 (1938).

(28a) Since submitting this paper for publication, Steiger and Reichstein, *Helv. Chim. Acta*, **21**, 164 (1938), have called attention to the possibility of an inverted configuration at C-9 in the cortical hormone series.

(29) Tschesche and Bohle, *Ber.*, **68**, 423 (1935); **69**, 793 (1936).

(30) Marker, Kamm, Wittle, Oakwood and Lawson, THIS JOURNAL, **60**, 1061 (1938).

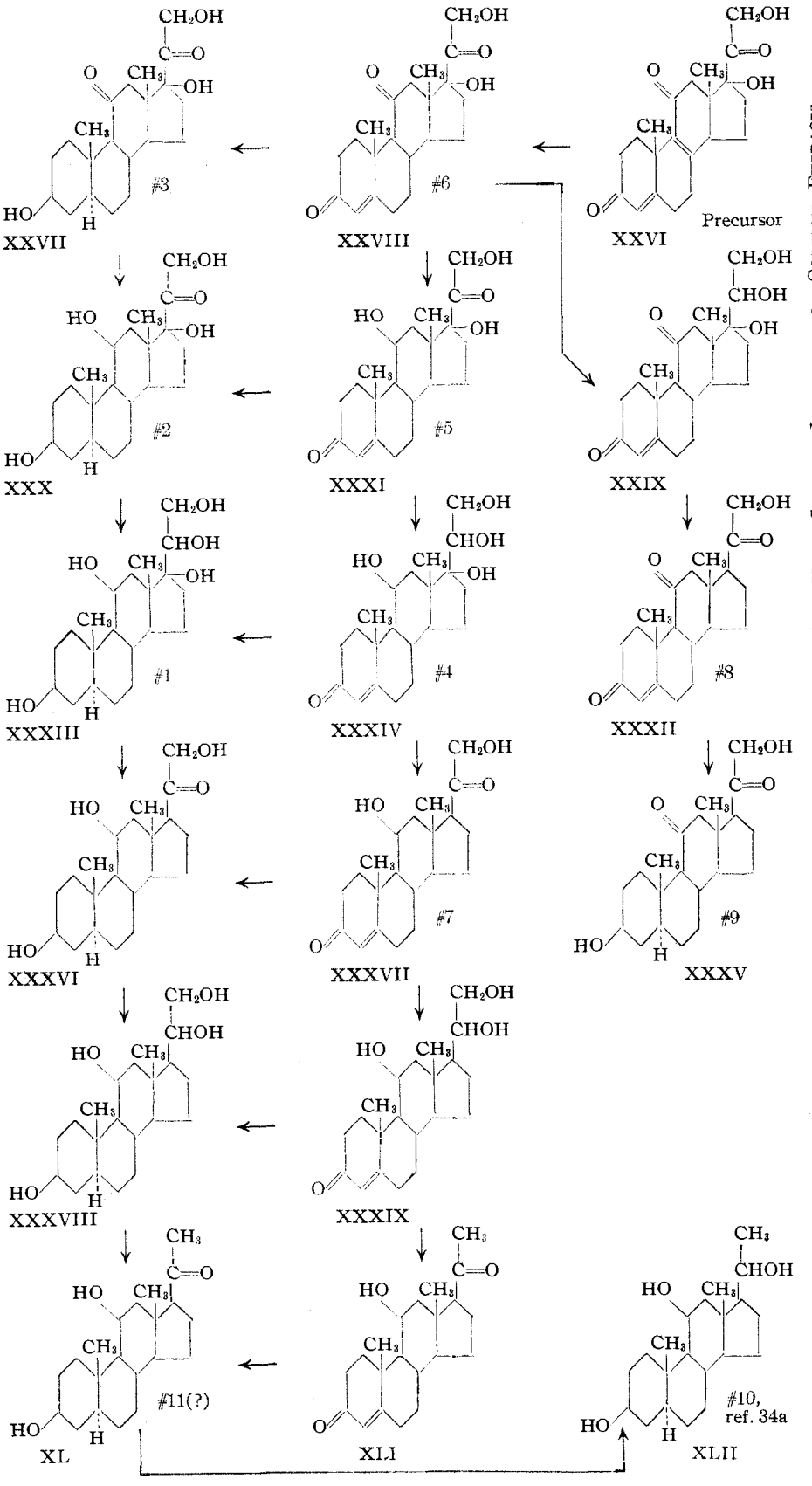

FIG. 4.—POSSIBLE COURSE OF REDUCTION (TYPES II AND III) OF PRECURSOR XXVI TO YIELD STEROIDS ISOLATED FROM CORTICAL EXTRACTS
The Arabic numbers given for the products isolated are those assigned by Reichstein in his Review Paper, Reference 27.

hydrogenation of compounds like corticosterone. While steroids having the cholestenone structure in Ring A usually give coprostane derivatives on catalytic hydrogenation,[31] the hydrogenation of the Δ^4-3-keto-cortical steroids yields only 3-β-hydroxy-*allo*-steroids. The presence of a 9β-configuration in Ring B may well account for the reduction of the Δ^4-double bond to give compounds of the *allo*-series rather than of the coprostane series, which are formed when cholestenone derivatives having a 9α-configuration are reduced.

While the suggestion that the cortical steroids may have a 9β-configuration cannot be proved definitely until diffraction and film measurements are made on cortical and urane derivatives, in this paper it will be assumed for consistency that the cortical steroids are *allo*-uranes. It is to be understood that the arguments for the theory of biogenesis of sex hormones presented in this paper are unaffected and remain valid regardless of the C-9 configuration of the cortical steroids.

In order to account for the inverted configuration at C-9 in urane and possibly in cortin derivatives, we assume that both types of substances are derived from the same hypothetical precursor, pregnadiene-4, 8-diol-17, 21-trione-3, 11, 20, (XXVI), or its hydrate at C-9, *which may be the as yet unisolated cortical hormone*. This substance would be expected to show the characteristic instability that cortin possesses, and upon reduction it could give rise to both C-9α and C-9β-compounds. This suggested precursor of the cortical and sex hormones can yield the compounds isolated from cortical extracts by an orderly scheme involving the application of definite rules in regard to the modes of reduction. Most of the compounds isolated from cortical extracts may be classified by the following statements: the structure of Ring A corresponds either to cholestenone or β-cholestanol; the asymmetric center C_9 probably has the β-configuration; the compounds possess carbonyl or β-OH groups at C-11, and have various oxygen-containing substituents at C-17 (as shown in Fig. 4). Figure 4 shows how these substances from cortical extracts may be formed in the course of the reduction of the hypothetical substance XXVI.

The first reduction product of the hypothetical parent hormone (XXVI) is assumed to be XXVIII a substance isolated by Wintersteiner and Pfiffner,[32] Kendall,[33] and Reichstein,[34] and designated respectively by them as compound F, E, and Fa. As has been mentioned previously, it is assumed that the asymmetric centers generated at C-8 and C-9 are of the normal (α) and *uro* (β) type, respectively.

The isolation of XXVII,[33,35] XXXI,[33,34] and XXXII[33] shows that further reduction of XXVIII to yield the other substances found in cortical extracts may follow at least three independent courses. According to the first mode of reduction a substance of the cholestenone type is reduced, in conformity with the rule of glandular reduction, to give the substances of the β-cholestanol type, while according to the second mode of reduction a C-11 carbonyl group is reduced to a 11-βOH-group. While this latter reaction proceeds with some difficulty in the laboratory, the large number of cortical derivatives with 11-OH groups indicate that *in vivo* the reaction proceeds readily. Alternatively, it is possible that the formation of 11-OH compounds occurs by a direct reduction of the hypothetical precursor XXVI, a reaction which might be expected to occur readily. According to the third mode of reduction the carbonyl group at C-20 is first reduced to a hydroxyl group. Although it is not possible yet to specify the configuration of the C-20-OH groups in cortical substances, it seems likely by analogy with the case of the reduction of progesterone that they will prove to be of the α-type. The substances of the glycerol type, XXXIII,[32,33,35] XXXIV,[34,35] and XXIX, are then assumed to be susceptible to ready biochemical dehydration, a reaction which may also be accomplished in the laboratory under not too vigorous conditions. The α-ketols XXXVI, XXXVII,[34,33] XXXII,[33] and XXXV[33] thus formed may be reduced further to the as yet unisolated α-glycols XXXVIII and XXXIX, which may suffer dehydration[34a] to yield the as yet unisolated ketone XLI, or the ketone XL which may be identical with Wintersteiner and Pfiffner's G.[32] Further reduction of the carbonyl group at C-20 will give rise to a 20-OH probably of the α-type.

(31) Grasshof, *Z. physiol. Chem.*, **223**, 249 (1934); **225**, 197 (1934).

(32) Wintersteiner and Pfiffner, *J. Biol. Chem.*, **111**, 599 (1935); **116**, 291 (1935).

(33) Mason, Myers and Kendall, *ibid.*, **114**, 613 (1936); **116**, 267 (1936).

(34) Reichstein, *Helv. Chim. Acta*, **19**, 1107 (1936); **20**, 953, 978 (1937).

(34a) Steiger and Reichstein, *ibid*, **21**, 546 (1938).

(35) Reichstein, *ibid.*, **19**, 29 (1936).

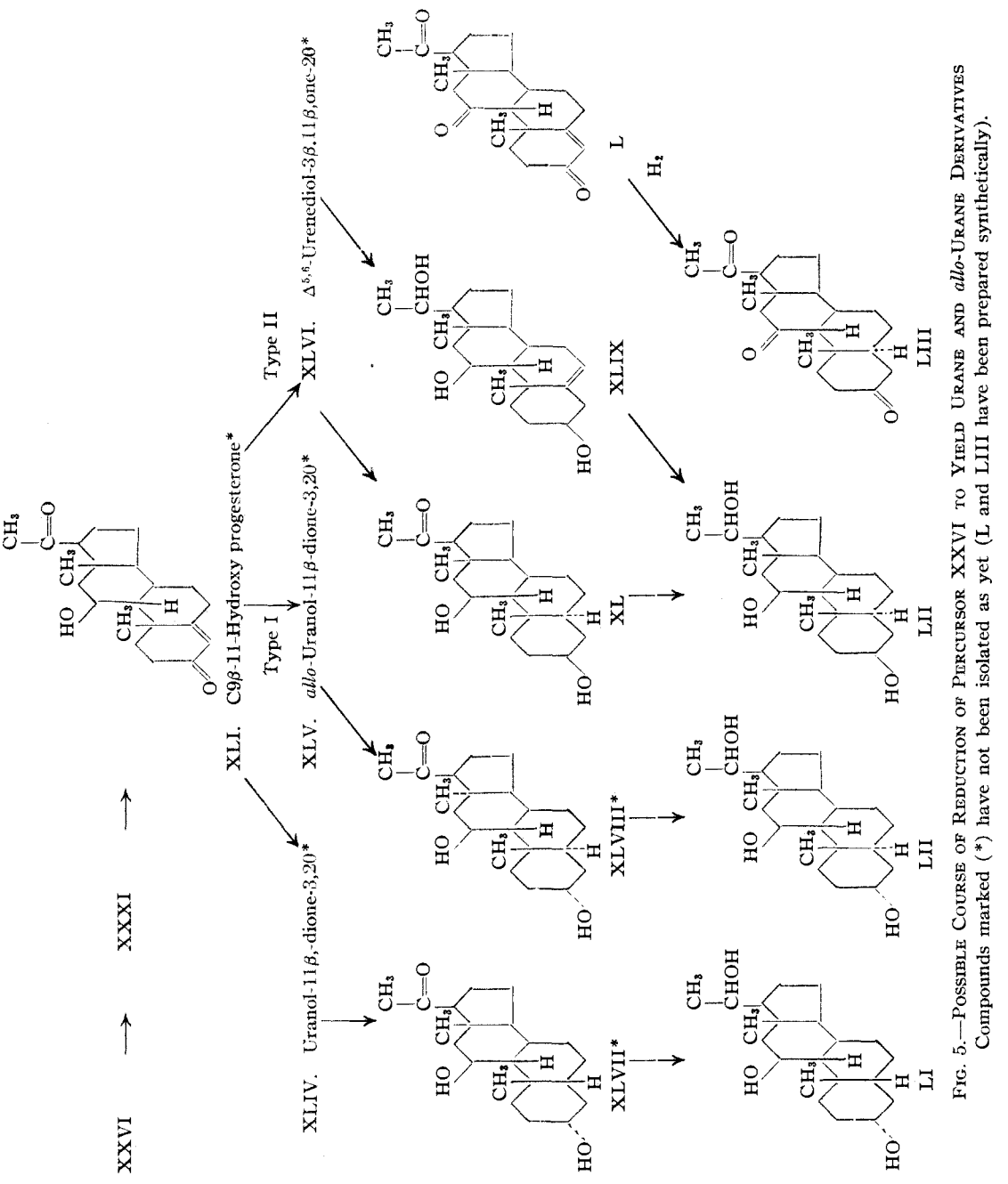

FIG. 5.—POSSIBLE COURSE OF REDUCTION OF PERCURSOR XXVI TO YIELD URANE AND *allo*-URANE DERIVATIVES
Compounds marked (*) have not been isolated as yet (L and LIII have been prepared synthetically).

The reduction of XXVI apparently proceeds further, for from urines there have been isolated a number of carbinols which appear to be derived from the cortical hormone, and evidence has been submitted of the occurrence of all other such compounds. Thus, from mares' pregnancy urine uranetriol-3α,11β,20α,[28] uranediol,[36] and uranolone[10] have been isolated and indications have been obtained of the occurrence of uranediol-3α,-11,[30] $\Delta^{5,6}$-urenetriol-3β,11,20 and of *allo*-uranetriol-3β,11,20.[37] Furthermore, it has been found that the carbinol fraction from mares' pregnancy urine yields ketones and acids on oxidation with periodic acid,[38] indicating the presence of the same characteristic glycol or glycerol residues that occur in the steroids from cortical extracts. Evidence has been obtained for the occurrence in stallions' urine of uranetriol-3α,11,20,[20] and *allo*-triol,[20] and an *allo*-tetrol.[20] These findings all

(36) Marker, Rohrmann and Wittle, THIS JOURNAL, **60**, 1561 (1938).

(37) Marker and Rohrmann, in press.

(38) Unpublished work of This Laboratory.

agree with the scheme given in Fig. 4. This seems to indicate that the cortical hormone, which may be identical with the hypothetical precursor XXVI, suffers the same type of biochemical reduction as progesterone and androstenedione. The formation of these urane and *allo*-urane derivatives from the hypothetical precursor XXVI may occur in the animal by several alternative or simultaneous processes. Thus the reduction of the conjugated system at 8, 9, 11 may yield either 11-keto-urane derivatives or 11-hydroxyurane derivatives, while at the same time the dihydroxyacetone residue may pass through the several stages of reduction and dehydration indicated in Fig. 4. The conjugated system at 3, 4, 5 at the same time may suffer reductions of Type I or Type II (Figs. 1 and 2).

In Fig. 5 a scheme is presented showing how all of the urane derivatives isolated to date may be formed by the reduction of the as yet unknown 11-hydroxy-9-β-progesterone XLI. It is to be understood, however, that several other intermediates, such as 11-keto-9β-progesterone, may, in accordance with the theory developed in this paper, also give rise to the urane derivatives found in urines. The reduction of 11β-hydroxy-9β-progesterone (XLI), if it proceeds according to Type I, will give rise, through the as yet unisolated compounds XLIV, XLV, XLVII and XLVIII, to uranetriol-3α,11β,20α (LI) and *allo*-uranetriol-3α,11β,20α (LII). The first of these compounds (LI) has been isolated from mares' pregnancy urine,[28] and its reactions and structure investigated.[30] Its presence in stallions' urine[20] has been demonstrated by the isolation of uranetrione-3,-11,20 from the oxidation of an *epi*-carbinol fraction. *allo*-Uranetriol-3α,11β,20α (LII) may be identical with a triol whose presence has been demonstrated in stallions' urine.[20] If the reduction of 11β-hydroxy-9β-progesterone (XLI) proceeds according to Type II, it may give rise to either XL or XLIX. The first of these has not been isolated or demonstrated to exist in urines, but it may be identical with Wintersteiner and Pfiffner's compound G. Urenetriol-3β,11β,20α has not been isolated as such but its presence in mares' pregnancy urine has been demonstrated.[37] The digitonin precipitable carbinols from mares' pregnancy urine yielded an unsaturated fraction which on addition of bromine, oxidation, and debromination, yielded $\Delta^{4,5}$-urenetrione-3,11,20 (L), a substance which had been prepared previously from uranetrione.[30] The further biochemical reduction of either XL or XLIX would give *allo*-uranetriol-3β,11β,20α (XLII). The latter, which may be identical with the 3β-OH-*allo*-triol which was prepared as described above from stallions' urine, has been demonstrated to occur in mares' pregnancy urine.

From cortical extracts there is obtained,[35] besides the C_{21} compounds, a substance, adrenosterone (LIX) which has been shown to be androstenetrione-3,11,17. This compound shows androgenic activity, and may therefore be considered to be a connecting link between the cortical substances and the C_{19} and C_{18} sex hormones. It should be mentioned, however, that adrenosterone may have a *uro*-(β-type) configuration at C-9. The idea, suggested here, that cortical and sex hormones may be interrelated, is supported by numerous facts. The structures of the C_{21} cortical steroids and progesterone, on the one hand, and of adrenosterone and androstenedione on the other hand, indicate their close relationship. The influence of cortical disturbances on sexual characteristics is well known, and several instances can be cited where abnormal products related to sex hormones have been isolated from the urines of patients suffering from some disorder of the suprarenal gland. For example, Marrian and Butler[39] isolated pregnanetriol-3,17,20 from the urine of female patients with adrenal tumors, while Burrows, Cook and Warren[40] isolated $\Delta^{3,5}$-androstadienone-17, originating presumably from *epi*-dehydroandrosterone, from the urine of a man with an adrenal tumor.

This connection between cortical and sex hormones may be accounted for by the assumption that both types of hormones have a common precursor. We propose to show now how the hypothetical precursor (XXVI) of the adrenal steroids may be also the precursor of the sex hormones of the androgenic (C_{19}), progestational (C_{21}), and oestrogenic (C_{18}) types. In order to make the course of the conversions clear, it is necessary first to recount some recent work on the reduction of uranetrione in this Laboratory.[41]

When uranetrione (LIV) is reduced catalytically, there is formed (Fig. 6) in addition to uranetriol-3,11,20 (LV), uranediol-3,11 (LVI) and a

(39) Marrian and Butler, *J. Biol. Chem.*, **119**, lxvi (1937).

(40) Burrows, Cook and Warren, *J. Soc. Chem. Ind.*, **55**, 1031 (1936).

(41) Marker, Wittle and Oakwood, THIS JOURNAL, **60**, 1561 (1938).

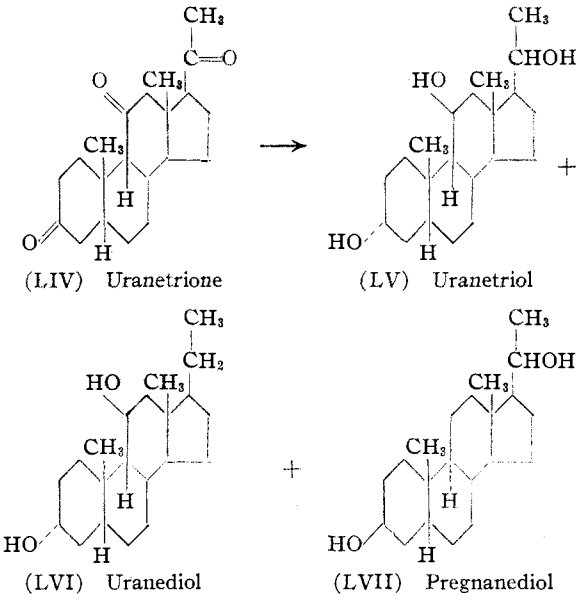

FIG. 6.—PRODUCTS FROM THE CATALYTIC REDUCTION OF URANETRIONE

mixture of pregnanediols (LVII). This implies that the carbonyl groups at C-11 and C-20, which are adjacent to tertiary hydrogens, are to some extent completely reduced to methylene groups, and, furthermore, that when the C-11-carbonyl is completely reduced, the molecular skeleton suffers inversion from the *uro-* (β) to the normal (α) form, although, as would be expected in view of the much greater stability of the normal (α), as compared to the *iso-* (β) type at C-17,[42] no inversion occurs when the C-20 carbonyl group is completely reduced. While at first sight this reduction may appear anomalous, it is by no means without parallel. Thus, when 7-keto-cholesteryl chloride is hydrogenated catalytically, α-cholestyl chloride is the major product.[43] It is evident, in view of the isolation from mares' pregnancy urine of uranediol, uranolone, and uranediol-3α,11β (the latter as uranedione from an *epi*-carbinol fraction), that this reduction of a C-20 carbonyl group to a methylene group occurs in the animal. Therefore, it is also likely that the reduction of a 9β-11-CO-system to a 9α-11-CH_2-system may occur in the animal as well as in the laboratory.

We are now able to understand how sex hormones may be formed from the hypothetical substance XXVI. This substance and a number of its possible reduction products contain dihydroxyacetone residues which are readily susceptible to oxidation to yield carbonyl groups at C-17. In the laboratory this oxidation may be accomplished by the use of lead tetraacetate, periodic acid, or almost any of the common oxidizing agents such as chromic acid or potassium permanganate; the fact that adrenosterone accompanies the C-21 steroids from cortical extracts shows that a similar type of oxidation occurs in the animal. Figure 7 illustrates how adrenosterone (LIX) or a precursor such as (LVIII) may give rise by biochemical reductions, to androstenedione (XIV) and therefore to the other male hormones. It will be evident, in view of the previous discussion, that the application of this theory will predict the possible existence in urines or glandular extracts of other related steroids, such as Δ^4-androstenol-11β-dione-3,17, which might arise by the various modes of reduction which

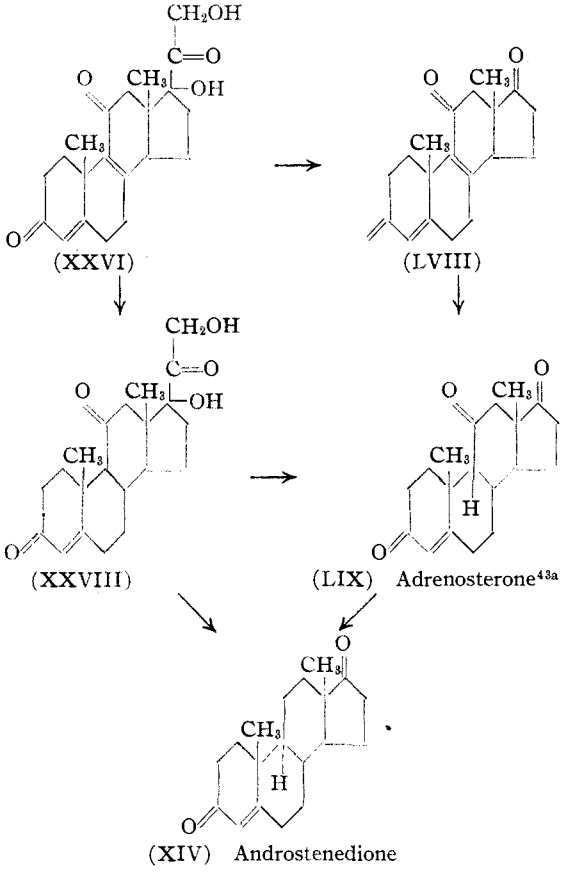

FIG. 7.—POSSIBLE FORMATION OF ADRENOSTERONE AND ANDROSTENEDIONE

(42) Butenandt and Mamoli, *Ber.*, **68**, 1854 (1935); Butenandt and Fischer, *ibid.*, **70**, 96 (1937).

(43) Marker, Kamm, Fleming, Popkin and Wittle, THIS JOURNAL, **59**, 619 (1937).

(43a) Since sending this paper to press H. L. Mason, *Proc. Staff Meet. Mayo Clin.*, **13**, 235(1938), has converted XXVIII into adrenosterone (LIX) by treatment of the former with a solution of calcium hydroxide.

have been proposed, and, conversely, it should be noted that this theory predicts the *absence* in urines or glandular extracts of such a steroid as aetiocholanediol-3β,11β,-one-17. It should be emphasized that the reduction of the 11-carbonyl group to give an inversion from the 9β to the 9α-type is supposed to occur in the animal as well as during the catalytic hydrogenation *in vitro*.

The same type of simultaneous reduction and inversion of a 11-CO-9β system to a 11-CH_2-9α system in conjunction with the degradation of a dihydroxyacetone residue will account for the formation of progesterone and related steroids from XXVI. In Fig. 8, which shows how this may be accomplished, a number of the steps already shown in Fig. 7 are omitted, and it is to be understood that several alternate reduction schemes, which already have been discussed in similar instances, are omitted here for the sake of brevity.

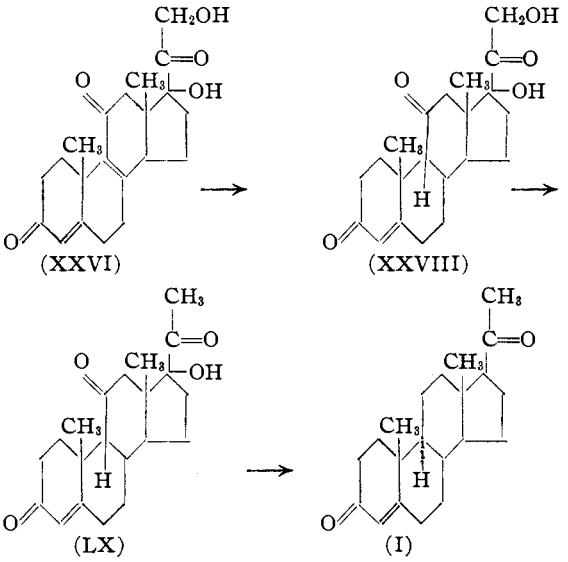

FIG. 8.—POSSIBLE FORMATION OF PROGESTERONE FROM (XXVI)

Not only is it possible to show how cortical steroids, male hormones, and progesterone derivatives may arise by orderly processes from XXVI, but it is also possible to show that the female hormones can be derived from the same parent compound. It is necessary to show first how the most characteristic feature of the female hormones, the occurrence of ring A, and sometimes B, in a benzenoid form, may arise. A number of examples are available to show that under drastic conditions, or because of the occurrence of an extended conjugated system, the methyl group at C-10 in many steroids may be lost as methane with the formation of benzenoid rings. One may cite the following examples: when apocholic acid is heated, methane is lost;[44] when ergopinacol is heated, neoergosterol is formed with the loss of methane;[45] when dianhydrostrophanthidin is treated with concentrated hydrochloric acid trianhydrostrophanthidin is formed;[46] when heptaacetyldesoxydihydroouabain is treated with hydrochloric acid and acetic acid the acetoxy lactone $C_{24}H_{30}O_4$, which contains a benzene ring, is obtained;[47] and finally when the debromination products of dibromoandrostanedione are heated, methane is evolved and phenolic compounds are formed.[48] These examples indicate that the elimination of methane with the formation of benzenoid rings is a type of reaction which may be expected to occur in the organism also if the parent molecule presents a sufficiently extended conjugated system, or some other structural feature which may be expected to facilitate the reaction. The hypothetical parent hormone (XXVI) has this type of molecular structure. In Fig. 9 a scheme is presented showing how the phenolic female hormones may be formed. It is not possible, of course, in

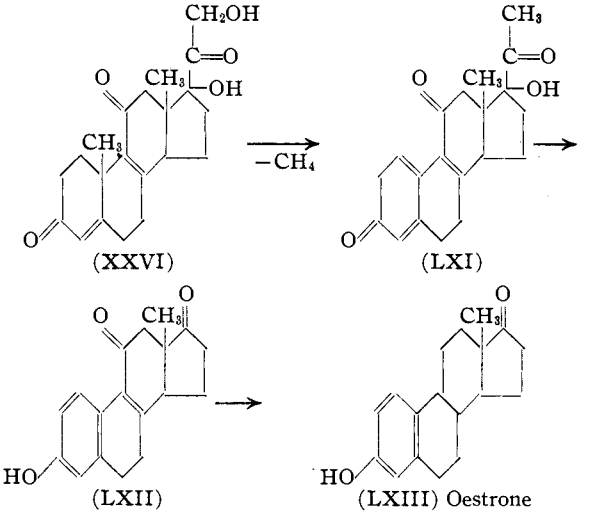

FIG. 9.—POSSIBLE FORMATION OF OESTRONE FROM XXVI

view of the meager amount of data available, to state which of the several probably independent processes of dihydroxyacetone oxidation, loss of methane, and reduction of the 11-carbonyl group

(44) Wieland and Dane, *Z. physiol. Chem.*, **212**, 263 (1932).

(45) Windaus and Borgeaud, *Ann.*, **460**, 235 (1928).

(46) Jacobs and Collins, *J. Biol. Chem.*, **63**, 123 (1925); see Fieser, *op. cit.*, p. 274 for a discussion of this transformation.

(47) For references and a critical discussion, see Fieser, *op. cit.*, pp. 292 ff.

(48) Inhoffen, *Naturwissenschaften*, **25**, 125 (1937).

may be expected to occur first. It should be noted that the mechanism indicates how equilin, as well as oestrone, may be formed. The formation of equilin by a migration of the double bond from the 8,9- to the 7,8-positions may occur in the animal, or it may occur in the course of the prolonged alkaline treatment of the urine. This drift of a double bond near a benzene ring is a familiar process in the chemistry of allyl- and propenyl-benzene derivatives, although usually the tendency is toward the establishment of an equilibrium mixture containing predominantly the propenylbenzene derivative. The formation of equilenin by the loss of hydrogen is also readily understandable as a tendency of the second ring to become aromatic. In fact, so great is the tendency for equilin to yield equilenin that when attempts are made to hydrogenate the former catalytically, the latter is always formed instead.[49] It is possible, furthermore, that a compound like LXII might suffer further loss of methane to yield, ultimately, *ar*-dihydroxycyclopentanophenanthrene derivatives, none of which have so far been found. In support of the hypothesis that oestrone and related female hormones are derived from a common precursor which is primarily not a sex hormone itself, we may note that oestrone is found in stallions' urine[50] as well as in mare and human female urines.

A discussion of the mode of biogenesis of the precursor XXVI is beyond the scope of this paper. It seems likely that the precursor may be formed from sugar units[51] in the pituitary or some other master gland, and supplied to the suprarenals, ovaries, corpus luteum, or testes for the production of the specific hormones elaborated by these glands. The reactions by which these syntheses are effected may be enzymatic, or they may be a part of some intramolecular oxidation and reduction in the glands, but it is not the purpose of this paper to discuss their nature. Since ascorbic acid occurs in the suprarenal glands, it may be that its function is the reduction of a precursor like XXVI to give steroidal hormones.

(49) Dirscherl and Hanusch, *Z. physiol. Chem.*, **233**, 13 (1935); **236**, 131 (1935).

(50) Zondek, *Nature*, **133**, 209, 494 (1934); Cortland, Meyer, Miller and Rutz, *J. Biol. Chem.*, **109**, 213 (1935).

(51) Reichstein, *op. cit.*, p. 368.

The author wishes to thank Dr. Elmer J. Lawson for his suggestions and assistance in the preparation of this manuscript, and all the members of his sterol group, Drs. Eugene L. Wittle, Elmer J. Lawson, Thomas S. Oakwood, Ewald Rohrmann, Harry M. Crooks, Stephen B. Binkley and David M. Jones for the use of their unpublished work.

Summary

1. Arguments are advanced to indicate that the steroidal hormones and bile acids do not originate from cholesterol.

2. It is suggested instead that the steroidal hormones, including the C-18, C-19, and C-21 sex hormones and the cortical steroids, may come from another common precursor, pregnadiene-4,8-diol-17,21-trione-3,11,20 (XXVI) or its hydrate at C-9.

3. Considerations of the interrelationships among the many steroids from urines and from gland extracts make it possible to propose a definite structure (XXVI) for the precursor of the steroidal hormones.

4. It is shown how the various steroids isolated may arise from this precursor by orderly processes following definite rules and having with few exceptions, their counterpart in the laboratory.

STATE COLLEGE, PENNA. RECEIVED MARCH 22, 1938