myoinositol, first at the 2- position and then at the 6-position of the ring.

ACKNOWLEDGMENT

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Evidence Favoring the Nonspecificity of 3-Hydroxysteroid Dehydrogenases in Relation to Steroid Conformation*

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The high-speed supernatant of homogenates from male rat liver contained enzymes which, after combination with TPN or DPN, were able to oxidize 3α- or 3β-hydroxysteroids derived from the hormone and cardenolide series. In the presence of a tenfold excess of cofactor the differences in the conformation of the substrates, i.e., A/B *cis*-, C/D *trans*- and A/B *trans*-, C/D *trans*-ring junction on the one hand and A/B *cis*-, C/D *cis*- and A/B *trans*-, C/D *cis*-ring junction on the other, did not influence the shapes of *p*H-activity curves and the positions of *p*H optima which were largely dependent on the steric configuration of the hydroxyl group at carbon 3. The *p*H optimum for the dehydrogenation of 3α-hydroxysteroids was at 9.6 with TPN or at 8.6 with DPN and that of 3β-hydroxysteroids was at 10.2 with DPN. Irrespective of the ring isomerization, the 3α-hydroxysteroids were dehydrogenated with TPN as cofactor much faster than when DPN was used. The 3β-hydroxysteroids were not significantly oxidized by the liver supernatant fraction in the presence of TPN. The *cis*- or *trans*- junction of the rings A and B or C and D influenced the reaction rate only. Extent and direction of this influence was not predictable from the conformation of the substrate. The present evidence favors the conclusion that in the supernatant fraction two types of 3α-hydroxysteroid dehydrogenases are involved, differing in their specificity to TPN and DPN, and one type of DPN-dependent 3β-hydroxysteroid dehydrogenase. No distinction, either in shape of *p*H curve, *p*H maximum, or nucleotide specificity could be observed between steroids of the hormone and cardenolide types having the same steric orientation of the hydroxyl group at C-3.

Metabolic experiments carried out with steroids of the hormone series possessing a C/D *trans* configuration have shown the presence of various 3-hydroxysteroid dehydrogenases in rat liver. Tomkins (1956) partially purified a soluble 3α-dehydrogenase. This enzyme used C_{19} and C_{21} compounds as substrates but did not attack two C_{27} steroids. A 3β-hydroxysteroid dehydrogenase has not yet been isolated; the existence of such an enzyme n rat liver may, however, be deduced from the results obtained in metabolic experiments using some C/D *trans*-steroids (Kochakian and Aposhian, 1952; Schneider, 1952; Harold *et al.*, 1956). These results indicated that rat liver contains 3β-hydroxysteroid dehydrogenases which may use C_{19}, C_{21}, and C_{27} steroids of the C/D *trans* series as substrates. The identity and the distribution of these 3β-enzymes in the soluble and particulate fractions of the liver is not yet clear. A 3α-hydroxysteroid dehydrogenase preparation obtained from the soluble fraction of a liver homogenate (105,000 × *g*) also used epiandrosterone as a substrate in addition to 3α-hydroxysteroids but did not metabolize testosterone (Hurlock and Talalay, 1958). The metabolism of the 3β-hydroxysteroid was probably due to the impurity of the enzyme preparation. Thus it is likely that the rat liver contains at least one soluble 3β-hydroxysteroid dehydrogenase which is distinctly different from the 17β-enzymes of the rat liver.

Oxidation-reduction reactions on C-3 are not limited to the C/D *trans*-steroid series but also have been observed with certain steroids of the cardenolide series distinguished by a C/D *cis* configuration. These C_{23} steroids show in addition to the difference in the C/D configuration a tertiary hydroxyl group at C-14 and a butenolide group on C-17 (Fig. 1). Upon incubation of 3β-hydroxycardenolide genins (especially digitoxigenin, gitoxigenin, digoxigenin, and diginatigenin) with liver slices, occasionally in addition to traces of 3-keto derivatives a substantial production of the 3α-epimers of these substrates could be detected (Lauterbach and Repke, 1960). Because of the peculiar constitution of the genins it has been supposed that

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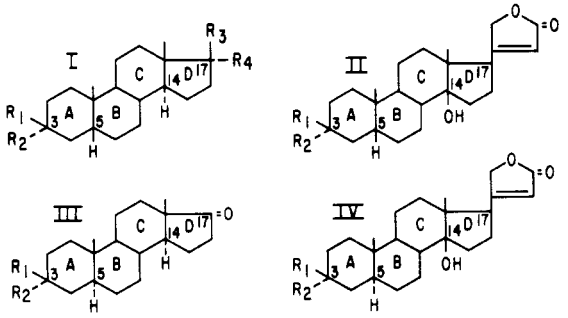

FIG. 1.—Chemical formulas for the steroids used. I: A/B *cis*, C/D *trans* junction. Pregnan-3α-ol-20-one: R_1 and R_4=H, R_2=OH, R_3=$COCH_3$; pregnan-3β-ol-20-one: R_1=OH, R_2 and R_4=H, R_3=$COCH_3$; etiocholan-3β-ol-17-one: R_1=OH, R_2=H, R_3 + R_4=O. II: A/B *cis*, C/D *cis* junction. 3-Epidigitoxigenin: R_1=H, R_2=OH; digitoxigenin: R_1=OH, R_2=H. III: A/B *trans*, C/D *trans* junction. Androstan-3α-ol-17-one: R_1=H, R_2=OH; androstan-3β-ol-17-one: R_1=OH, R_2=H. IV: A/B *trans*, C/D *cis* junction. 3-Epiuzarigenin: R_1=H, R_2=OH; uzarigenin: R_1=OH, R_2=H.

specific 3α- and 3β-hydroxycardenolide genin dehydrogenases may exist (Repke and Lauterbach, 1960).

In the present work it has been shown that the enzymes carrying out the above oxidations and reductions can be found in the supernatant fraction from rat liver homogenates. Furthermore, we have tried to determine whether soluble 3α- and 3β-hydroxysteroid dehydrogenases acting on the C/D *trans*-steroids differ from those acting on the C/D *cis* series.

EXPERIMENTAL PROCEDURE

Steroids.—Cardenolide genins,[1] when chromatographed on paper, were either pure or contained at most 5% foreign substances. Other steroids did not contain any measurable impurities on paper chromatography. The steroids were dissolved in pyridine (spectroscopic grade, Eastman Kodak) and made up to a concentration of 0.15 μmole/10 μl of solvent.

Pyridine Nucleotides.—DPN (98–100%, Sigma) and TPN (95–100%, Sigma) were placed in a volumetric flask and dissolved in a small volume of water. Sodium hydroxide (0.1 N) was then added to bring the solution to *p*H 5.5, and finally water was added to give a concentration of 1.5 μmoles/100 μl of solution.

Buffer Solutions.—Glycine and Tris buffers were prepared according to Rauen (1956). To both buffers EDTA[2] was added to give a final concentration of 2 mM. The *p*H of these buffers was checked with a glass electrode *p*H meter at 20°.

Preparation of Supernatant at 105,000 × g.—Sprague-Dawley male rats with body weights of 200–250 g were killed by decapitation. The liver was removed as rapidly as possible and weighed. All operations thereafter were carried out at 0°. The homogenization of the liver was performed in a Potter-Elvehjem type glass homogenizer using a 0.25 M sucrose–2 mM EDTA solution (*p*H 7.5). The relationship between tissue and this solution was 1:1 (w/v). The homogenate obtained from the livers of 10 rats was centrifuged for 30 minutes at 6500 × *g*, and the supernatant fraction then was centrifuged for 60 minutes at 105,000 × *g*. The second supernatant was dialyzed in cellophane, first for 4 hours against 4 liters 10 mM Tris–2 mM EDTA solution (*p*H 7.5) and then for 14 hours against 2 liters 10 mM EDTA solution (*p*H 7.0). The resulting turbidity was removed from the nondialyzable fraction by recentrifugation for 60 minutes at 105,000 × *g*. The pellets obtained during the different centrifugations were carefully kept. The final clear supernatant fraction was diluted with water to a volume corresponding to the original liver weight and kept at 0°. Under these conditions the activity in this fraction remained essentially unchanged for about 1 week. No attempts were made to isolate the 3-hydroxysteroid dehydrogenases supposed to be in this fraction because this might change the original ratio of the different enzymatic activities. The knowledge of this ratio was needed for a subsequent study on the mechanism of epimerization of the hydroxyl group at carbon 3 (Repke and Samuels, 1964, accompanying paper).

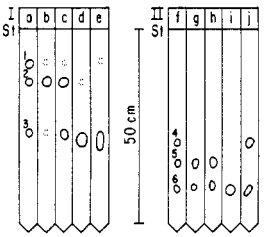

FIG. 2.—Identification by paper chromatography of the metabolites formed by incubating cardenolide derivatives and nucleotides with the soluble fraction from rat liver homogenates obtained at 105,000 × *g* for 1 hour. On strips (a) and (f) are shown the following authentic compounds: (1) 3-epidigitoxigenin, (2) digitoxigenin, (3) 3-dehydro-digitoxigenin, (4) uzarigenin, (5) 3-epi-uzarigenin, (6) 3-dehydro-uzarigenin. After the enzymatic reaction had approximately reached its equilibrium it was stopped by extraction with chloroform. Incubation conditions: digitoxigenin and DPN at *p*H 7.4 (b), *p*H 9 (c), and *p*H 10.1 (d); 3-epi-digitoxigenin and TPN at *p*H 9.6 (e); 3-epi-uzarigenin and DPN (g) or TPN (h) at *p*H 7.4; 3-epi-uzarigenin and TPN at *p*H 9.6 (i); uzarigenin and DPN at *p*H 10.1 (j). The thickness of the circles on these chromatograms indicates a rough measurement of the amount found. St = Start. Chromatographic systems: I: xylol/formamide, 11 hours; II: benzene-chloroform (7:5)/formamide, 4.5 hours.

Protein determinations were carried out by the method of Gornall *et al.* (1949), using a standard calibration curve established with bovine serum albumin.

Determination of Enzyme Activity.—Enzymatic activity was estimated by the optical method of Warburg and Christian (1936). The optical density of the reduced pyridine nucleotide was measured at 340 mμ in a Beckman DU spectrophotometer. The enzymatic reaction was carried out at 37.5° in a total volume of 3 ml using a cuvet with a 1-cm light path. The control cuvet contained 2.5–2.95 ml of the buffer solution and 0.5–0.05 ml of the enzyme preparation. To the test cuvet 0.10 ml (1.5 μmoles) of DPN or TPN solution and correspondingly less buffer were added. The addition of DPN led to an increase in the optical density of the solution. Velocity and extent of this increase depended upon the amount of enzyme added to the cuvet and upon the *p*H of the solution. When this change of optical density had come to an equilibrium, 0.01 ml of either a steroid solution in pyridine or of pyridine alone was mixed with the contents of the cuvets and the optical density was measured every 30 seconds thereafter. The difference in optical density during the first 30 seconds, after correcting for the pyridine blank, was taken as a measurement of enzymatic activity.

[1] The authors express their gratitude to Professors T. Reichstein, Ch. Tamm, and R. Tschesche, and Dr. G. Baumgarten for the gift of the cardenolide genins.

[2] Abbreviation used in this work: EDTA, ethylenediaminetetraacetic acid.

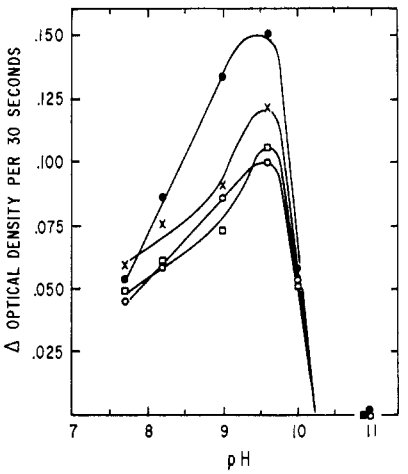

FIG. 3.—The influence of *p*H on the speed of TPN reduction by 3α-hydroxysteroids in the presence of the soluble liver fraction obtained at 105,000 × *g* for 1 hour. Increased optical density as a function of production of TPNH (λ = 340 mμ and *d* = 1 cm). The final volume of 3 ml contained a mixture of the following components: 1.5 μmoles of TPN, 143 μmoles of Tris buffer (*p*H 7.6) or 286 μmoles of glycine–sodium hydroxide buffer (*p*H 8.2–10.9), respectively, 2 mg of protein from the soluble liver fraction and 0.15 μmole of steroid. ●—●, 3-epidigitoxigenin; O—O, pregnan-3α-ol-20-one; ×—×, 3-epi-uzarigenin; □—□, androstan-3α-ol-17-one.

The enzymes were differentiated by the stereospecificity shown toward the hydroxyl group on C-3 of the substrate. With the unfractionated enzyme preparation, oxidation of DPNH or TPNH by 3-ketosteroids would give a mixture of 3α- and 3β-hydroxysteroids. Where DPN or TPN was reduced by 3α- or 3β-hydroxysteroids the steric course of the reaction was predetermined, thus allowing the separate measurement of the different types of enzymatic activities without isolation of the proper enzymes. The reverse reaction, which can occur near *p*H 7 and which would disturb the accuracy of the measurement, was negligible in an alkaline medium (*p*H 8.5) (Talalay and Marcus, 1956).

The increases of optical density measured at 340 mμ represented in fact the formation of reduced nucleotide, since the addition of an excess of acetaldehyde or 3-ketosteroid brought about a complete disappearance of the observed increases in optical density.

Identification of Reaction Products.—The contents of the cuvets were extracted with chloroform, and the residue obtained after evaporation of the solvent was chromatographed on paper. The chromatographic systems used for the separation of cardenolide derivatives (Fig. 2) were xylol/formamide[3] (Kaiser, 1955) and benzene-chloroform (7:5)/formamide (Schmid *et al.*, 1959). The derivatives of the pregnane, etiocholane, and androstane series were analyzed by using the well-known standard solvent systems of the Zaffaroni type (for details see Neher, 1958). The localization of the cardenolides was identified by the fluorescence which these compounds give with trichloracetic acid and "chloramine" (Kaiser, 1955). The other steroids were identified by their reactions with $SbCl_3$ or phosphomolybdic acid, or by the Zimmermann reaction (for applications see Neher, 1958). The paper chromatographic studies demonstrated that an increase of optical density was associated with the formation of the proper 3-ketosteroid reaction product.

[3] The solvent before the (/) is the moving phase while that after it is the stationary phase.

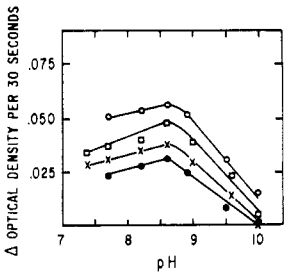

FIG. 4.—The influence of *p*H on the speed of DPN reduction by 3α-hydroxysteroids and the soluble liver fraction obtained at 105,000 × *g* for 1 hour. Increased optical density is due to production of DPNH (λ = 340 mμ and *d* = 1 cm). The final volume of 3 ml contained a mixture of the following components: 1.5 μmoles of DPN, 135 μmoles of Tris buffer (*p*H 7.35 and 7.7) or 270 μmoles of glycine–sodium hydroxide buffer (*p*H 8.2–10.0), respectively, 8 mg of protein from the soluble liver fraction, and 0.15 μmole of steroid. O—O, pregnan-3α-ol-20-one; ●—●, 3-epidigitoxigenin; □—□, androstan-3α-ol-17-one; ×—×, 3-epi-uzarigenin.

RESULTS

*p*H *Optima.*—The *p*H optimum of the dehydrogenation of 3α-hydroxysteroids was determined using two representatives of each series: C/D *cis-* and C/D *trans-*. With TPN as a cofactor the optimum *p*H of the reaction was at 9.6 regardless of the C/D configuration (Fig. 3). When DPN was used the greatest rate of dehydrogenation was found at *p*H 8.6 (Fig. 4). The C/D configuration again had no influence.

The *p*H optimum for dehydrogenation of 3β-hydroxysteroids was established for two C/D *cis-* and three C/D *trans*-steroids. With DPN as cofactor an optimum of approximately *p*H 10.2 was found in every case (Fig. 5). In certain instances (oxidation of 3-epi-digitoxigenin and pregnan-3α-ol-20-one with TPN or DPN; digitoxigenin and pregnan-3β-ol-20-one with DPN) the *p*H activity curves were repeated after storage of the supernatant at 0° up to 10 days. There was no measurable difference between C/D *cis-* and C/D *trans*-steroids in relation to the position of the *p*H optima and the relative reaction velocity.

Nucleotide Specificity.—As already shown, the supernatant fraction dehydrogenated the 3α-hydroxysteroids in the presence of both TPN and DPN independently of the C/D configuration. The reaction rates were always greater with TPN than with DPN. 3β-Hydroxysteroids, however, were oxidized only with DPN. Androstan-3β-ol-17-one, pregnan-3β-ol-20-one, and digitoxigenin were ineffective as substrates when TPN was cofactor, even though 50–500 μl of the supernatant solution (2–20 mg protein) was used as enzyme source, and *p*H was varied over a range 7.1–10.1. The oxidation of 3β-hydroxysteroids by supernatant, therefore, seems to be DPN-specific not only for C/D *trans-* but also for the C/D *cis*-steroids.

Influence of the A/B Configuration.—The relative metabolic rates of A/B *cis-*, C/D *trans-* and A/B *trans-*, C/D *trans*-steroids under these experimental conditions were also compared with those of A/B *cis-*, C/D *cis-* and A/B *trans-*, C/D *cis*-steroids. Unfortunately it was not possible to carry out this comparison properly in relation to the oxidation of 3α-hydroxysteroids in the presence of TPN and DPN since the groups on C-17 of the only C/D *trans* pair available differed.[4] With DPN 3β-hydroxysteroids

[4] Etiocholan-3α-ol-17-one and allopregnan-3β-ol-20-one were not soluble enough in the test solution and were therefore not used. We were unable to obtain allopregnan-3α-ol-20-one.

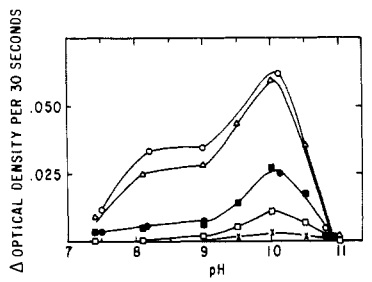

FIG. 5.—The influence of *p*H on the speed of reduction of DPN by 3β-hydroxysteroids and the soluble liver fraction obtained at 105,000 × *g*. The increased optical density is due to production of DPNH (λ = 340 mμ and *d* = 1 cm). The final volume of 3 ml contained a mixture of the following components: 1.5 μmoles of DPN, 143 μmoles of Tris buffer (*p*H 7.5) or 286 μmoles of glycine–sodium hydroxide buffer (*p*H 8.2–11.0), respectively, 2 mg of protein from the soluble liver fraction, and 0.15 μmole of steroids. O—O, pregnan-3β-ol-20-one; ●—● and ■—■, digitoxigenin (different preparations of supernatant); △—△, etiocholan-3β-ol-17-one; □—□, androstan-3β-ol-17-one; ×—×, uzarigenin.

of the A/B *cis* series were always dehydrogenated faster than their A/B *trans* isomers regardless of whether the C/D configuration was *trans*- or *cis*-.

DISCUSSION

As shown in this paper, the supernatant fraction of male rat liver contains enzymatic activities which may dehydrogenate both 3α- and 3β-hydroxysteroids of the different series. The nucleotide specificity, shape of the *p*H curves, and *p*H optima in the presence of a 10-fold excess of cofactor were found to be dependent only on the steric configuration of the hydroxyl group at position 3. The steric conformation of the C/D or A/B ring junctures did not influence these features but did affect the reaction rates. Comparing 3β-hydroxysteroids with the same C/D juncture (*cis* or *trans*), those with A/B *cis* configuration appeared to be dehydrogenated more rapidly with DPN as cofactor than those with A/B *trans* junction. When 3α-hydroxysteroids were dehydrogenated in the presence of either DPN or TPN, the effect of A/B junction did not appear to be consistent; the apparent discrepancies, however, may be explained by the fact that the C/D *trans* pair also differed in respect to the substituent on C-17. A comparison of the steroids with the same A/B juncture (*cis* or *trans*) indicates that a change from C/D *cis* to C/D *trans* configuration accelerated the rate of reaction of 3α- and 3β-hydroxysteroids with DPN as coenzyme but reduced that of 3α-hydroxysteroids in the presence of TPN.

These observations considered together suggest that in the supernatant fraction of rat liver three types of enzyme are present: TPN-dependent 3α-hydroxysteroid dehydrogenases, DPN-dependent 3α-hydroxysteroid dehydrogenases, and DPN-dependent 3β-hydroxysteroid dehydrogenases, which are acting on the 3α- and 3β-hydroxysteroids irrespective of the configuration of the C/D and A/B ring junctions.

The dependence of the effect of stereoisomerism at the C/D ring juncture on the type of enzyme also extends to the A/B configuration. Talalay (1957) found that the DPN-dependent 3α-hydroxysteroid dehydrogenase from *Pseudomonas testosteroni* showed a markedly more rapid rate of reaction with A/B *trans* than A/B *cis* isomers. A corresponding order of reaction velocities was found for the DPN-dependent 3β-hydroxysteroid dehydrogenase from the same source. On the contrary, Tomkins (1956) observed that the DPN-dependent 3α-hydroxysteroid dehydrogenase of rat liver reduced 3-ketosteroids of the A/B *cis* series more rapidly than the *trans* isomers, and the DPN-dependent 3β-hydroxysteroid dehydrogenase has been found to oxidize A/B *cis*-faster than A/B *trans*-steroids under the conditions used in these studies. The rate of reaction is apparently dependent on the relation of the rings in the steroid molecule, but the structure of the enzyme protein determines the direction of this influence. The same is true for the axial and equatorial positions of the hydroxyl group.

Certain results of the present work do not support the hypothesis that the soluble 3α-hydroxysteroid dehydrogenases of rat liver have a double pyridine nucleotide specificity (Tomkins, 1956; Hurlock and Talalay, 1958). First, the relative speeds of reaction with C/D *trans*-steroids versus those with C/D *cis* isomers are reversed by change of the pyridine nucleotides (Figs. 3 and 4). In addition, the *p*H optima were definitely different. The *p*H optimum for the DPN reduction by 3α-hydroxysteroids, approximately 8.6, was in agreement with Tomkins (1956); for the TPN reduction it was at 9.6. This difference in *p*H optima cannot, on the basis of present information, be attributed to an influence of the corresponding coenzymes since *p*H optima over 9.5 were also found for DPN-dependent enzymes such as the estradiol-17β-dehydrogenase from human placenta (Langer and Engel, 1958) and the testosterone dehydrogenase of guinea pig liver (Endahl *et al.*, 1960). It is also unlikely that in the presence of a large excess of cofactor the interaction of the cofactor with the enzyme would be limiting.

The present evidence, although not conclusive, favors the hypothesis that the dehydrogenation of 3α-hydroxysteroids with TPN and DPN is caused by distinct enzymes with different cofactor specificity.

ACKNOWLEDGMENTS

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Enzymatic Basis for Epimerization of Cardiotonic Steroids at Carbon 3 in Rat Liver*

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The conversion of 3β-hydroxysteroids to their 3α epimers, denoted as epimerization, has been studied using various fractions of liver homogenates from male or female rats. The greatest epimerization capacity was found to reside primarily in the clear supernatant and next in the microsomal fraction. The oxidation rates of a large set of 3-hydroxysteroids by the soluble fraction and DPN or TPN were determined using a spectrophotometric method. Both cardioactive and hormone-type steroids were effectively dehydrogenated to their 3-keto derivatives. Much parallelism between the two steroid types was found in the influence of structure variations on the oxidation velocities. The reduction of 3-ketosteroids of both types by DPNH or TPNH in the presence of the soluble fraction was also followed. The results suggest that the rat liver contains soluble as well as particulate 3β- and 3α-hydroxysteroid dehydrogenases which differ in their dependency upon DPN or TPN and in their relative affinity for DPNH or TPNH. On the assumption that DPN is the common hydrogen acceptor and TPNH is the major hydrogen donor for the interconversions of 3-hydroxy- and 3-ketosteroids in the liver cell, it has been concluded that the epimerization process involves an oxidation of the cardioactive steroids by the DPN-dependent 3β-hydroxysteroid dehydrogenase and an immediately subsequent reduction of the 3-keto derivatives produced by the TPN-dependent 3α-hydroxysteroid dehydrogenases. Both β- and α-enzymes showed certain sex differences in their activities. An explanation for the sex differences in the steric course of the 3-keto-(5α-H-)-steroid reduction has been offered. The biological significance of epimerization is discussed with regard to the mechanism of action of cardioactive steroids and to their detoxification in the animal body.

Cardiotonic steroids, probable intermediary products in the degradation of cardiac glycosides in the animal body (Repke, 1963), are themselves rapidly metabolized. The main locus of the biotransformation of genins is the hydroxy group at carbon 3. In the glycosides this group is involved in the binding of the sugar component to the steroid nucleus and hence becomes freely accessible only after glycoside fission. This hydroxy group is in the β position in nearly all natural representatives, but it is rapidly inverted to the α position in the animal body (Repke and Lauterbach, 1959). This inversion, denoted hereafter as epimerization, has been considered to be a major pathway for the detoxification of genins (Repke and Lauterbach, 1959) and therefore deserved further investigation. The present paper aims at a closer analysis of this reaction.

In addition to the genins, other 3β-hydroxysteroids like Δ^4-pregnen-3β-ol-20-one, Δ^4-androstene-3β,17β-diol (for review see Samuels, 1960), and cholesterol (Samuelsson, 1959) are metabolized by epimerization at C-3. For the sake of comparison, the study of this type of steroids is included in this paper.

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In the course of *in vitro* studies with tissue preparations from the rat, using digitoxigenin as substrate, it has been shown that the epimerization may be effected by isolated erythrocytes (Portius and Repke, 1960), pieces of diaphragm,[1] and slices from cardiac muscle[1] or liver (Lauterbach and Repke, 1960). It is probable that other tissues are also able to bring about the same reaction, but the liver seems to possess by far the highest activity (Repke, 1963). In addition to the rat tissue, liver slices of other animal species will invert the hydroxy group at C-3 from β to α position, the following order of increasing activities having been found: frog, pigeon, toad, guinea pig, cat, rat, mouse, and rabbit (Repke, 1960). Slices of human liver likewise have a distinct capacity for epimerization of digitoxigenin.[2] Because of accessibility and relatively high activity, rat liver was chosen as enzyme source in the present study. Cell fractions instead of slices have been used to analyze the enzymatic basis for epimerization at a subcellular level and to prevent side reactions like conjugation.

Experimental Procedure

Steroids.—Uzarigenin, digitoxigenin, strophanthidin, sarmentogenin, digoxigenin, gitoxigenin, bufalin, scillarenin (Tamm, 1956); 3-dehydrouzarigenin, 3-epiuzarigenin (Kuritzkes *et al.*, 1959); 17β-H-digitoxigenin

[1] F. Lauterbach and K. Repke, unpublished experiments.
[2] I. Herrmann and K. Repke, to be published.