Oxidation of 21-Dehydrocorticosteroids to Steroidal 20-Oxo-21-oic Acids by an Aldehyde Dehydrogenase of Sheep Adrenal*

(Carol Monder) and Ping Tu Wang

From the Research Institute of the Hospital for Joint Diseases and Medical Center New York, New York 10035 and the Mount Sinai School of Medicine, New York, New York 10029

SUMMARY

Sheep adrenal cortex contains an enzyme which catalyzes the oxidation of steroidal 20-oxo-21-al derivatives to 20-oxo-21-oic acids. The enzyme was purified 60- to 10-fold and retained broad specificity for a variety of aliphatic and aromatic aldehydes. The aldehyde dehydrogenase was localized in the cytosol. It was protected from inactivation by NAD+, which it uses as specific coenzyme, but was otherwise unstable. Reagents that react with —SH groups inactivated the enzyme. Molecular weight was estimated as 164,000. The product of 21-dehydrocorticosterone oxidation was 11β-hydroxy-3,20-diketo-4-pregnen-21-oic acid. For each mole of product made or substrate used, 1 mole of NAD+ was reduced.

Studies in a number of laboratories have shown that corticosteroids are oxidized to steroidal carboxylic acids by human and other mammalian tissues in vitro and in vivo. The three classes of acidic steroid which have been isolated after exposure of corticosteroids directly to tissues or to partially purified enzymes are 17β-carboxylic (1-6), 20-hydroxy-21-oic (5, 7), and 20-oxo-21-oic (8) acids. The isolation and characterization of radioactive steroid acids from the urine of patients given tracer doses of corticosteroids has been accomplished (9-12). The extent of conversion of corticosteroids to these various metabolites is quantitatively significant in all cases. A possible pathway leading to some of these end products may involve the following sequence: (R)-20-oxo-21-al + (R)-20-oxo-21-al + (R)-20-oxo-21-oic acid → R-20-oic acid, where (R) represents the fused ring system. This pathway requires that the 21-dehydrocorticosteroids (i.e. steroids containing the 20-oxo-21-al side chain) be metabolic intermediates in the synthesis of the 20-oxo-21-oic acids or the 20-oic acids (5, 13). In liver, the 21-dehydrosteroids are oxidized by an NAD+-dependent enzyme to keto acids (8). A similar enzymic conversion may in part account for the keto acids found in the urine of patients who were given synthetic corticosteroid alcohols (9). Oxidative decarboxylation of the keto acids would lead to 20-oic (etienic) acids, and thus provide an explanation for reports that corticosteroids perfused through liver are converted to 20-carbon acids (2, 5).

A similar enzymic activity has been found in the adrenal gland (1, 3, 4, 14, 15). It was anticipated, therefore, that the adrenal would have enzyme activity corresponding to that found in the liver. In this paper we describe the properties of an enzyme of broad specificity obtained from the cortices of sheep adrenal glands, which catalyzes the oxidation of 21-dehydrocorticosteroids to 20-oxo-21-oic acids.

MATERIALS AND METHODS

Sheep adrenals were obtained from a local slaughterhouse and kept frozen until use. Ammonium sulfate, enzyme grade, was bought from Schwarz-Mann. Sephadex G-25, G-100, G-200, and A-50, purchased from Pharmacia Fine Chemicals, were prepared as recommended by the manufacturer. All water was twice distilled with a Corning all glass still, model AG-2. NADH, NADPH, NAD+, and NADP+ were obtained from P-L Biochemicals. Broken cell suspensions of adrenal cortex were prepared and fractionated as described by Sweat et al. (16) using a Teflon pestle fitted into a smooth wall glass tube. Each fraction was reconstituted to the original volume of homogenate with cold 0.25 M sucrose.

Activity was measured in a system containing 0.75 μmole of NAD+, 0.29 μmole of 21-dehydrocorticosterone, and 0.1 ml of enzyme in 0.2 M Tricine, pH 8.4. It is important that this sequence of adding the components be followed, for in the absence of NAD+, the dilute enzyme was slowly inactivated, and the initial rates were not consistently reproducible. Final vol-

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‡ Recipient of Career Development Award AM06841 from the United States Public Health Service. To whom all inquiries should be addressed.

§ Present address, 211-B Landsdown Street, Blacksburg, Virginia 24060.
the medullary enzyme was 1.0 ml. Rate of reduction of NAD\(^+\) was measured continuously at 340 nm with a Gilford model 2000 multiple absorbance spectrophotometer. Concentrations of other substrates used are indicated under “Results.” Steroids were bought from Steraloids, Inc. The 21-dehydrocorticosteroids were synthesized as described previously (17). Methylglyoxal was purified (18) and the concentration determined by the method of Racker (19). Other aldehydes used in this study were purchased commercially and used without further purification. Disc gel electrophoresis was performed in a Canaco apparatus. The running gel consisted of 1 part of an aqueous mixture of 30% acrylamide and 0.8% bis-acrylamide; 2 parts of a solution of 0.15 ml of tetramethylenediamine in 100 ml of 0.75 M Tris-HCl, pH 8.9; and 1 part of 0.0004% riboflavin. The spacer gel contained 1 part of an aqueous mixture of 16% acrylamide and 1.52% bis-acrylamide; 2 parts of a solution of 20 g of sucrose and 0.05 ml of tetramethylenediamine in 100 ml of 0.01 M Tris-HCl, pH 7.4. The gels were photopolym- erized with fluorescent lamps. A current of 1.25 ma per tube was applied until the bromphenol blue marker had entered the spacer gel. Electrophoresis was performed at 2.5 ma per tube. Protein was stained at the end of the electrophoresis with Coo- massie blue by the procedure of Chrambach et al. (20). Ald- hyde dehydrogenase was located after incubating the gel in 0.1 M Tris, pH 8.3, containing NAD\(^+\) (0.2 mg per ml), nitro blue tetrazolium (0.8 mg per ml), phenazine methosulfate (0.14 mg per ml), and the appropriate aldehyde substrate. Substrate concentrations were methylglyoxal, 12 mM; acetaldehyde, 12 mM; benzaldehyde, 12 mM; 21-dehydrocorticosterone, 5.5 mM. Permanent records of the gels were made by scanning them on a Gilford model 2000 recording spectrophotometer with a gel scanning accessory. Synthesis of 11β-hydroxy-3,20-dioxo-4-pregnene-21-carboxylic acid was performed as described by Monder and Wang (8).

**RESULTS**

Preparation of Enzyme—Sheep adrenals were found to have 5 to 10 times more activity than those of pig, cow, or rat. Initial attempts to purify the enzyme from whole sheep adrenals were frustrated by the irreversible inactivation of some preparations by an unidentified component in the medulla. The inhibitor was not epinephrine. Therefore, before fractionation was started, chilled adrenal glands from sheep (or lambs) were split and the cortical tissue was carefully separated from the medulla and capsule. Subsequent steps in the purification were done at 3°. A combined total of 13 g of cortical tissue was homogenized in 25 ml of 0.1 M sodium phosphate, pH 7.5, containing 0.0025 M EDTA. The suspension was centrifuged at 27,000 \(\times g\) for 30 min. Solid ammonium sulfate was added to 25 ml of the resulting supernatant fluid to a final concentration of 35% of saturation (1.4 M). The preparation was stirred for 15 min, then centrifuged at 10,000 \(\times g\) for 10 min. The precipitate was discarded. Additional ammonium sulfate was slowly added with stirring to the supernatant fluid until 60% of saturation (2.4 M) was reached. Fifteen minutes later, the suspension was centrifuged at 10,000 \(\times g\) for 10 min, and the supernatant layer was discarded. The precipitate was dissolved in 6 ml of 0.1 M sodium phosphate, pH 7.5. The solution was passed through a column (50 x 0.9 cm) of Sephadex G-100 which had previously been equilibrated with 0.2 M Tricine, pH 7.5. Flow rate was 7.5 ml per hour. Each fraction contained 4.5 ml. The enzyme emerged in fractions 12 to 18. Those with the highest specific activities were combined, and an aliquot of 6.3 ml was applied to a column (35 x 0.9 cm) of Sephadex A-50 previously equili- brated with 0.2 M Tricine, pH 7.5. Fractions of 2 ml were collected. Elution was initiated with 0.2 M Tricine, pH 7.5, followed by a linear gradient of sodium chloride in the same buffer. The reservoirs contained 100 ml of buffer, and 100 ml of 0.5 M sodium chloride in buffer, respectively. The fractions of highest specific activity (fractions 27 to 36) were combined. Further attempts at purification with CM-cellulose, DEAE-cellulose, alumina gel CY, calcium phosphate gel, ethanol, or acetone invariably led to losses in total activity and specific activity. The initial ammonium sulfate fractionation removed inhibitory material since the total activity increased 6.5-fold as a result of this step. Specific activity increased 60- to 70-fold during the purification. Activity of the enzyme was also followed with 7.6 mM methylglyoxal. Table I summarizes the purification sequence with both substrates. Methylglyoxal was oxidized about 10 times faster than 21-dehydrocortico sterone, but the purification was similar with respect to both substrates.

**Table II**

| Fraction | Isolation conditions | Total enzyme activity | μmoles/min |
|----------|----------------------|-----------------------|------------|
| Whole homogenate | 700 \(\times g\), 15 min | 0.524 | 0 |
| Nuclear and “debris” | 14,500 \(\times g\), 60 min | 0.012 | 0 |
| Mitochondrial | 105,000 \(\times g\), 60 min | 0.402 | 0 |
FIG. 1. Heat inactivation of aldehyde dehydrogenase. One milliliter of substrate and 1 ml of enzyme (120 µg of protein) were incubated in a water bath at the indicated temperatures. Samples were removed at intervals for estimation of activity. Rates of inactivation in the presence of 21-dehydrocorticosterone (3 × 10^{-3} M) dissolved in 10% (final concentration) of propylene glycol (○—○) were compared with controls containing 10% propylene glycol (□—□). Rates of inactivation with NAD⁺ (1.5 × 10^{-3} M; △—△) were compared with controls containing water.

to 0.005 M EDTA, 0.001 M β-mercaptoethanol, 0.002 M glutathione, 20% glycerol, or suspension in 3.0 mM ammonium sulfate. A solution of 0.005% metal-free gelatin stabilized enzyme activity for at least 3 days, but the solutions were viscous and difficult to pipette. For this reason and because gelatin represented a source of additional protein contamination, this method of preserving the dehydrogenase was discarded. Enzyme was always prepared fresh and used as quickly as possible.

FIG. 2. pH activity curves of adrenal aldehyde dehydrogenase. System contained 140 µmoles of buffer, 0.75 µmole of NAD⁺, 0.29 µmole of 21-dehydrocorticosterone, and 11 µg of enzyme protein in a final volume of 1.0 ml at 30°C. The pH of each mixture was determined immediately after measuring the initial reaction rate at 340 nm. □—□, Tricine; ○—○, carbonate; ×—×, barbital; △—△, borate. Control mixtures contained no enzyme.

FIG. 3. Dependence of rate on enzyme concentration. The assay system contained the indicated quantity of enzyme solution (5 µg of protein per 0.1 ml) in 0.2 M Tricine, pH 8.4, 0.75 µmole of NAD⁺, and either 0.75 µmole of 21-dehydrocorticosterone (○—○) or 0.75 µmole of methylglyoxal (●—●) in a final volume of 1.0 ml.

Effect of Enzyme Concentration—Fig. 3 shows that the rate of oxidation of either methylglyoxal or 21-dehydrocorticosterone was proportional to enzyme concentration. The lack of curvature indicated that the purified preparations contained no detectable reversible inhibitors or activators.

Cofactor Specificity—With 21-dehydrocortisol or methylglyoxal as substrate, NAD⁺ was totally inactive up to a concentration of 0.26 µmole per ml final concentration. The NAD⁺ had no effect on the reduction of NAD⁺.

Other Enzymes of Steroid Metabolism—No 21-hydroxysteroid dehydrogenase activity was detected in purified enzyme preparations. Also absent were 20α- and 20β-hydroxysteroid dehydro-
genases, 11β-hydroxysteroid dehydrogenase, 5-reductase, and 3-ol-dehydrogenase.

Substrate Specificity—Table III shows that steroidal keto aldehydes were readily oxidized by the enzyme. The corresponding steroidal ketols were found to be totally inactive. Under the conditions used, a 5-fold difference in rate was seen between the most active (21-dehydrocorticosterone) and least active (3β-hydroxy-20-oxo-5-pregnene-21-ol). No correlations could be made between the oxidation rates and the steroid structures.

Km Values—the apparent Km values for several substrates were determined at an NAD+ concentration of 0.75 mM. The double reciprocal plots did not deviate from linearity (Fig. 4).

Relative rates of oxidation for the various substrates studied did not change significantly during enzyme purification, although the Km values for NAD+ of 5.1 ± 1.3 × 10−5 M was obtained.

Relative activity of the enzyme with respect to formaldehyde was determined by gel filtration. The position at which enzyme activity emerged was compared with absorbance peaks at 280 nm of known standard proteins emerging concurrently, according to the method of Andrews (21). From the relationships shown in Fig. 6, the mean molecular weight of the enzyme was estimated to be 164,000 ± 9.500 for three determinations.

Identification of Steroid Product—A mixture containing 5 mg of 21-dehydrocorticosterone and 25 mg of NAD+ in 0.2 M Tri-
cine, pH 9.2, was incubated with 2.5 ml of purified adrenal aldehyde dehydrogenase at 37°C in a volume of 18 ml. Progress of the reaction was followed spectrophotometrically. At intervals, more enzyme or NAD⁺ was added as required to stimulate the reaction. After 24 hours, the pH was adjusted to about 4 with glacial acetic acid and the incubation mixture was extracted with ethyl acetate. The organic extract was washed with water, dried by the addition of anhydrous sodium sulfate, and evaporated to a small volume. The acid was isolated by preparative layer chromatography on silica gel using ethyl acetate-formic acid (99:1, v/v) as the developing solvent. Steroid was extracted from the silica gel with methanol-chloroform (1:1) containing a few drops of glacial acetic acid and dried under a stream of nitrogen. The residue was dissolved in 5% potassium bicarbonate, then acidified with 10% hydrochloric acid. White needles separated out overnight at 3°C. The crystals were washed with dilute acetic acid and dried in vacuo over phosphorus pentoxide. From 10 mg of 21-dehydrocorticosterone, a total of 6 mg of crystalline product was obtained. A control incubation containing no NAD⁺ yielded no acid product. Mobility of the product on thin layer plates of silica gel corresponded with that of authentic 11β-hydroxy-3,20-diketo-4-pregnen-21-one acid when chromatographed in water-saturated ethyl acetate-formic acid (99:1) ($R_f = 0.32$), and water-saturated ethyl acetate ($R_f = 0.00$). The melting point of the authentic steroid acid was 212-216°C and this was not depressed by the enzymically prepared steroid. Both enzymatically and chemically synthesized steroids had identical infrared spectra (Fig. 7). In sulfuric acid, maxima at 412, 370, 365 nm, shoulder at 420 nm, and minimum at 382 nm, corresponded to the spectrum of authentic steroid.

$$C_{18}H_{18}O_6$$

Calculated: C 69.9, H 7.6

Found: C 69.7, H 8.0

The free steroid acid was esterified by reaction with diazomethane. Mobility of the derivative on thin layer plates was identical with that of 11β-hydroxy-3,20-diketo-4-pregnen-21-one acid methyl ester in carbon tetrachloride-ethanol (19:1), $R_f = 0.29$; benzene-ethanol (9:1) $R_f = 0.37$; chloroform-ethanol (19:1) $R_f = 0.95$. In these systems the free acid remained at the origin. Conversion to ester was quantitative. The infrared

![Absorbance profiles of aldehyde dehydrogenase subjected to disc gel electrophoresis.](image)

**Fig. 5.** Absorbance profiles of aldehyde dehydrogenase subjected to disc gel electrophoresis. 1, acetaldehyde, 12 mM; 2, benzaldehyde, 12 mM; 3, methylglyoxal, 12 mM; 4, 21-dehydrocorticosterone, 5.5 mM; 5, dimethylsulfoxide, 5% aqueous; 6, water blank; $P$, protein, stained with Coomassie blue. All substrates were dissolved in 5% aqueous dimethylsulfoxide (final concentration). Electrophoresis, staining, and scanning were performed as described in the text.

**Table IV**

| Substrate                                | Concentration | Relative activity at stage of enzyme purification (acetaldehyde = 100) |
|------------------------------------------|---------------|------------------------------------------------------------------|
| 21-Dehydrocorticosterone                | 0.3           | 9.3                                                              |
| Benzaldehyde                             | 0.3           | 42                                                               |
| Acetaldehyde                             | 5             | 100                                                              |
| Methylglyoxal                            | 5             | 100                                                              |
| Formaldehyde                             | 5             | 3.6                                                              |
| Glyoxylic acid                           | 7             | 0                                                                |
| o-Aminobenzaldehyde                      | 2.5           | 0                                                                |

**Fig. 6.** Estimation of molecular weight of aldehyde dehydrogenase by gel filtration on Sephadex G-200. ▲, cytochrome c; ○, ovalbumin; X, bovine serum albumin; ▼, catalase. Values for individual determinations of standard proteins indicated by dots. Range for individual determinations of adrenal aldehyde dehydrogenase indicated by limit lines.
spectra of the methyl esters of the enzymically and chemically prepared steroids were identical.

**Stoichiometry**—In order to establish the quantitative relationships between the components of the reaction, a complete system containing 2.9 μmoles (2.0 × 10^5 cpm) of 21-dehydro-[4-14C]-corticosterone, 1.5 μmoles of NAD^+, 0.2 m Tricine, pH 9.5, and enzyme in a volume of 3.0 ml was prepared. A parallel control contained no NAD^+. At intervals, 0.3 ml of the incubation mixture was pipetted into 0.2 ml of glacial acetic acid. The mixture was diluted to 1.0 ml with water and extracted repeatedly with ethyl acetate to remove all radioactivity from the aqueous phase. The organic layer was dried over sodium sulfate, concentrated, and transferred to thin layer plates. Steroids were resolved with ethyl acetate-formic acid (99:1). The radioactivity on developed plates was measured on a Packard model 7201 radiochromatogram scanner. The incubation was allowed to proceed for 24 hours with periodic additions of enzyme or NAD^+. The final absorbance of the complete system was 1.64, and for the control, 0.083 absorbance units.

Fig. 8 shows the profile of radioactivity during the course of the oxidation. Initially two radioactive peaks were present in incubations performed in Tricine buffer; one of these was highly polar. Re-examination of the 21-dehydrocorticosterone confirmed that the steroid was chromatographically homogeneous and contained no impurities. The polar peak did not appear in carbonate buffer. Tricine was analyzed on an amino acid analyzer and was found to contain 2.1 μmoles of glycine per mmole of buffer. This amount of glycine added to the carbonate buffer containing 21-dehydrocorticosterone resulted in the appearance of the polar material on thin layer plates. This is, therefore, most probably a condensation product of steroid aldehyde and amino acid. As the reaction proceeded, the glycine-bound steroid dissociated. At the end of the incubation, no complex was left. The stoichiometry of the reaction is presented in Table V. It is concluded that all components reacted in an equimolar relationship. No keto acid was formed in the control incubations.

**Alternative Pathways**—In an earlier paper (18) it was shown that sheep liver ketoaldehyde dehydrogenase converts methylglyoxal to pyruvic acid by direct oxidation and not via prior isomerization to lactic acid. The oxidation of 21-dehydrocorticosterone to keto acid could also theoretically occur through a hydroxy acid intermediate. The following observations support a pathway of direct oxidation: (a) oxidation of 21-dehydrocorticosterone to keto acid proceeded with no chromatographic evidence of an intermediate; (b) glutathione did not influence...
the rate of reaction (c) incubation of enzyme, substrate, and glutathione with or without NAD⁺ led to no formation of a hydroxy acid intermediate; (d) incubation of the steroid keto acid, enzyme, and NADH did not result in the formation of a steroid hydroxy acid; (e) neither 11β,20α- nor 11β,20β-dihydroxy-3-keto-4-pregnen-21-one acids were oxidized to the corresponding 20-keto acids in the presence of enzyme and NAD⁺; (f) with these steroid hydroxy acids, rabbit muscle and bovine heart lactate dehydrogenases were completely inactive.

**Reversibility**—Enzyme was incubated with 11β-hydroxy-3,20-diketo-4-pregnen-21-one acid and NADH for periods up to 24 hours over a range of pH values and substrate concentrations. No evidence was found for reversibility of the reaction.

**Inhibition Studies**—Table VI summarizes the effects of a number of reagents on enzyme activity. Compounds that react with enzyme sulfhydryl groups were generally effective inhibitors, although iodoacetate was not. Sodium azide did not inhibit. That inhibition by sodium bisulfite and flavin adenine dinucleotide was due to their combination with the substrate was supported by chromatographic evidence of altered mobility of the steroid in the presence of these compounds.

The effects of a number of metal ions is shown in Table VII. Inhibition by mercuric and silver ions is consistent with requirement of free sulfhydryl groups by the enzyme. Lead was also effective.

The inhibitory effects of arsenite on activity is shown in Table VIII. Arsenite is a reagent which inhibits enzymes whose activity depends on the presence of contiguous sulfhydryl groups (22). Its effects are demonstrated in the presence of exogenous mercaptans. It is concluded, therefore, that the mechanism of action of the aldehyde dehydrogenase requires the presence of two closely spaced sulfhydryl groups.

A number of compounds containing sulfhydryl groups including glutathione, β-mercaptoethanol, α-lipoic acid, cysteine, coenzyme A, and dithiothreitol up to 1 mM had no effect on enzyme activity with 21-dehydrocorticosterone, methylglyoxal, or acetaldheyde as substrates. Glutathione partially overcame the inhibition of the enzyme by p-hydroxymercuribenzoate.

**Discussion**

The enzyme described in this paper was discovered as a result of our attempts to explain how corticosteroids may be oxidized to steroids is hydroxy acids by the adrenal gland (1, 3, 4, 14, 15). It was postulated that the transformations require the oxidation of the ketol side chain through intermediate ketoaldehydes which are subsequently oxidized to acidic metabolites. The properties of the enzyme described here are consistent with such a scheme.

Unlike liver ketoaldehyde dehydrogenase (18), the adrenal enzyme has broad substrate specificity and utilizes both unsubstituted and α-substituted aldehydes.

The enzyme was present as a single peak when examined by gel electrophoresis on polyacrylamide supports. The activity

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**Table V**

**Stoichiometry of oxidation of 21-dehydrocorticosterone**

| Incubation time | Steroid a remaining | Steroid b acid product | NADH c formed | Ratio of product to NADH |
|-----------------|---------------------|------------------------|---------------|-------------------------|
| 0 hr            | 1.11 μmoles         | 0.05 μmoles            | 0.01 μmoles   | 1.27                    |
| 0.5 hr          | 1.11 μmoles         | 0.156 μmoles           | 0.123 μmoles  | 1.37                    |
| 1 hr            | 0.849 μmoles        | 0.261 μmoles           | 0.191 μmoles  | 1.37                    |
| 2 hr            | 0.705 μmoles        | 0.405 μmoles           | 0.303 μmoles  | 1.22                    |
| 3 hr            | 0.557 μmoles        | 0.555 μmoles           | 0.540 μmoles  | 1.02                    |
| 4 hr            | 0.464 μmoles        | 0.656 μmoles           | 0.657 μmoles  | 1.00                    |
| 24 hr           | 0.286 μmoles        | 0.824 μmoles           | 0.754 μmoles  | 1.09                    |

a Sum of free steroid and steroid-glycine adduct.

b Determined from area under curves reproduced in Fig. 8.

c Calculated from absorbance change at 340 nm.

**Table VI**

**Effects of some organic reagents on 21-dehydrocorticosterone oxidation**

| Addition                  | Inhibitor concentration | Inhibition |
|---------------------------|-------------------------|------------|
|                           | X 10⁻⁴                  | %          |
| p-Mercuribenzoate         | 1.24 X 10⁻⁴             | 93         |
| N-Ethylmaleimide          | 1.36 X 10⁻⁴             | 95         |
| Diethylthiocarbamate      | 1.12 X 10⁻⁴             | 79         |
| Iodoacetate               | 1.43 X 10⁻⁴             | 0          |
| Sodium azide              | 3.14 X 10⁻⁴             | 0          |
| Sodium bisulfite          | 3-60 X 10⁻⁴             | 100        |
| Flavin adenine dinucleotide | 3-20 X 10⁻⁴             | 100        |

**Table VII**

**Effect of metal ions on enzyme activity**

| Additions | Concentration (mM) | Inhibition |
|-----------|-------------------|------------|
| None      |                   |            |
| Mercuric chloride | 0.190      | 100        |
| Silver nitrate | 0.257     | 88         |
| Lead acetate | 0.065    | 48         |
| Zinc acetate | 0.089      | 12         |
| Zinc chloride | 0.263     | 8          |
| Magnesium chloride | 0.129 |              |
| Magnesium acetate | 0.099 |            |
| Barium acetate | 0.108     | 0          |
| Nickel chloride | 0.115    | 0          |
| Cobalt chloride | 0.116     | 0          |
| Manganese chloride | 0.105 |            |
| Lithium chloride | 0.268     | 0          |
| Calcium chloride | 0.109     | 0          |
| Aluminum chloride | 0.066   | 0          |

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**Table VIII**

**Effect of sodium arsenite on adrenal aldehyde dehydrogenase**

To the complete system containing 100 μg of enzyme protein, 0.75 μmole of NAD⁺, 0.3 μmole of 21-dehydrocorticosterone or 0.75 μmole of methylglyoxal in 0.2 mL Tricine, pH 8.4, were added sodium arsenite and β-mercaptoethanol to the final concentrations indicated.
profile was the same for all of the substrates examined. Single peaks also emerged after gel filtration and ion exchange chromatography. No evidence was obtained for any other enzymes acting on any substrate. The ratios of activity for several substrates remained constant during purification, and when the enzyme was treated with inhibitors. This broad range of substrate action is similar to that of other aldehyde dehydrogenases (23-26), although the specific substrates oxidized and their relative rates differ. It is not yet possible to draw quantitative conclusions about relative rates of substrate oxidation, since this is probably determined by the fraction of substrate present as the unhydrated free aldehyde. The percentage of hydration differs for each substrate and depends on the nature of the substituent next to the carbonyl (27).

The enzyme was not homogeneous with respect to protein. All attempts at further purification led to loss in activity and substantial decrease in stability. The instability is similar to that of other purified mammalian aldehyde dehydrogenases. The enzyme was stabilized against inactivation by heat or high pH by the addition of NAD+ or aldehyde substrate. Other aldehyde dehydrogenases are similarly protected (28). Protection of enzyme against sulphydryl group inhibitors suggests that active site interaction involves thiol groups (29). The effects of arsenite on activity indicate that the activity or stability of the enzyme requires dithiol groups (22, 30). Glutathione and other compounds containing sulfhydryl groups did not affect enzyme activity, although other aldehyde dehydrogenases were affected (31, 32).

The stoichiometric relationships during the reaction are like that of other aldehyde dehydrogenases (33, 34), as is the irreversibility of the oxidation (24, 35). The enzyme is found in the cytosol, as are other aldehyde dehydrogenases of the same category (28).

The molecular weight of approximately 165,000 is close to that which has been reported for horse liver dehydrogenase (36, 37).

Whether the ability of the dehydrogenase to utilize a steroid substrate is a unique one, or is a general property of aldehyde dehydrogenases, will require further investigation. The ability of the enzyme to oxidize steroid aldehydes may be physiologically significant. This reaction occurs in an organ whose unique ability is directed to the synthesis and metabolic alteration of corticosteroids. The transformation described in this paper supports the pathway which has been proposed for the biosynthesis of known steroidal carboxylic acids.

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