[7] Wilkins, M. H. F., *Cold Spring Harbor Symp. Quant. Biol.*, **21**, 75 (1956).
[8] Schwartz, D., *Nature*, **181**, 769 (1958).
[9] Morgan, R. S., *J. Mol. Biol.*, **2**, 243 (1960).
[10] We had previously confirmed the significance of the 1–4 and certain other intervals by a new method, *variable-span pair-order analysis*, to be described elsewhere. The method is straightforward and does not depend on a prior classification of the amino acids such as employed by Morgan and ourselves.[1]

STEROID HORMONE ACTIVATION OF L-ALANINE OXIDATION CATALYZED BY A SUBUNIT OF CRYSTALLINE GLUTAMIC DEHYDROGENASE

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Glutamic dehydrogenase, obtained in crystalline form by Olson and Anfinsen[1] and Strecker[2] from beef liver, has a molecular weight of 1,000,000. Sedimentation data[1] suggested the possibility that on dilution the molecule disaggregated. Frieden[3, 4] later made the interesting observations that the enzyme dissociates into four subunits (of molecular weight 250,000) in the presence of a number of compounds, including high concentrations of DPNH, which also inhibit the enzymic conversion of glutamate to α-ketoglutarate. The adenosine nucleotides, AMP and ADP, prevent this disaggregation and overcome the DPNH inhibition. On this basis, Frieden[3, 4] proposed a model in which the tetramer is enzymically active while the subunits are inactive in catalyzing the glutamic dehydrogenase reaction.

During a study of the inhibition of this enzyme by various steroid hormones,[5] we found that these compounds also promoted disaggregation of the macromolecule into subunits[6] and that ADP can, again, prevent both inhibition of the reaction and dissociation of the enzyme.

Struck and Sizer[7] had shown that, in addition to glutamate, a number of monocarboxylic L-amino acids would serve as substrates for crystalline glutamic dehydrogenase, although at considerably slower rates. In the present work, we have studied the reversible oxidative deamination of L-alanine to form pyruvate:

$$\text{L-alanine} + \text{D(T)PN} + H_2O \rightleftharpoons \text{pyruvate} + NH_4^+ + \text{D(T)PNH}$$

catalyzed by the crystalline enzyme. The effects of steroids and ADP on this reaction turned out to be the converse of those observed with glutamate oxidation. That is, diethylstilbestrol (DES) and estradiol accelerate the interconversion of pyruvate and L-alanine, while ADP inhibits it. Furthermore, it appears generally true that conditions which promote enzyme dissociation (thereby interfering with the glutamate reaction) favor alanine dehydrogenation. In contrast, alanine dehydrogenase is inhibited, and glutamate oxidation stimulated, under circumstances where the enzyme is associated.

We were thus led to the conclusion that the dissociated enzyme catalyzes the alanine dehydrogenase reaction, while, as previously established, the tetramer is active as glutamic dehydrogenase.

Materials and Methods.—Crystalline beef liver glutamic dehydrogenase, steroid hormones, DES, and nucleotides were obtained from the Sigma Chemical Company. DPN analogs were purchased from the Pabst Laboratories.

Millimolar extinction coefficients for the pyridine nucleotides and their analogs[8] are as follows: DPN, TPN, and deamino* DPN, 6.22 at 340 mμ; thionicotinamide* DPN, 11.3 at 395 mμ; acetyl pyridine* DPN, 9.1 at 363 mμ; 3-pyridine aldehyde* DPN; 9.3 at 359 mμ.

Spectrophotometric assays for the glutamic and alanine dehydrogenase reactions were carried out at room temperature in quartz cuvettes with a 1.0 cm light path. Initial rates for amino acid oxidation were calculated from the change in optical density of the pyridine nucleotides, at 340 mμ, between 15 and 30 seconds. The rate of keto acid reduction was determined in the same way, although this reaction was linear for at least several minutes. Sedimentation studies were performed in the Spinco Model E analytical ultracentrifuge under the conditions described in a previous communication.[6] In experiments where steroid hormones were used, they were added as solutions in ethanol and control rates were obtained with the solvent alone.

Results.—Evidence that the alanine and glutamic dehydrogenase reactions are catalyzed by the same protein: Struck and Sizer,[7] in studying the oxidation of L-leucine by crystalline glutamic dehydrogenase, performed several types of experiments to demonstrate that both glutamate and leucine were attacked by the same protein. With respect to paper curtain electrophoresis, heat inactivation, and differential centrifugation, both activities responded identically. Leucine was oxidized at 1.7 per cent of the rate of glutamate. On these grounds alone, it seemed likely that the alanine and glutamic dehydrogenase activities are also catalyzed by the same protein. Since Struck and Sizer[7] examined only leucine oxidation in detail, it was important to establish this point more definitely. They did state that L-alanine was oxidized at 0.27 per cent of the rate of glutamate.

The enzyme used in our studies was recrystallized several times and found to be homogeneous in the ultracentrifuge. Furthermore, when the protein was chromatographed on DEAE cellulose (Fig. 1), protein concentration and enzyme activities for both reactions followed each other very closely.[9] The absence of contaminating protein in the enzyme preparations was also indicated

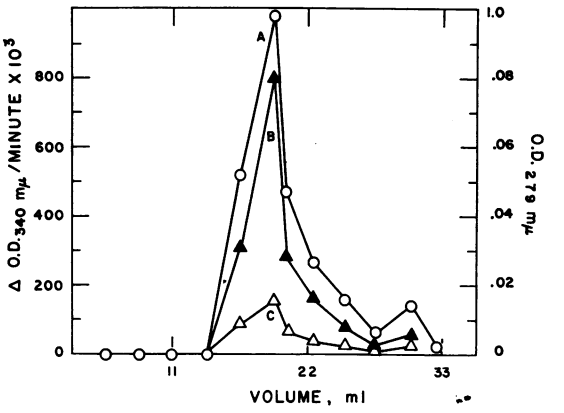

FIG. 1.—Chromatography of glutamic dehydrogenase. 10 mg. of enzyme were dissolved in 10 ml of 0.05 *M* Tris buffer pH 7.4 containing 1×10^{-4} *M* EDTA and applied to a 1 × 9 cm column of diethylaminoethyl cellulose. The column was washed with 50 column volumes of Tris-EDTA before linear gradient elution was begun. The reservoir contained 20 ml of a solution 0.5 *M* in KCl, 1×10^{-4} *M* in EDTA and buffered at pH 7.4 with 0.05 *M* Tris HCl. The mixing flask contained 20 ml of 0.05 *M* Tris buffer with 1×10^{-4} *M* EDTA. The flow rate was adjusted to 1 ml/min.

Pyruvate reduction was assayed in a volume of 1.0 ml containing the following components: sodium pyruvate, 0.01 *M*; ammonium chloride, 0.1 *M*; DPNH, 1.4×10^{-4} *M*; and 0.05 *M* Tris buffer. The reaction was run at pH 8.4 and either column effluent or Tris buffer were added to complete the mixture. The α ketoglutarate reaction mixture was similar except that it was run at pH 8.0.

Pyruvate reduction (Curve B) is expressed as Δ $O.D._{340}$/min/ml of effluent. α Ketoglutarate reduction (Curve C) as Δ $O.D._{340}$/min/μl of effluent. Curve A shows the protein concentration by indicating $O.D._{279}$.

by the fact that the specific activity for the glutamate reaction did not vary significantly from fraction to fraction. The data showed that, under the present conditions, pyruvate was reduced 0.5 per cent as rapidly as α-ketoglutarate.

As another test for the identity of alanine and glutamic dehydrogenase, the enzyme crystals were centrifuged and dissolved in 0.05M Tris buffer, pH 8.0 containing $1 \times 10^{-4}M$ EDTA, so that the protein concentration was 4.0 mg/ml. The enzyme was fractionated with absolute ethanol at $-5°$. The ratio of alanine to glutamic dehydrogenase did not vary significantly in each of the four fractions obtained (Table 1).

TABLE 1

ALCOHOL FRACTIONATION OF GLUTAMIC ACID AND ALANINE DEHYDROGENASE

Fraction	Δ O.D.$_{340}$/min/ml enz		Ratio: glutamate oxidation/ pyruvate reduction
	Glutamate	Pyruvate	
Original	264	5.44	48.6
Ppt. 0–20%	122	2.79	43.7
Ppt. 20–30% ethanol	34.5	0.69	50.0
Supernatant 20–30% ethanol	122	2.5	48.8

The fractions were redissolved in 0.05 M Tris buffer pH 8 containing 1×10^{-4} M EDTA. Glutamate oxidation was measured with the following reaction mixture: glutamate 0.05 M, DPN 1×10^{-4} M, and 0.025 N Tris buffer, pH 8, with 1×10^{-4} M EDTA, in a volume of 1.0 ml. Pyruvate reduction was also measured in a 1.0 ml volume containing 0.025 M Tris buffer pH 8.6, 1×10^{-4} M EDTA, 0.05 M pyruvate 1×10^{-4} M DPNH, and 0.1 M NH_4Cl.

TABLE 2

INACTIVATION OF ALANINE AND GLUTAMIC DEHYDROGENASES BY HEAT

Time (minutes)	Per Cent of Original Activity	
	α-ketoglutarate	Pyruvate
0	100	100
10	78.2	83.5
20	62.5	57.5
30	47.0	51.5
40	34.4	36.4
50	20.3	22.8
60	3.1	3.2

A solution of enzyme in 0.05 M Tris buffer pH 7.2 with a protein concentration of 4.0 mg/ml was heated at 48° for the times indicated. Pyruvate reduction was assayed as described in Figure 2 except the pH was 8.4 and ethanol and DES were omitted. α-Ketoglutarate reduction was assayed in a 1.0 ml mixture containing Tris buffer, pH 8, 0.025 M, EDTA, 1×10^{-4} M, NH_4Cl, 0.1 M, α-ketoglutarate, 0.02 M. Appropriate enzyme dilutions were used so that the Δ O.D.$_{340}$ remained about 0.200/min for each substrate.

In heat inactivation experiments, carried out at 48°, both activities declined at exactly the same rate so that the ratio of one activity to the other was constant at the intervals examined (Table 2).

On the basis of all these criteria, it was concluded that both the alanine and glutamic dehydrogenase reactions were catalyzed by the same protein molecule.

Characteristics of the alanine dehydrogenase reaction: When L-alanine was incubated with the beef liver enzyme, and DPN (or TPN), there was prompt reduction of the pyridine nucleotide. By contrast, D-alanine was not oxidized. The product of L-alanine oxidation was pyruvic acid as shown by the rapid fall in optical density, at 340 mμ, which occurred on addition of crystalline lactic dehydrogenase to the reaction mixture. Moreover, pyruvic acid caused the oxidation of either DPNH or TPNH, catalyzed by glutamic dehydrogenase, only in the presence of ammonia. Therefore, the alanine-pyruvate interconversion is analogous to the glutamate α-ketoglutarate reaction.

There was a linear dependence of the rate of both pyruvate amination and L-alanine oxidation on protein concentration over the range used in the kinetic experiments.

TABLE 3

KINETIC CONSTANTS FOR THE ALANINE AND GLUTAMIC DEHYDROGENASE REACTIONS

Compound	Alanine dehydrogenase K_m (M)	Glutamate dehydrogenase (10)
Alanine	3.6×10^{-2}	—
Pyruvate	3.3×10^{-3}	—
Glutamate	—	1.1×10^{-3}
α-ketoglutarate	—	0.7×10^{-3}
DPN	3.6×10^{-4}	1.01×10^{-4}
TPN	6.6×10^{-4}	5.7×10^{-5}
DPNH	8.4×10^{-5}	9.6×10^{-5}
NH_3	0.125	0.056

Alanine oxidation was measured in a volume of 1.0 ml containing 1.0 mg of enzyme and buffered with Tris EDTA, 0.025 M, pH 8.4. The reactants were varied to obtain the appropriate data. When fixed, the alanine concentration was 2.5×10^{-2} M and DPN was 2.8×10^{-4} M. Pyruvate reduction was assayed at pH 8.0, as described in Figure 2 except for variation in reactant concentration necessary to compute the constants, and ethanol and DES were omitted.

Table 3 gives kinetic constants for the participants in the pyruvate-alanine reaction which were obtained by conventional reciprocal plots (10) of reaction rate as a function of substrate concentration. The data depicted in this way were linear over the concentration ranges examined except for the case of the oxidized pyridine nucleotides, which showed substrate inhibition at higher concentrations (see below). For comparison, the data of Olson and Anfinsen for the glutamate reaction are also shown.[11]

Evidence that the subunit catalyzes the alanine dehydrogenase reaction: DES is a powerful inhibitor of the glutamic dehydrogenase reactions[5] as a result of its ability to promote the disaggregation of the protein molecule into four enzymically inactive subunits.[6] Figure 2 shows that increasing the concentration of DES stimulates the rate of the reductive amination of pyruvate catalyzed by this enzyme so that at $4 \times 10^{-5}M$, the hormone analog produces a doubling of the rate. DES stimulates alanine oxidation as well. Estradiol and progesterone, which can also promote the dissociation of the enzyme,[6] likewise stimulate alanine dehydrogenase, but cortisone, which is not effective in dissociating the enzyme, does not stimulate the alanine reaction. Therefore, the ability of the steroids to produce protein disaggregation is correlated both with their capacity to enhance the interconversion of alanine and pyruvate or inhibit the glutamate reaction.[6] These observations suggest that the alanine reaction is catalyzed, primarily, by the maximally dissociated form of the enzyme, while the tetramer is much less active in this regard.

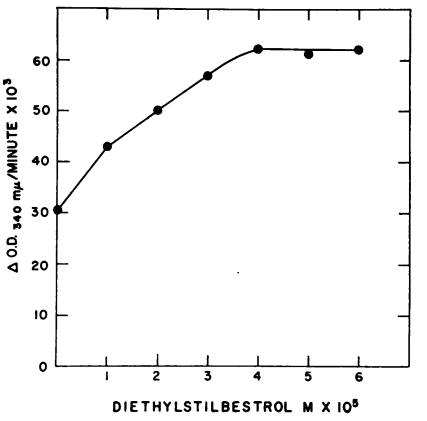

FIG. 2.—Influence of diethylstilbestrol on pyruvate reduction. The reaction mixture contained 0.05 M sodium pyruvate, 0.1 M NH_4Cl, 5×10^{-5} M DPNH, 0.025 M Tris buffer pH 8.0, and 1×10^{-4} M EDTA, in a volume of 1.0 ml. Enzyme concentration was 0.2 mg/ml. DES was added in ethanol solution and the final concentration of ethanol was maintained at 1.0%.

To examine this possibility further, we studied the effect of ADP, which, Frieden showed,[4] stimulates glutamic dehydrogenase by favoring aggregation of the protein. Enzyme dissociation and the consequent inhibition of the reaction by either DPNH[4] or steroid[5, 6] are also prevented by ADP. We would predict, therefore, that the alanine dehydrogenase reaction should be inhibited by ADP and that this inhibition ought to be reversed by steroids. The data confirming this prediction are shown in Figures 3 and 4. Figure 3 shows that ADP is a very effective inhibitor of alanine dehydrogenase, causing 72 per cent inhibition at $2.5 \times 10^{-4}M$. Figure 4 shows that DES can overcome the inhibition produced by $10^{-3}M$ ADP. The data are, therefore, consistent with the hypothesis that the glutamic dehydrogenase subunit catalyzes the alanine reaction. The fact that high concentrations of ADP do not completely inhibit the reaction indicates that the associated enzyme probably has some alanine dehydrogenase activity as well. Olson and Anfinsen[1] first observed that DPN, at high concentrations, enhanced the oxidation of glutamate, and Frieden[3] interpreted these findings in terms of the ability of DPN to promote aggregation. It might be expected, therefore, that DPN at high concentrations would inhibit the alanine dehydrogenase reaction. That this is the case is shown in Figure 5. Likewise, TPN, which can also produce enzyme aggregation, inhibits the reaction at higher concentrations.

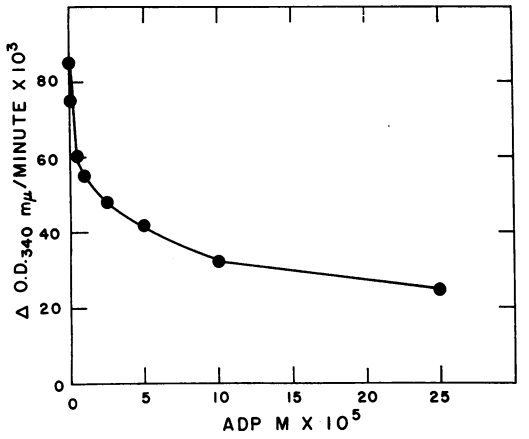

FIG. 3.—Effect of ADP on pyruvate reduction. Reaction conditions were the same as in Figure 2 except for the absence of DES and ethanol and the addition, as noted, of ADP.

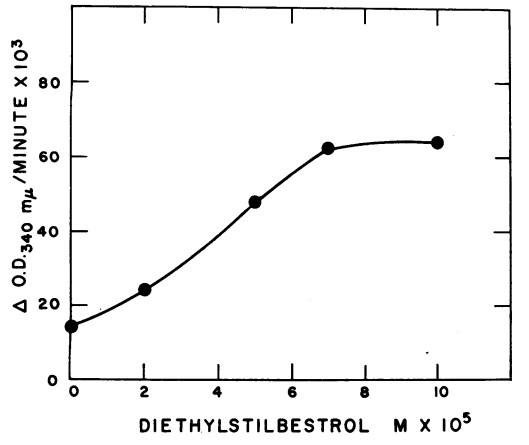

FIG. 4.—Reversal of ADP inhibition of pyruvate reduction by diethylstilbestrol. Reaction conditions as in Figure 2 except ADP, $10^{-3}M$ was present in all mixtures.

Additional experiments also pointed to the conclusion that the subunit is the active alanine dehydrogenase. Frieden[4] observed that ATP enhanced the ability of DPNH to split the enzyme, thereby inhibiting the glutamic dehydrogenase reaction. We have, accordingly, found the converse to hold for alanine dehydrogenase; namely, in the presence of DPNH, ATP stimulates the alanine reaction (Table 4). It was found[4] that ATP did not affect the glutamate reaction when TPNH, in the same concentration as DPNH, was used, and, as expected, ATP does not enhance the rate of the alanine reaction when run with TPNH[12] (Table 4).

1,10-phenanthroline, which has

been used[4] to dissociate glutamic dehydrogenase and which, accordingly, inhibited the glutamate reaction, should, therefore, stimulate alanine dehydrogenase. Table 5 shows that, at relatively low concentrations, phenanthroline is an effective activator of the initial rate of the alanine reaction, provided the enzyme is not preincubated with it.

The effect of pH on the alanine dehydrogenase reaction was studied. The pH optimum for the amination of pyruvate was about 8.7, about 1 pH unit above that reported for the reduction of α-ketoglutarate.[11] Alanine oxidation also has a higher pH optimum than that of glutamate. Although an analysis of the effect of changing pH on the velocity of enzymic reactions is complicated, it seemed worth while to investigate the effect of pH on the sedimentation characteristics of the protein. Figure 6 shows the behavior of the enzyme in the ultracentrifuge at pH 8 and 9. It can be seen that at the higher value there was a tendency for the

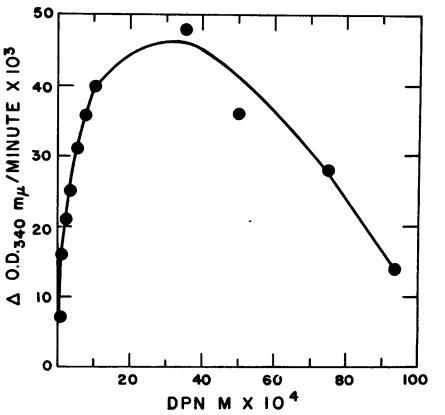

FIG. 5.—Effect of DPN on L-alanine oxidation. The reaction mixture contained: Tris buffer 0.025 M, pH 8.4; L-alanine, 0.025 M; EDTA, 1×10^{-4} M; enzyme, as noted in previous figures, and DPN indicated above.

TABLE 4

EFFECT OF ATP ON PYRUVATE REDUCTION

Reduced Pyridine Nucleotide	ATP	Δ O.D.340/min
DPNH (2×10^{-4} M)	0	0.248
	5×10^{-4} M	0.428
TPNH (2×10^{-4})	0	0.192
	5×10^{-4}	0.192

The reactions were carried out, with additions noted, at pH 8.4, as described in Figure 2 and in the absence of DES and ethanol.

TABLE 5

EFFECT OF 1,10 PHENANTHROLINE ON REDUCTIVE AMINATION OF PYRUVATE

Additive	Δ O.D.340/min × 10³
0	51.5
1,10 phenanthroline 5×10^{-3} M	125.8

Experiments were carried out as in Figure 2. Phenanthroline was dissolved in ethanol and the final concentration of ethanol was 1.0%.

enzyme to disaggregate. Although this finding may not account for the effect of alterations in hydrogen ion concentration on kinetic properties, it is consistent with the other evidence which suggests that the state of aggregation of the molecule determines whether it functions as glutamic or alanine dehydrogenase.

Table 6 summarizes the effects of various experimental conditions on the state of aggregation of the glutamic dehydrogenase molecule and its catalytic behavior as either glutamic or alanine dehydrogenase.

A question of some importance is whether the monomer has some residual glutamic dehydrogenase activity. When the effect of DES on the glutamate reaction was examined closely, it was found that a maximum of 98–99% inhibition can be obtained. On this basis, it seems that the monomer subunit has about 1 per

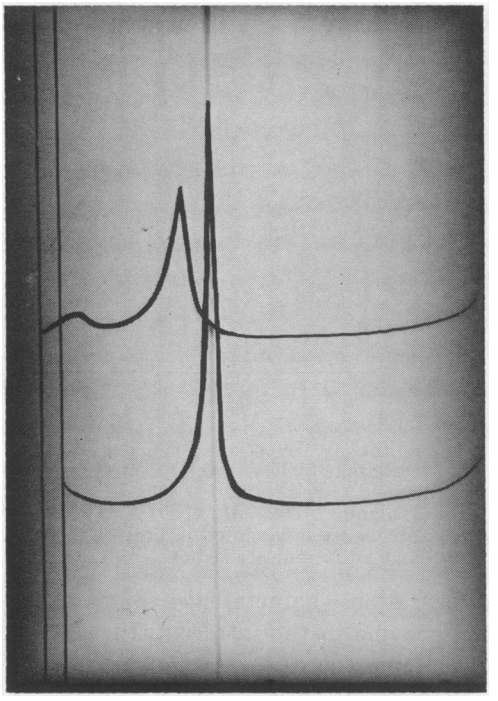

FIG. 6.—Sedimentation of glutamic dehydrogenase. The upper diagram was run at pH 9.0 and the lower at pH 8.0.

cent of the activity of the tetramer in catalyzing the interconversion of glutamate and α-ketoglutarate.

Comparison of active sites of glutamic and alanine dehydrogenase: Because the substrate specificity of the enzyme depends on its state of aggregation, it is of interest to determine whether the glutamate and alanine sites are the same. It is possible that disaggregation either alters glutamate sites or opens up new centers for alanine. Alanine does not inhibit glutamate oxidation. Pyruvate, although somewhat inhibitory for both glutamate oxidation and α-ketoglutarate reduction, is not competitive with these substrates. D-glutamate, which strongly inhibits L-glutamate oxidation, catalyzed by both the tetramer and the monomer, does not affect L-alanine oxidation. It thus appears that the glutamate and alanine sites are different.

The glutamic and alanine dehydrogenase reactions were also examined with several pyridine nucleotide analogs (Table 7). There is a striking difference between the two enzyme activities with respect to these compounds. The values

TABLE 6

CORRELATION OF THE STATE OF AGGREGATION OF CRYSTALLINE GLUTAMIC DEHYDROGENASE AND ITS CATALYTIC PROPERTIES

Experiment	Protein structure	Glutamic dehydrogenase	Alanine dehydrogenase
Estrogenic steroids	Dissociate	Inhibit	Activate
ADP	Associates	Activates	Inhibits
DPN	Associates	Activates	Inhibits
TPN	Associates	Activates	Inhibits
ATP + DPNH	Dissociates	Inhibits	Activates
1,10 phenanthroline	Dissociates	Inhibits	Activates
pH 9	Dissociates	Inhibits	Activates

TABLE 7

COMPARISON OF GLUTAMATE AND ALANINE DEHYDROGENASE ACTIVITIES WITH DPN ANALOGS

Pyridine nucleotide	Reaction: Glutamate	Reaction: Alanine
DPN	1	1
TPN	0.1	0.58
Deamino DPN	0.37	0.68
Acetyl pyridine DPN	0.93	0.20
Pyridine-3-aldehyde DPN	1.50	0.29
Thionicotinamide DPN	0.45	1.90

Alanine oxidation was measured as described in Table 3. DPN and analogs were 1.4×10^{-3} *M*. Glutamate oxidation was measured in 1.0 ml buffer at pH 8.0 with 0.025 *M* Tris-EDTA, containing glutamate 0.025 *M*, 5 μg of protein, and DPN or analogs as above.

in the table are corrected for the differences in extinction coefficients between the analogs and differences in the turnover numbers of the alanine and glutamic dehydrogenase activities so that the rate of amino acid oxidation with DPN is designated as 1. TPN, while less active than DPN in both cases, is much closer to the DPN rate with alanine. The same is true for deamino DPN. The acetyl pyridine and pyridine-3-aldehyde analogs are much less active than DPN with alanine but equal to or better than DPN with glutamate. The thionicotinamide analog is almost twice as active as DPN with alanine but only half as active with glutamate. These results suggest that the active sites for the pyridine nucleotides of glutamic and alanine dehydrogenase are also in some way different.

Discussion.—The data in this paper allow us to conclude that the L-alanine and L-glutamic acid dehydrogenase reactions are catalyzed by different forms of the same protein molecule.

This conclusion is based on the fact that procedures which promote the disaggregation of the enzyme invariably result in loss of glutamic dehydrogenase and concomitant increase in alanine dehydrogenase activity. Presumably, the most active form of alanine dehydrogenase is the subunit of molecular weight 250,000, although there is evidence that the associated molecule also has some activity of this kind. It should also be mentioned that the subunit, although much less active than the tetramer as glutamic dehydrogenase, appears to have some residual glutamate activity.

The existence of enzymically active dimers and trimers as intermediates in the degradation of the tetramer cannot be excluded in this scheme. However, our finding that maximum dissociation of the enzyme favored maximum activity toward alanine suggests that the smallest subunit is the most active alanine dehydrogenase.

Although single enzymes capable of catalyzing several types of reactions (glyceraldehyde-3-phosphate dehydrogenase for example[13]) have been reported, the present case is somewhat different because the state of aggregation of the enzyme determines its substrate specificity. Since steroids regulate this aggregation, this system may provide a mechanism for hormonal control of metabolic processes. In essence, low concentrations of some hormones convert glutamic dehydrogenase to alanine dehydrogenase. This molecular alteration might produce changes in amino acid or protein metabolism. Since many other enzymes have been shown to consist of subunits, it seems quite likely that other examples of this phenomenon will be discovered.

The present findings also provide a basis for speculations on how new biochemical characteristics might evolve, since a mutation could allow subunits with one function to polymerize and produce a qualitatively different molecule.

Summary.—The ability of crystalline glutamic dehydrogenase of beef liver to catalyze the reversible oxidative deamination of L-alanine has been studied.

Certain steroid hormones and diethylstilbestrol, 1,10-phenanthroline, and high pH, all of which promote disaggregation of the protein into subunits, stimulate the alanine and inhibit the glutamic dehydrogenase reactions.

ADP, DPN, and TPN, which cause association of the enzyme, inhibit alanine and stimulate glutamic dehydrogenase.

It appears that the associated form of the enzyme (molecular weight 1,000,000)

is principally concerned with the glutamic dehydrogenase reaction. A subunit catalyzes primarily the alanine-pyruvate interconversion.

Some characteristics of the alanine dehydrogenase reaction were studied.

These findings suggest a means by which steroid hormones by changing the physical properties of an enzyme can alter both its kinetic properties and its substrate specificity.

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[8] These values were obtained from Pabst Circular OR-10.

[9] In the experiment shown in Figure 1, there was a small protein peak which was eluted after the main component. This did not appear in other experiments and probably does not indicate heterogeneity of the crystalline enzyme.

[10] Lineweaver, H., and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).

[11] Olson, J. A., and C. B. Anfinsen, *J. Biol. Chem.*, **202**, 841 (1953).

[12] We have, however, observed that TPNH, as well as DPNH, can promote disaggregation of the enzyme. The higher concentration of TPNH required, 1.0×10^{-3} *M*, may account for Frieden's report[3] that only DPNH can split the protein.

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*INHIBITION OF VIRAL RNA SYNTHESIS BY PARAFLUOROPHENYLALANINE**

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Protein synthesis is required to initiate the synthesis of deoxyribonucleic acid (DNA) of bacteriophages in infected host cells, but not to continue it.[1-5] The demonstration of phage specific DNA in these experiments was facilitated by the fact that the DNA of T-even bacteriophages is characterized by the presence of 5-hydroxymethylcytosine (HMC) in place of cytosine.[6]

In order to study the possible relationship between protein synthesis and the formation of viral ribonucleic acid (RNA) in infected animal cells, it is essential to identify the synthesized viral RNA. At present, there are no chemical markers available like HMC in phage DNA; however, the isolation of viral RNA in an infectious form makes it possible to characterize this material by means of its biological activity, which implies specific chemical and physical properties as well (cf. ref. 8).

An infectious RNA preparation can be obtained from tissue culture cells of