

the second r.f. field,  $H_2$ , are shown in Fig. 1, C and D. In Fig. 1, C, line d decreases in intensity while the remaining lines are essentially unchanged. When  $H_2$  is increased, line d almost disappears while line c splits into a broad doublet. The double resonance spectra obtained by irradiation of line i are shown in Fig. 1, E and F. The behavior of these spectra is complementary to those for irradiation of line h.

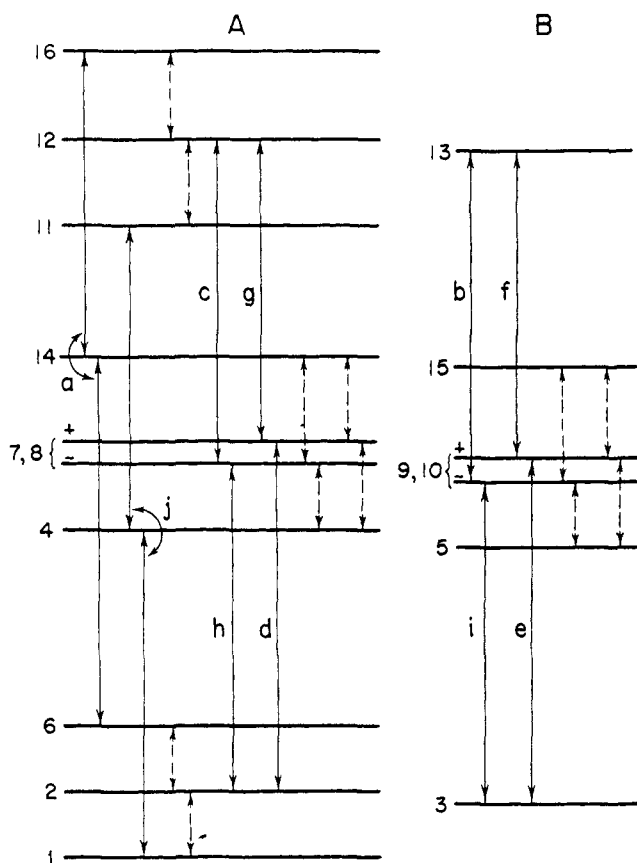


Fig. 2.—Schematic energy level diagram for an AA'XX' system of spins  $1/2$ .

A schematic energy level diagram for an AA'XX' system, assuming K, L, M and N positive, is shown in Fig. 2. The diagram is separated into states belonging to the  $A_1$  and  $B_1$  representations of the symmetry group  $C_{2v}$ . The transitions indicated by solid lines represent proton single resonance transitions and are labeled a through j in correspondence with Fig. 1, A. Fluorine single resonance transitions are indicated by dashed lines. Since transitions a and j have no energy levels in common with proton transitions of different frequencies, an Overhauser effect on the remaining proton lines when line a or j is irradiated can occur only if the fluorine relaxation times are much shorter than the proton relaxation times. If line a is irradiated, the population of level 6 will be decreased and that of level 16 will be increased. Fluorine relaxations can restore the Boltzmann distribution among levels 1, 2 and 6, and independently among levels 11, 12 and 16. Thus the populations of levels 1, 2 and 6 will be decreased, while the populations of levels 11, 12 and 16 are increased and transitions c, g, h, d and j will all be decreased in intensity as in Fig. 1, B. Since transition a belongs to the  $A_1$  symmetry representation, this result unambiguously identifies the other transitions belonging to the  $A_1$  representation. The assignment of the lines to symmetry representations enables the parameters K and M to be distinguished, and shows that  $J_{HH}$  and  $J_{FF}$  are opposite in sign.<sup>9</sup>

Spectra C-F of Fig. 1 are consistent with the above assignment. For these experiments some of the pertinent transitions have energy levels in common. Thus, in the estimation of intensities from peak heights, perturbations of individual spin systems must be considered.<sup>7</sup> For instance, when line h is irradiated, the intensity of line c should increase, but for sufficiently large  $H_2$  it splits into a broad doublet. Thus, while the intensity may be increased by the Overhauser effect, the relation of peak height to intensity is obscured by the change in line shape. On the other hand, line d should decrease in intensity when line h is irradiated, and for larger  $H_2$ , line d should be split into a sharp doublet. In this case the Overhauser effect is clear. The interpretation of spectra E and F of Fig. 1 is entirely complementary to that of spectra C and D.

From the qualitative interpretation of these experiments it is possible to obtain the relative sign of two coupling constants and information on the relative fluorine and proton relaxation times. It is apparent from these spectra that even in the liquid phase there is no important relaxation mechanism connecting states belonging to different symmetry representations.<sup>11</sup>

The sample of *cis*-1,2-difluoroethylene was kindly supplied by Professor Norman C. Craig of Oberlin College. The financial support of the National Science Foundation and an Alfred P. Sloan Grant to J. D. B. are gratefully acknowledged.

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IDENTITY OF SYNTHETIC N<sup>6</sup>-β-GLYCERYLLYSINE AND THE C<sup>14</sup>-LABELED AMINO ACID OBTAINED ON SODIUM BOROHYDRIDE REDUCTION AND HYDROLYSIS OF A COMPLEX FROM C<sup>14</sup>-FRUCTOSE 6-PHOSPHATE-TRANSALDOLASE INTERACTION<sup>1</sup>

Sir:

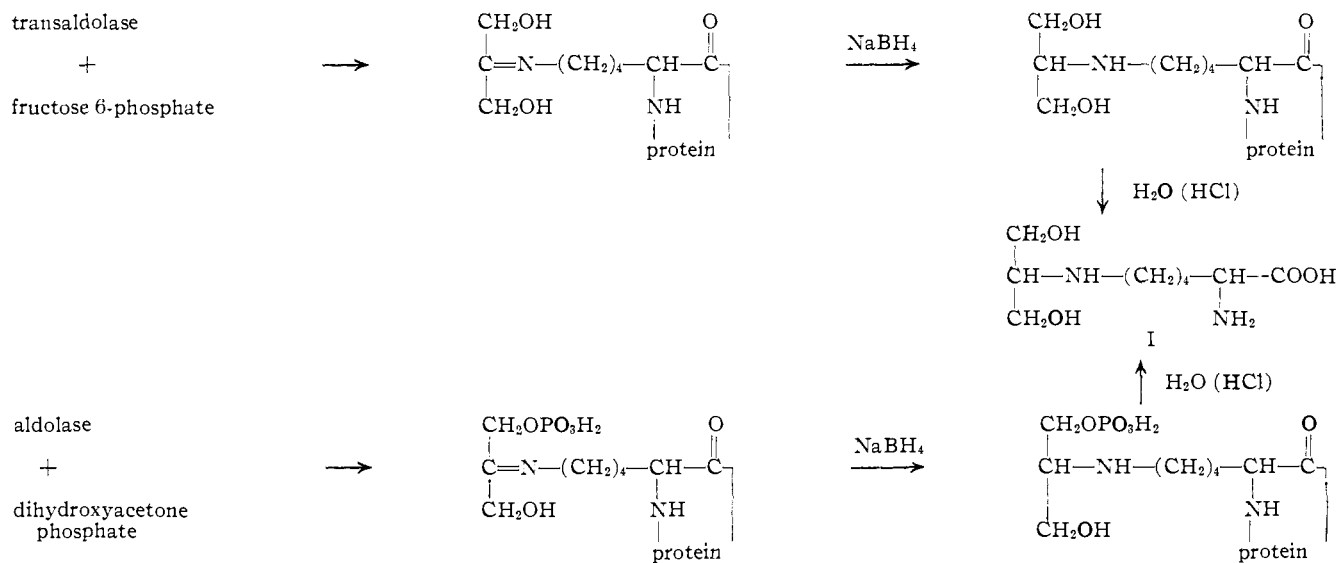
The results of recent investigations of the interaction of rabbit skeletal muscle aldolase and yeast (*Candida utilis*) transaldolase with dihydroxyacetone phosphate and fructose 6-phosphate, respectively, have indicated that certain intermediates in these reactions are Schiff bases—formed in each instance by reaction of an ε-amino group of a lysine residue of the enzyme and the carbonyl function of the substrate.<sup>2-4</sup> Thus, for example, sodium borohydride reduction of a protein complex from the reaction of C<sup>14</sup>-fructose 6-phosphate with the yeast transaldolase, followed by complete acid hydrolysis of the resulting modified protein, gave a radioactive amino acid which was identical in two-dimensional chromatography with the labeled amino acid obtained by the same treatment of a C<sup>14</sup>-dihydroxyacetone phosphate aldolase complex.<sup>2,3</sup> Periodate oxidation of this C<sup>14</sup>-amino acid gave C<sup>14</sup>-formaldehyde and a non-radioactive amino acid which was indistinguishable from lysine on chromatography, electrophoresis, or bioassay with a lysineless mutant of *Escherichia coli*.<sup>4</sup> The behavior of the radioactive amino acid toward periodate was that expected of N<sup>6</sup>-β-glyceryllysine (I) (N<sup>2</sup>-β-glyceryllysine was ruled out as a possibility because neither the transaldolase nor the aldolase appears to have an N-terminal lysine residue), and the reactions indicated in the following scheme were suggested for the formation of this substance.

(1) Supported by grants-in-aid from the National Institutes of Health.

(2) B. L. Horecker, S. Pontremoli, C. Ricci and T. Cheng, *Proc. Natl. Acad. Sci. U. S. A.*, **47**, 1942 (1961).

(3) E. Grazi, T. Cheng and B. L. Horecker, *Biochim. Biophys. Res. Commun.*, **7**, 250 (1962).

(4) E. Grazi, P. T. Rowley, R. Cheng, O. Tchola and B. L. Horecker, *ibid.*, **9**, 38 (1962).



We wish now to report the synthesis of  $\text{N}^6$ - $\beta$ -glyceryl-D,L-lysine and the results of comparison of this synthetic substance with the radioactive amino acid obtained on acid hydrolysis of the sodium borohydride-reduced complex from the  $\text{C}^{14}$ -fructose 6-phosphate-transaldolase reaction.

Reaction of 5- $\delta$ -bromobutylhydantoin with a tenfold excess of 2-amino-1,3-propanediol for 37 hr. in boiling, absolute ethanol gave 5- $\delta$ -( $\beta$ -glyceryl)-butylhydantoin; this product was separated from unchanged 2-amino-1,3-propanediol on the hydroxide form of Amberlite IRA-400 (Rohm and Haas Co.) and isolated in the form of a heavy sirup by eluting the resin with 1 *M* acetic acid and evaporating this eluate at reduced pressure. Hydrolysis of the crude 5- $\delta$ -( $\beta$ -glyceryl)-butylhydantoin in 2 *M* sodium hydroxide for 17 hr. at 100° under nitrogen gave the  $\text{N}^6$ - $\beta$ -glyceryl-D,L-lysine from which any unhydrolyzed hydantoin was removed by acidifying the mixture to pH 5 and equilibrating it with the hydroxide form of Amberlite IRA-400. After desalting the resulting solution on Amberlite CG-120 by the method of Dreze, *et al.*,<sup>5</sup>  $\text{N}^6$ - $\beta$ -glyceryl-D,L-lysine dihydrochloride was isolated as an exceedingly hygroscopic glass by evaporating the portion of the 4 *M* hydrochloric acid eluate in which it appeared in the desalting operation and drying the residue over barium oxide. Equilibration of an aqueous solution of this dihydrochloride with the hydroxide form of Amberlite IRA-400, followed by elution of the resin with 1 *M* acetic acid and vacuum evaporation of the eluate, gave  $\text{N}^6$ - $\beta$ -glyceryl-D,L-lysine free base in the form of a heavy sirup. Adding ethyl ether to an absolute ethanol solution of this product precipitated it as a colorless solid, m.p. 155–57° (with decomposition). *Anal.* Calcd. for  $\text{C}_9\text{H}_{20}\text{N}_2\text{O}_4$ : C, 49.07; H, 9.11; N, 12.72. Found: C, 48.66; H, 8.97; N, 12.41; ash, 0.61.<sup>6</sup>

As shown in Fig. 1, the migration of synthetic  $\text{N}^6$ - $\beta$ -glyceryl-D,L-lysine during electrophoresis was identical with that of the radioactive amino acid obtained from the  $\text{C}^{14}$ -fructose 6-phosphate-transaldolase complex; further, synthetic  $\text{N}^6$ - $\beta$ -glyceryl-D,L-lysine underwent complete conversion to lysine under the same conditions of periodate treatment that oxidized the radioactive amino acid to lysine. These data provide final proof for the structure of the radioactive amino acid obtained in the experiments with aldolase and transaldolase; thus, these findings are completely in accord

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(6) Microanalysis by Spang Microanalytical Laboratory, Ann Arbor, Mich.

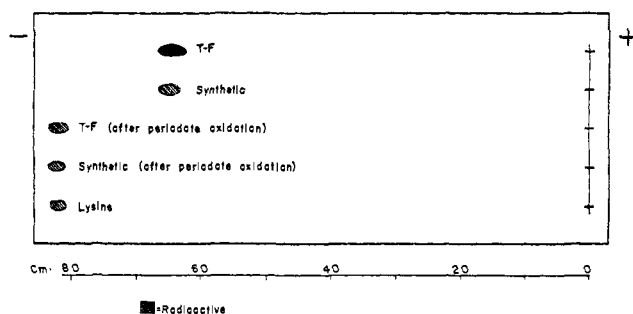


Fig. 1.—Electrophoresis pattern of the radioactive amino acid (T-F) (from the transaldolase-fructose 6-phosphate system) and synthetic  $\text{N}^6$ - $\beta$ -glyceryl-D,L-lysine (synthetic) before and after periodate oxidation. Electrophoresis was carried out in 4% acetic acid, 4% formic acid (pH 1.8) at 5000 v. and at 20–30° for 2 hr. The quantities of synthetic  $\text{N}^6$ - $\beta$ -glyceryllysine and T-F used for electrophoresis were  $3 \times 10^{-8}$  and approximately  $5 \times 10^{-9}$  mole, respectively. The periodate-oxidized samples were equivalent to  $1 \times 10^{-8}$  mole of the synthetic amino acid and approximately  $4 \times 10^{-9}$  mole of T-F;  $1 \times 10^{-8}$  mole of lysine was used for reference.

with the concept of Schiff base intermediates in these reactions and with an earlier hypothesis of operation of immonium ion catalysis (specific amine catalysis) in aldolase-catalyzed reactions.<sup>7</sup>

- (7) J. C. Speck, Jr., and A. A. Forist, *J. Am. Chem. Soc.*, **79**, 4459 (1957).  
 (8) Given Foundation-National Research Fellow in Academic Medicine.

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#### EVIDENCE FOR THE EXISTENCE OF GROUP VI METAL PENTACARBONYLS

Sir:

In a recent communication<sup>1</sup> we presented evidence for the photoproduction of  $\text{W}(\text{CO})_5$ , which is believed<sup>2–4</sup> to be an intermediate in the formation of tungsten carbonyl derivatives through the replacement of one or

(1) I. W. Stolz, G. R. Dobson and R. K. Sheline, *J. Am. Chem. Soc.*, **84**, 3589 (1962).

(2) L. E. Orgel, *Nature*, **191**, 1387 (1961).

(3) G. R. Dobson, M. A. ElSayed, I. W. Stolz and R. K. Sheline, *Inorg. Chem.*, **1**, 526 (1962).

(4) W. Strohmeyer, *Chem. Ber.*, **94**, 3337 (1961).