An enzyme which promotes the degradation of insulin has been isolated from beef liver (2, 3). This enzyme, which has been designated glutathione-insulin transhydrogenase (4), catalyzes the reductive cleavage of the disulfide bonds of insulin by a simple sulfhydryl compound such as glutathione (4, 5). Because the process of degradation may be important in the etiology of diabetes mellitus, experiments were initiated to determine whether or not a similar enzyme exists in human liver. Ensink, Coombs, Williams, and Vallance-Owen (6) have recently reported that B chain formed from insulin by the transhydrogenase binds to serum albumin, and that this bound B chain may be the syndialbumin antagonist which has been proposed by Vallance-Owen et al. (7-9) to be present in excess in plasma of obese diabetics and prediabetes. The results presented in this paper show that the insulin-degrading enzyme, glutathione-insulin transhydrogenase, is present in human liver.

EXPERIMENTAL PROCEDURE AND RESULTS

Crystalline beef insulin and amorphous beef insulin were generous gifts of Dr. W. W. Bromer of the Lilly Research Laboratories. Specially labeled 131I-insulin, List 4750, was purchased from the Abbott Laboratories. This insulin, which is prepared by iodination with I2, was found to be better suited for these studies than the regularly catalogued preparation, List 6797, which although formerly prepared by iodination with I2, is now made by ICI iodination. Glutathione reductase and TPNH were purchased from the Sigma Chemical Company. Protein values were determined by the method of Lowry et al. (10).

Enzyme Purification—This was carried out by the procedure described for isolation of the transhydrogenase from beef liver (2, 3). Frozen liver which contained a large amount of blood was used for early attempts to purify the human enzyme. However, the dark brown acetone-dried powder prepared from such livers proved to be unsatisfactory for this purpose. Therefore, powder was prepared from fresh normal liver after it had been perfused with water to remove blood from the main vessels. The liver was obtained from a normal 23-year-old female who had died instantly of a severe head injury. Autopsy was performed 2 hours after death, and processing of the liver was begun within 18 hours after death. Liver, 1.8 Kg, yielded 236 g of a tan acetone-dried powder. Enzyme activity was followed by use of the 131I-insulin assay (2). In brief, liver protein was incubated for 5 min at 37° with 0.1 mg of amorphous insulin, a tracer amount of 131I-insulin, and 2 pmoles of GSH in 2 ml of 0.1 M potassium phosphate-5 mM EDTA, pH 7.5. The incubation was terminated by the addition of trichloroacetic acid to a final concentration of 5%. A unit of enzyme was defined as the amount which causes 1% of the radioactivity to become soluble in trichloroacetic acid. Specific activity may, therefore, be expressed as the percentage of total radioactivity solubilized in trichloroacetic acid per mg of enzyme protein.

RESULTS

Results of the final step in the purification, starch block electrophoretic separation of the enzyme, are shown in Fig. 1. There was some contamination of the enzyme with protein which had a slightly faster mobility but this contaminant was cut away from the enzyme-containing portion of the block prior to its elution. Table I summarizes the details of the purification. The finding that the human liver enzyme can be purified by essentially the same procedure developed for the isolation of the beef liver GSH-insulin transhydrogenase indicated that the physical properties of the two are very similar.

Identification of Trichloroacetic Acid-soluble Product Formed with Insulin—With beef liver transhydrogenase, the enzymatically produced trichloroacetic acid-soluble peptide from insulin was shown to be the A chain (4, 5). In the current study, the incubation mixture consisted of 10 mg of beef insulin, 15 pmoles of 2-mercaptoethanol (substituted for GSH), and 0.5 mg of human liver enzyme, brought to a volume of 10 ml with 0.1 M potassium phosphate-5 mM EDTA, pH 7.5. The rate of reaction with 15 pmoles of 2-mercaptoethanol is approximately the same as that with 10 pmoles of GSH. Turbidity, an indication of the reductive cleavage of insulin (4, 5), was noted after 10 min of incubation at 37°. After incubation for 1 hour, the reaction was terminated by the addition of an equal volume of 10% trichloroacetic acid.

With the use of the procedures described earlier (5), the resultant trichloroacetic acid-soluble product was purified and desalted with use of Dowex 50-X2 resin, and its NH2-terminal and constituent amino acids were determined. By the 2,4-dinitro-
fluorobenzene procedure, the NH$_2$-terminal amino acid of the product was found to be glycine, which is the NH$_2$-terminal amino acid residue of the A chain of insulin. The product was treated with performic acid, subjected to acid hydrolysis, and its amino acid composition was determined with a Beckman/Spinco amino acid analyzer. The constituent amino acids of the product were the same as those present in the A chain of beef insulin and the number of residues per molecule was comparable (Table II). Amino acids which occur in only the B chain of beef insulin, namely, lysine, histidine, arginine, threonine, proline, and phenylalanine, were not present in measurable amounts in the hydrolysate. These results provided evidence that the human enzyme functions in the same manner as does the beef liver GSH-insulin transhydrogenase.

**Kinetic Studies**—In order to facilitate experiments with the human liver enzyme, an assay was used in which the reaction promoted by the enzyme was coupled with that catalyzed by glutathione reductase. The procedure used was similar to those of Narahara and Williams (11) and Katz and Stetten (4) in which rat or beef liver GSH-insulin transhydrogenase and gluta-

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**TABLE II**

**Amino acid analysis of enzymatically produced peptide**

The values recorded for the enzymatic product were calculated on the basis of 2 aspartic acid residues per mole. Contaminating amino acids, previously observed in the product (5), were present in amounts too small to be measurable. Loss of tyrosine because of its reaction with residual mercaptoethanol was also noted before (5).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Product residue/molecule</th>
<th>A chain residue/molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteic acid</td>
<td>3.7</td>
<td>4</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>2.0</td>
<td>2</td>
</tr>
<tr>
<td>Serine</td>
<td>2.3</td>
<td>2</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>4.0</td>
<td>4</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>Valine</td>
<td>1.4</td>
<td>2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.7</td>
<td>2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.7</td>
<td>2</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Starch block electrophoretic purification of the insulin-degrading enzyme from ethanol-fractionated liver protein. The arrow indicates the point of application of sample. •—•, protein; ○—○, activity.

**Fig. 2.** Cellulose polyacetate electrophoresis of beef liver GSH-insulin transhydrogenase and human liver insulin-degrading enzyme. The buffer was 0.026 M potassium phosphate, pH 8.0. Electrophoresis was carried out at 450 volts for 2½ hours at room temperature. The strips were stained with Buffalo black.

**Fig. 3.** Relationship of concentration of insulin-degrading enzyme to reaction rate. Each cuvette contained 0.07 mM TPNH, 0.14 mM GSH, 0.71 mM insulin, 200 µg of glutathione reductase, and various amounts of the insulin-degrading enzyme in a total volume of 1.4 ml; 0.1 M potassium phosphate, pH 7.5, containing 5 mM EDTA was used to make all solutions.
The capability, even if very much smaller, be capable, in vitro, of destroying about 1000 units (40 mg) of insulin per min. The fact that secreted insulin must first pass through the liver, the reports that 20 to more than 50% of the insulin entering the human liver is removed in a single passage through this organ (13, 14), the observation that liver can rapidly inactivate insulin (15), and the finding that reduced insulin has no insulin-like activity (16) are all in accord with the concept that liver GSH-insulin transhydrogenase provides a physiologically important mechanism for inactivation of the hormone. Further corroboration and extension of the investigations on synalbumin by Vallance-Owen and Ensink cited earlier (see “Introduction”) would strengthen the concept that the transhydrogenase may be of importance in the etiology of diabetes mellitus. Recently, Alp and Recant (17) have reported confirmation of Vallance-Owen’s work on synalbumin.

A group of livers obtained at autopsy from normal and unselected diabetic humans, kept frozen until use, were tested for total insulin-degrading activity. GSH-insulin transhydrogenase itself was not determined since the present isolation procedure yields a variable recovery of enzyme. There was no significant difference between the two groups of livers in the ranges of values for total insulin-degrading activity. If a difference does exist between the concentration of active hepatic GSH-insulin transhydrogenase in normal individuals and certain diabetics, detection of this difference would seem to be dependent upon reduction of several variables by such measures as careful selection of livers, immediate processing, and development of a procedure which will give reproducible recovery of the transhydrogenase free of GSH.

A similarity between the properties of the beef liver GSH-insulin transhydrogenase and enzyme systems investigated by Anfinsen et al. (18, 19) deserves mention. They have described a liver microsomal enzyme system which accelerates the oxidation of reduced lysozyme and ribonuclease to biologically active forms. Katzen, Tietze, and Stetten (20) reported results which suggest that the insulin-transhydrogenase reaction can be reversed to promote the formation of insulin from reduced insulin and GSSG. Both beef enzymes have Svedberg constants of about 3 (2, 4, 19). However, the methods employed for purification of the two beef enzymes were very different, and ribonuclease was not found to be a substrate for GSH-insulin transhydrogenase in normal individuals and certain diabetics.

Michaelis constants for the human liver enzyme and the beef liver transhydrogenase were determined from Lineweaver-Burk plots relating the rate of disappearance of TPNH to changes in the initial concentration of insulin. Fig. 4 shows the Lineweaver-Burk plots for the human liver enzyme and the beef liver transhydrogenase. The values, therefore, are of the same order of magnitude.

**DISCUSSION**

The human liver enzyme is like the beef liver GSH-insulin transhydrogenase in its behavior during purification, its electrophoretic mobility on cellulose acetate, the nature of the reaction it catalyzes, and its kinetic properties. These findings show that the human liver enzyme is a GSH-insulin transhydrogenase.

If it were assumed that this transhydrogenase was the only insulin-degrading activity of human liver, the entire liver would be capable, in vivo, of destroying about 1000 units (40 mg) of insulin per min. The capability, even if very much smaller in vivo because of such factors as restricted cell permeability and limited GSH, is significant since the average daily secretion of insulin in an adult human is only 55 units (12). The fact that secreted insulin must first pass through the liver, the reports that 20 to more than 50% of the insulin entering the human liver is removed in a single passage through this organ (13, 14), the observation that liver can rapidly inactivate insulin (15), and the finding that reduced insulin has no insulin-like activity (16) are all in accord with the concept that liver GSH-insulin transhydrogenase provides a physiologically important mechanism for inactivation of the hormone. Further corroboration and extension of the investigations on synalbumin by Vallance-Owen and Ensink cited earlier (see “Introduction”) would strengthen the concept that the transhydrogenase may be of importance in the etiology of diabetes mellitus. Recently, Alp and Recant (17) have reported confirmation of Vallance-Owen’s work on synalbumin.

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**SUMMARY**

Data are presented which demonstrate the presence of glutathione-insulin transhydrogenase in human liver and some of its properties are described.

**Acknowledgments**—We wish to thank Mrs. Maryan Stanfield, Mrs. Mella Hood, and Miss Carol Colombo for technical assistance. Thanks are due to Drs. J. M. Bloodworth, Jr., and B. J. Katchman for supplying the livers.

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