ISOLATION, PURIFICATION, AND CHARACTERIZATION
OF ALDOLASE FROM HUMAN HEART

APPROVED:

[Signatures]

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Aldolase from human heart has been purified 128-fold to a final specific activity of 11.52 units per mg. The purification procedure employed column chromatography on phosphocellulose. The final purified enzyme appeared to be homogeneous on disc gel electrophoresis, sedimentation velocity ultracentrifugation, and sedimentation equilibrium ultracentrifugation.

The molecular weight of human heart aldolase as determined by gel filtration on Sephadex G-200, sedimentation velocity ultracentrifugation, and high speed sedimentation equilibrium ultracentrifugation was 158,000. The subunit molecular weight, determined by sedimentation equilibrium ultracentrifugation after dissociation in guanidinium chloride and by sodium dodecyl sulfate disc gel electrophoresis, was 40,000. Therefore, the native enzyme is a tetramer.

The physical properties of human heart aldolase were very similar to those of other muscle aldolases. Sedimentation velocity ultracentrifugation yielded a value for $s^{20,w}_{20}$ of $7.79 \times 10^{-13}$ sec. A value for $D^{w}_{20}$ of $4.58 \times 10^{-7}$ cm$^2$ sec$^{-1}$ was obtained by gel filtration.
The partial specific volume of the enzyme calculated from the amino acid composition was 0.73 ml/g. Stokes' radius, determined by gel filtration, was 46 Å.

A comparison of the amino acid analysis of human heart aldolase and other mammalian muscle aldolases yielded minor differences in the content of some residues; however, a basic overall similarity was observed. This was substantiated by tryptic fingerprints of human heart aldolase and rabbit muscle aldolase, which indicated that one-third to one-half of the peptides showed almost identical migration. The migration of the active site peptides in both enzymes was also identical.

Kinetic studies yielded Michaelis constants which were similar to those determined for other muscle aldolases. However, the FDP/FrP ratio was significantly lower than the reported value for most muscle aldolases. This is proposed to be the result of the proteolytic removal of tyrosine from the C-terminal of the enzyme.
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THESIS

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INTRODUCTION

Aldolase catalyses the reversible cleavage of fructose 1,6-diphosphate into two triose phosphates, as illustrated:

\[
\begin{align*}
\text{CH}_2\text{OPO}_3 & \quad \text{C}==\text{O} \\
\text{HO-C}^{-}\text{H} & \quad \text{H-C}==\text{OH} \\
\text{H-C}==\text{OH} & \quad \text{CH}_2\text{OH} \\
\text{CH}_2\text{OPO}_3 & \quad \text{CH}_2\text{OPO}_3 \\
\text{Fructose 1,6-diphosphate} & \quad \text{Dihydroxy-acetone phosphate} \\
\end{align*}
\]

Aldolases are divided into two different classes, based on their requirement for a metal ion and their molecular weight (Rutter, 1964). Class I aldolases have molecular weights of from 120,000 to 160,000 (Fluri et al., 1967, Sia and Horecker, 1968b, Alarcon et al., 1971), and they do not require the presence of a metal ion, since the reaction is carried out through the formation of a Schiff base with a lysine residue in the active site (Horecker et al., 1963, Grazi et al., 1962). Class I aldolases are found in higher plants and animals (Rutter, 1964, Rutter and Groves, 1964). Class I aldolases are further divided into three distinct types, according to the tissue in which they are predominantly found. These types are designated by letter, with
A representing muscle tissue enzyme (Taylor et al., 1948), B representing the enzyme from liver (Rajkumar et al., 1967), and C representing the brain enzyme (Penhoet et al., 1966). These isoenzymes have been demonstrated to be the result of three different genes (Penhoet et al., 1961), and they are distinguished by their electrophoretic mobility and the ratio of the maximum velocity of the enzyme with fructose 1,6-diphosphate (FDP) to the maximum velocity with fructose-1-phosphate (F1P). This ratio is fifty for type A, one for type B, and ten for type C (Penhoet et al., 1966).

Aldolase is present in normal serum, with values of 7.14 units per ml of serum considered to be average. (One unit equals 1 c.mm. of FDP split per gm (wet weight) per hour at 38°; one μmole of FDP equals 22.4 c.mm. of FDP.) Values above 10.5 units per ml are regarded as abnormal (Van Rymenant and Tagnon, 1959). The enzyme in human blood serum is thought to be a typical, Class I, mammalian aldolase. Chelating agents and metal ions have little to no effect on its activity (Foranini et al., 1964). In addition, Michaelis constants, kinetic data, and electrophoretic studies indicate that human serum aldolase is a type A, muscle aldolase (Dikow, 1969).

The enzyme was discovered by Meyerhof and Lohmann in 1934, and it was first obtained in crystalline form by
Warburg and Christian in 1943, who also observed increased serum aldolase levels in tumor-bearing rats. Thus, aldolase was one of the first enzymes demonstrated to be present in serum and elevated in pathological conditions. Sibley and Lehninger (1949) determined that humans with malignancies had significantly increased serum aldolase levels, and it is now known that increased serum aldolase activity accompanies a variety of conditions, including pulmonary infarction, peripheral gangrene, severe pneumonia, hemorrhagic pancreatitis, severe hemolytic anemia (Sibley and Fleisher, 1954), acute hepatitis, and liver necrosis (Bruns and Puls, 1954). In addition, infectious mononucleosis, pericarditis, alcoholic psychosis with delerium tremens (Van Rymenant and Tagnon, 1959), and progressive muscular dystrophy (Neibroj-Dobosz et al., 1970) have all been shown to raise the level of serum aldolase in affected patients. In all cases, the increased serum levels are attributed to release of aldolase from tissue. It has been shown that experimental nerve transection is followed by an increase in serum aldolase in conjunction with a decline in the activity of this enzyme in muscle tissue (Fischer, 1948). The increase in serum aldolase found in patients with progressive muscular dystrophy has also been shown to parallel a decrease of this enzyme in striated muscle tissue (Dreyfus et al., 1954,
Niebroj-Dobosz et al., 1970). The common characteristic in diseases producing a rise in serum aldolase is acute and extensive destruction or necrosis of tissue. This releases the enzyme at a rate faster than it can be removed from the bloodstream (Sibley and Fleisher, 1954). Heart muscle is the second most abundant source of aldolase from human tissues (Agress and Estrin, 1963). Volk and coworkers (1956) determined that hyperaldolasmia accompanied myocardial infarction in dogs, and they attempted to correlate the extent of necrosis and the serum aldolase level. Petersen (1959) applied these experiments to human subjects and found a correlation between severity of infarct and rise in serum aldolase. In spite of these positive results, serum aldolase activity proved to be only a limited diagnostic tool. Although it is highly sensitive, it is relatively nonspecific, since it is affected by a wide variety of diseases and conditions. It is present in erythrocytes, and even slight hemolysis of these red blood cells will introduce significant error in the determination of aldolase activity (Agress and Estrin, 1963, Petersen, 1959).

A myocardial infarction results in considerable disturbance of the carbohydrate metabolism of cardiac tissue (Chazov and Savina, 1958). Under normal conditions, myocardial cell metabolism is almost entirely aerobic.
(Sarnoff et al., 1958), and the enzyme systems involved in the utilization of oxygen and lactate appear to be most sensitive to the effect of anoxia (Wallace, 1959). Adult heart muscle cannot regenerate itself, so that when cells are destroyed by anoxia they can only be replaced by connective tissue (Rabinowitz, 1971). The heart is able to overcome this problem with a highly regulated metabolism which adapts oxidative processes to energy requirements, primarily through respiratory control (Charce et al., 1967). The glycolytic rate is adjusted to the oxidative rate through the allosteric control of three key enzymes, phosphofructokinase, pyruvate kinase, and glyceraldehyde phosphate dehydrogenase (Newsholme and Randle, 1961, Wollenberger and Krause, 1968, Williamson, 1965). Although aldolase has not been shown to be one of the key enzymes in regulation following myocardial infarct, the sensitivity of its rise in response to infarct reveals that it is at least a major indicator of the metabolic damage.

Although serum aldolase and its relation to myocardial infarction have been studied, no work has been done on the heart enzyme, and there are a number of questions such a study should answer. A characterization of the myocardial enzyme and a comparison of the characteristics of the two aldolases would determine if these enzymes are identical, or if modification of heart aldolase occurs either during
myocardial infarction or during the release of the enzyme into the circulation. If such a modification were found, it might help to explain the altered biochemistry of the infarcted heart. The characterization of heart muscle aldolase could also be the basis for a study of the movement of the enzyme through the cellular membranes and its ultimate fate. Aldolase levels do not remain elevated following myocardial infarction. The serum level reaches its peak 24-48 hours after cell death and returns to normal in approximately four days (Volk et al., 1956). The removal of the large amounts of aldolase released in a severe infarct in that amount of time cannot be explained by the three customary routes for elimination of aldolase. These are renal excretion, biliary excretion, and destruction in the liver (Sibley and Fleisher, 1954). A study of normal human heart aldolase would be valuable in a comparative study of the abnormal enzyme from infarcted hearts. A kinetic study including inhibition data, combined with binding constants and substrate availability studies of normal aldolase versus aldolase from infarcted tissue, should help reveal the effect of infarction on the biochemical pathway of glycolysis.

A large number of Americans die each year from heart disease, and a majority of others suffer from arteriosclerosis. In the United States today, the coronary fate
of most men is established by the time they reach their twenties (Hinkle et al., 1968), and afterward, only extensive coronary care, including diet, planned, regular exercise, removal of stress factors, and even climate can alter the preset plan (Frank, 1968). The need for more knowledge concerning the biochemistry of myocardial infarction has prompted this study, a presentation of the characteristics of aldolase from normal human heart. This study will provide a basis for future studies probing myocardial metabolism and the effect of infarction on the carbohydrate metabolism of the human heart.
MATERIALS AND METHODS

Materials

Substrates and Enzymes

The disodium salt of the reduced nicotinamide adenine dinucleotide (NADH), fructose-1-phosphate, and a mixture of α-glycerophosphate dehydrogenase (EC 1.1.1.8, specific activity 100 units/mg), and triose phosphate isomerase (EC 5.3.1.1, specific activity 2,500 units/mg), were obtained from Sigma. Fructose 1,6-diphosphate was from Boehringer-Mannheim. Fructose 1,6-diphosphate-C14 was the product of New England Nuclear. Rabbit muscle aldolase (EC 4.1.2.13, specific activity 18.6 units/mg), was purchased from Calbiochem. TPCK-trypsin was obtained from Worthington Biochemical Corporation, Freehold, N. J.

Materials for Column Chromatography

Cellulose phosphate (coarse, exchange capacity, 0.86 meq/mg) was purchased from Sigma. Chromatography columns, Sephadex G-200, and calibration kit were supplied by Pharmacia, Upsala, Sweden. Tris(hydroxymethyl)aminomethane was supplied by Fisher.
Others

Ethylenediamine tetraacetate (EDTA) was the product of J. T. Baker Chemical Co., guanidinium chloride and sodium dodecyl sulfate (SDS), both "Sequanal grade", and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from Pierce Chemical Company. Crystalline bovine serum albumin, phenazine methosulfate, MTT tetrazolium [3(4,5-dimethyl thiazolyl-2)-2-5-diphenyl tetrazolium bromide], and 2-mercaptoethanol were from Sigma. Iodoacetic acid and pyridine were purchased from Eastman. Toluene, 2,5-diphenyloxazole (PPO), and p-bis[2-(5-phenyloxazolyl)] benzene (POPOP), all scintillation grade, were obtained from New England Nuclear. All other chemicals were reagent grade and were purchased from Sigma.

Methods

Enzyme Assays and Definition of Units of Activity

Aldolase was assayed spectrophotometrically at 340 nm following the oxidation of NADH through a modification of the method of Blostein and Rutter (1963). Cuvettes of 1.0-cm light path were used. Tris-Cl, 0.01 M, pH 7.5, was used as a buffer. The routine reaction mixture consisted of 10 mM FDP, 1.3 mM NADH, and 20 μg of the mixture of α-glycerol phosphate dehydrogenase and triose phosphate isomerase. Enzyme was added to initiate the reaction, and initial
velocities were measured. The results reported are the average of at least two measurements. The reaction was carried out on a Beckman Model DB-GT Spectrophotometer attached to a Beckman Ten-Inch Laboratory Potentiometric Recorder. For kinetic studies the full scale of the recorder was set to equal 0.1 optical density unit. A chart speed of 0.5 inch per minute was used for routine assays, while a speed of 5 inches per minute was used for more precise measurement of initial velocity in the kinetic studies. Temperature was maintained at 25° by a circulating water bath surrounding the assay chamber. One unit of enzyme activity is defined as the amount of enzyme catalyzing the reaction of one micromole of substrate per minute under initial velocity conditions at 25°. The specific activity is expressed as units of enzyme per milligram of protein.

**Protein Determination**

The biuret method of protein analysis was employed on crude samples using crystalline bovine serum albumin as the standard (Gornall et al., 1949). Protein concentration in eluted fractions from column chromatography was estimated spectrophotometrically by measuring the absorbance at 280 nm. Protein concentration of the purified enzyme was determined using the calculated extinction coefficient for
human heart aldolase of 0.904 for a concentration of 1 mg per ml in a 1-cm light path.

**Ion Exchange and Gel Filtration Chromatography**

Phosphocellulose was washed repeatedly with acid and base according to the procedure suggested by Peterson and coworkers (1962) and equilibrated with the desired buffer prior to use. After the column was packed, it was washed with buffer in a cold room maintained at 0-4°C. The eluant was tested to ensure the desired pH had been attained before the sample was applied. Fractions were collected at the same temperature, with constant flow rates maintained by a peristaltic pump.

Sephadex G-200 was equilibrated with the desired buffer as suggested by Pharmacia (1970). A 1.5 x 110 cm column was packed with a hydrostatic head pressure of 10 cm. Sucrose (20% w/v) was added to all samples to allow the eluting buffer to be layered above the column without mixing of the sample. The column was calibrated with blue dextran, tryptophan, and proteins of known physical parameters. The elution volumes were correlated with the molecular weights (Andrews, 1965, 1970) or with Stokes' radii (Ackers, 1964).
Electrophoresis

Polyacrylamide disc gel electrophoresis was carried out according to Davis (1964) utilizing the Canalco Model 200 apparatus. Gels were prepared with a 7.5 per cent monomer concentration in 25 mM Tris-glycine buffer, pH 8.8, and stained with 0.25 per cent (w/v) Coomassie Brilliant Blue (Weber and Osborn, 1969).

Sodium dodecyl sulfate gel electrophoresis was performed according to the method of Weber and Osborn (1969). The mobilities of the standard proteins were plotted against the log of their subunit molecular weight.

Ultracentrifugation

Ultracentrifugation experiments were conducted in a Beckman-Spinco Model E analytical ultracentrifuge equipped with RTIC temperature control and electronic speed control. Sedimentation coefficients were determined by the usual method (Chervenka, 1970), and corrected to values in water at 20°. Plots of values obtained at several protein concentrations were extrapolated to infinite dilution to obtain $s^{0}_{20,w}$.

Sedimentation equilibrium ultracentrifugation was conducted by the meniscus depletion method (Yphantis, 1964, Van Holde, 1967). Densities and viscosities of buffers were determined as described previously (Rozacky et al.,
1971). Analysis of fringes was performed with a Nikon Model 6 C Shadow Graph equipped with digital X,Y encoders.

**Amino Acid Analysis**

Samples were analyzed on a Beckman Model 120 C automatic amino acid analyzer (Spackman *et al.*, 1958) following hydrolysis in sealed, evacuated tubes in 5.7 N hydrochloric acid for 24, 48, and 72 hours at 110° (Moore and Stein, 1963). Tryptophan was determined by the spectrophotometric method of Edelhoch (1967). Cysteine was determined by titration with DTNB according to the method of Eilman (1959).

**Tryptic Fingerprints**

Samples of aldolase (10 mg/ml) were uniformly labeled with $^{14}$C-fructose 1,6-diphosphate by reduction of the Schiff base intermediate with sodium borohydride according to Lai *et al.*, 1967. The γ-glycerophosphate aldolases were precipitated with 5 per cent trichloroacetic acid (TCA) and the pellets were washed successively with 5 per cent TCA, acetone, and ether and air-dried. The samples were then dissolved in 8 M urea, reduced and carboxymethylated (Crestfield *et al.*, 1963). After dialysis and lyophilization the proteins were digested with trypsin at 25° and pH 7.5-8.0 (trypsin/aldolase weight ratio of 1/50). The reaction
mixture was maintained at the desired pH with 2 per cent trimethylamine. After digestion the samples were lyophylized, dissolved in water, and applied to an Eastman chromatogram sheet, 20 x 20 cm, 160 μ cellulose layer, at a spot 2 cm from one edge and 4.5 cm from the adjoining edge. Electrophoresis was carried out under varsol at pH 5.5 in pyridine: acetic acid: water (100: 30: 3000) at 300 volts (Tarui et al., 1972). Cellulose sponges, cut to fit the electrophoresis tank and saturated with buffer, were used as wicks. The plate was sprayed with buffer, placed face down, resting on the sponges, and subjected to electrophoresis for two hours. Following electrophoresis, the sheet was air dried. Ascending chromatography was carried out in the second dimension in n-butanol: pyridine: acetic acid: water (150: 100: 30: 120) until the solvent front was five mm from the edge of the chromatographic sheet. After drying, the peptides were located by spraying with ninhydrin: ethanol: acetic acid: collidine (50 mg: 30 ml: 10 ml: 4 ml) and incubating in an 85° oven for five to ten minutes. Tracings were made of each plate immediately after development, although the plates gave no indication of fading for more than three weeks, if kept under refrigeration. The active site peptide was located by scraping the spots from the chromatographic plate,
suspending the scrapings in 10 ml of toluene: PPO: POPOP (1000 ml: 6 g: 0.01 g) and counting in a Beckman LS-100 Liquid Scintillation Counter.
RESULTS

Isolation of Aldolase from Human Heart

Step I - Crude Extract

Human heart muscle (296 grams) was allowed to thaw in a refrigerator overnight before use. The heart was cut into 10-20 gram sections with shears and ground in a meat grinder. The ground tissue was then homogenized with a Waring blender in 400 ml of 10 mM Tris-Cl buffer, pH 7.5, containing 1.0 mM EDTA, 10 mM 2-mercaptoethanol, 10 mM sucrose, and 100 mM sodium chloride. The use of a high ionic strength homogenizing buffer prevents the binding of aldolase to muscle proteins (Arnold and Pette, 1970, Amberson and Bauer, 1971). The homogenate was centrifuged for thirty minutes at 1020 x g in a Sorvall RC-2B refrigerated centrifuge. The supernatant solution was filtered through glass wool and collected, and the pellet was rehomogenized in 400 ml of buffer. After centrifugation as before, the supernatant was again filtered and pooled with the supernatant from the first centrifugation. The pellet was discarded. The pooled fractions were then spun at 11,700 x g for one hour. The supernatant was filtered through glass wool and the pellet was discarded. A total of 900 ml was obtained as the crude extract. This
was dialyzed for twenty-four hours against three changes, 8 liters each, of 5 mM Tris-Cl, pH 7.5, 10 mM 2-mercaptoethanol, 1.0 mM EDTA (Buffer A).

**Step II - First Cellulose Phosphate Chromatography with Substrate Elution**

The dialyzed crude extract was applied to a cellulose phosphate column, 2.5 x 95 cm, that was previously equilibrated with Buffer A. The elution pattern from this column is shown in Figure 1. The flow rate was maintained at 0.5 ml per minute, and the column was washed with buffer until the eluant was protein free. At this point 50 mM Tris-Cl, 10 mM 2-mercaptoethanol, 1.0 mM EDTA, pH 7.5 (Buffer B) was applied, and the column was washed until the eluant contained no contaminating protein. Aldolase was then eluted with 2.5 mM fructose 1,6-diphosphate in the same buffer. Fractions of fifteen ml were collected. Protein and catalytic activity appeared together in fractions 362-365 (first phosphocellulose eluate). The fractions were pooled and concentrated by dialysis against a saturated solution of ammonium sulfate. The precipitated protein was collected by centrifugation and dialyzed in Buffer A. The protein concentration was adjusted to 25 mg/ml.
Fig. 1. First column chromatography of human heart aldolase on cellulose phosphate. The dialyzed crude extract (900 ml) was applied to a cellulose phosphate column (2.5 x 95 cm). Buffer A was pumped through the column at a flow rate of 0.5 ml/min, and 15 ml fractions were collected. After the first large peak of protein had washed through the column, Buffer B was applied, as indicated by a. When no more protein could be eluted from the column, 2.5 mM FDP in Buffer B was applied, as indicated by b. After elution of activity c—0, a 1 M NaCl solution was applied at c to remove all remaining protein.
Step III - Second Cellulose Phosphate Chromatography with Substrate Elution

The dialyzed fractions from the first phosphocellulose column were applied to a cellulose phosphate column, 1.5 x 30 cm, previously equilibrated with Buffer A. The flow rate was maintained as before. The column was washed with the same buffer until the eluant was protein free. Approximately 150 ml of Buffer B were washed through the column, but no protein was eluted from the column. To selectively remove aldolase from the column, 2.5 mM FDP was added to Buffer B. Seven ml fractions were collected, and protein and activity appeared simultaneously in fractions 93-97, as illustrated in Figure 2. These fractions were collected and concentrated as described above. Human heart muscle aldolase isolated by this procedure was found to be homogeneous by sedimentation analysis and disc gel electrophoresis. As shown in Table 1, this procedure using ion exchange chromatography results in a high overall recovery (75%). The enzyme is stable for months when stored in ammonium sulfate at 4°.

Physical Properties of Heart Aldolase

Absorbance Index

The absorbance of isolated heart aldolase at 280 nm was determined, and a value of 0.904 was obtained. This
Fig. 2. Second column chromatography of human heart aldolase on cellulose phosphate. The first phosphocellulose eluant (60 ml) was applied to a cellulose phosphate column (1.5 x 30 cm). Buffer A was pumped through the column at a flow rate of 0.5 ml/min, and seven ml fractions were collected. After the initial protein peak had washed through, Buffer B was applied, beginning at a. After washing thoroughly with Buffer B, 2.5 mM FDP in Buffer B was applied, as indicated by b. After elution of activity , a 1 M NaCl solution was applied at c to remove any remaining protein.
TABLE I

FRACTIONATION OF ALDOLASE FROM HUMAN HEART

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total Protein (mg)</th>
<th>Total Activity (units)</th>
<th>Specific Activity (Units/mg)</th>
<th>Purification</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>10980.0</td>
<td>990</td>
<td>0.09</td>
<td>--</td>
<td>100</td>
</tr>
<tr>
<td>First phosphocellulose</td>
<td>74.7</td>
<td>843</td>
<td>11.28</td>
<td>125.3</td>
<td>85</td>
</tr>
<tr>
<td>column</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second phosphocellulose</td>
<td>64.8</td>
<td>746</td>
<td>11.52</td>
<td>128.01</td>
<td>75.4</td>
</tr>
<tr>
<td>column</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
value is in close agreement with that found for rabbit muscle aldolase (Baranowski and Niederland, 1949).

**Molecular Weight of Native Enzyme**

When subjected to ultracentrifugation at 60,000 rpm, human heart aldolase sedimented as a single, symmetrical boundary throughout the cell, as shown in Figure 3. Velocity runs were made as a function of enzyme concentration, and analysis of the data yielded the relationship, 

$$s_0 \text{ at } \infty - 0.008 c$$

A value for $s_{20,w}$ at infinite dilution ($s_{20,w}^0$) of $7.79 \times 10^{-13}$ sec was obtained, as illustrated in Figure 4. The diffusion coefficient calculated from the spreading of the boundary (Schachman et al., 1957) yielded a $D_{20,w}$ value of $4.58 \times 10^{-7}$ cm$^2$ sec$^{-1}$. When both of these values were substituted into the Svedberg equation, the molecular weight was determined to be 158,000.

The results of high speed equilibrium ultracentrifugation experiments, as diagramed in Figure 5, confirmed the homogeneity of the preparation. The partial specific volume calculated from the amino acid composition was 0.73 ml gm$^{-1}$. The molecular weight of human heart aldolase calculated from the sedimentation equilibrium experiments was 158,000.

Quantitative gel filtration was used as a third method of estimating the molecular size of human heart aldolase.
Fig. 3. Sedimentation velocity patterns of human heart aldolase. The enzyme was dialyzed against 10 mM Tris-Cl, 1.0 mM EDTA, 100 mM NaCl, 0.05 % 2-mercaptoethanol, pH 7.5. The protein concentration was 5.5 mg/ml. The pictures were taken at five minute intervals after attaining top speed (60,000 rpm) with a bar angle of 65° and a temperature of 20°. Sedimentation is from left to right.
Fig. 4. Extrapolation of the apparent sedimentation coefficients \( s_{20,w} \) of human heart aldolase to zero protein concentration. The samples were dialyzed against 10 mM Tris-Cl, 1.0 mM EDTA, 100 mM NaCl, 0.05 % 2-mercaptoethanol, pH 7.5. All samples were run at 20° and 60,000 rpm.
Fig. 5. Fringe displacement obtained from sedimentation equilibrium experiment with native human heart aldolase. The protein (0.5 mg/ml) was dissolved and dialyzed against 50 mM Tris-Cl, 0.1 M NaCl, 10 mM 2-mercaptoethanol, pH 8.0. Sedimentation was at 17,000 rpm in the An-D rotor in a 12-mm double sector cell with quartz windows for 24 hours at 20°. The protein concentration was calculated from the fringe displacement ($Y_i - Y_0$) in millimeters as measured on the microcomparator. The abscissa represents the square of the distance (centimeters) from the center of rotation.
Figure 6 illustrates that when the elution volume of human heart aldolase was correlated with elution volumes and molecular weights of known proteins (Andrews, 1970), a value of 160,000 was obtained for the molecular weight. When the calibrated Sephadex column was used to correlate exclusion volume with the Stokes' radius as in Figure 7 (Ackers, 1964), a value of 46 Å was obtained. A diffusion coefficient (D_{20,w}) of 4.43 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1} was calculated from gel filtration data. This value is in good agreement with those determined by ultracentrifugation.

**Subunit Molecular Weight of Human Heart Aldolase**

The enzyme was dissociated into subunits by extensive dialysis in guanidinium chloride. Figure 8 presents the sedimentation equilibrium studies of the dissociated aldolase which yielded a subunit molecular weight of 40,000.

For sodium dodecyl sulfate gel electrophoresis, the protein standards and aldolase enzyme sample were dissolved in 1.0 ml of 10 mM sodium phosphate buffer, pH 7.0, containing 1% (w/v) SDS, and 1% (v/v) 2-mercaptoethanol and incubated at 37° for five hours (Bryce and Crichton, 1971). Electrophoresis was carried out as indicated in the "Methods", and the results, presented in Figure 9, yield a value of 42,500 for the subunit molecular weight.
Fig. 6. Determination of molecular weight of human heart aldolase by gel filtration. The ordinate represents the ratio of $V_e$ (elution volume) to $V_0$ (void volume) and the abscissa the molecular weight plotted on a log scale. The column was prepared and calibrated as described under "Methods". Chymotrypsin A, MW 25,000 is represented by a, ovalbumin, MW 45,000, is represented by b, and rabbit muscle aldolase, MW 158,000, is represented by c. Human heart aldolase is shown as an open circle. Fractions of 1.0 ml were collected and monitored by their absorbance at 280 nm.
Fig. 7. Calibration plot for the determination of Stokes' radius by gel filtration. The ordinate represents the ratio of $V_e$ (elution volume) minus $V_0$ (void volume) to $V_i$ (internal volume) and the abscissa the Stokes' radius in angstroms. The column was prepared and calibrated as described under "Methods". Chymotrypsin A (20.9 Å) is represented by a, Ovalbumin (27.3 Å) is represented by b, and rabbit muscle aldolase (45 Å) is represented by c. Human heart aldolase is shown as an open circle.
$K_d$ vs. Molecular Radius (Å)
Fig. 8. Fringe displacement of human heart aldolase in guanidinium chloride. The protein was dissolved in 6 M guanidinium chloride, 0.1 M NaCl, 10 mM 2-mercapto-ethanol and dialyzed against the same solution for 48 hours. Sedimentation was at 34,000 rpm; otherwise all conditions were as in Fig. 5.
Fig. 9. Sodium dodecyl sulfate gel electrophoresis of human heart aldolase. Human heart aldolase and standard proteins were prepared as described in the "Methods" and subjected to electrophoresis on separated gels (10% monomer concentration) (8 ma, 5 hours). The mobility of each protein was plotted against the log of its molecular weight. Albumin, MW 68,000, is represented by a, ovalbumin, MW 43,000, is represented by b, pepsin, MW 35,000, is represented by c, and trypsin, MW 23,000, is represented by d. Human heart aldolase is shown as an open circle.
the value obtained by this method agrees very well with the value of 40,000 obtained by sedimentation equilibrium ultracentrifugation. Like other mammalian aldolases, human heart aldolase is a tetramer (Gracy et al., 1970, Kawahara and Tanford, 1966, Penhoet et al., 1966).

**Electrophoresis**

Polyacrylamide disc gel electrophoresis was performed according to the method of Davis (1964), and the results are shown in Figure 10. A single band of protein was obtained which showed identical migration as compared to human heart aldolase when the gel was stained for catalytic activity (Penhoet et al., 1966).

**Chemical Properties of Human Heart Aldolase**

**Amino Acid Composition**

The amino acid composition of human heart aldolase, calculated on the basis of a molecular weight of 158,000, is shown in Table II. The data for human skeletal muscle (Dikow et al., 1971) and rabbit muscle aldolases (Gracy et al., 1969) are presented for comparison. Threonine, serine, and tyrosine destruction were corrected by extrapolation to zero time. Valine and isoleucine values were taken from the 72-hour hydrolysates. Although the overall composition of human heart muscle aldolase appears to be similar to rabbit muscle aldolase, there are
Fig. 10. Polyacrylamide disc gel electrophoresis of human heart aldolase. Fifty micrograms of human heart aldolase were applied to a 7.5% standard 0.6 x 7 cm polyacrylamide gel. Electrophoresis was carried out at 50° in Tris-glycine buffer, pH 8.8. The gel shown on the left was stained in a developer specific for aldolase activity, while the gel on the right was stained for protein as indicated in "Methods".
TABLE II

A COMPARISON OF THE AMINO ACID COMPOSITIONS OF HUMAN HEART ALDOLASE, HUMAN SKELETAL MUSCLE ALDOLASE, AND RABBIT MUSCLE ALDOLASE

<table>
<thead>
<tr>
<th>Residue</th>
<th>Human Heart</th>
<th>Skeletal Muscle</th>
<th>Rabbit Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>92</td>
<td>142</td>
<td>104</td>
</tr>
<tr>
<td>His</td>
<td>28</td>
<td>38</td>
<td>44</td>
</tr>
<tr>
<td>Arg</td>
<td>56</td>
<td>69</td>
<td>56</td>
</tr>
<tr>
<td>Asp</td>
<td>112</td>
<td>102</td>
<td>116</td>
</tr>
<tr>
<td>Thr</td>
<td>100</td>
<td>83</td>
<td>88</td>
</tr>
<tr>
<td>Ser</td>
<td>112</td>
<td>86</td>
<td>84</td>
</tr>
<tr>
<td>Glu</td>
<td>180</td>
<td>170</td>
<td>164</td>
</tr>
<tr>
<td>Pro</td>
<td>80</td>
<td>113</td>
<td>80</td>
</tr>
<tr>
<td>Gly</td>
<td>144</td>
<td>130</td>
<td>124</td>
</tr>
<tr>
<td>Ala</td>
<td>156</td>
<td>186</td>
<td>172</td>
</tr>
<tr>
<td>Val</td>
<td>92</td>
<td>74</td>
<td>80</td>
</tr>
<tr>
<td>Met</td>
<td>12</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Ile</td>
<td>56</td>
<td>83</td>
<td>76</td>
</tr>
<tr>
<td>Leu</td>
<td>132</td>
<td>156</td>
<td>140</td>
</tr>
<tr>
<td>Tyr</td>
<td>41</td>
<td>54</td>
<td>48</td>
</tr>
<tr>
<td>Phe</td>
<td>40</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>Trp</td>
<td>14</td>
<td>--</td>
<td>12</td>
</tr>
<tr>
<td>Cys</td>
<td>27</td>
<td>--</td>
<td>28</td>
</tr>
</tbody>
</table>
significant differences in the content of some residues. Thus, for example, the content of histidine, alanine, and isoleucine appear low, while phenylalanine, serine, and threonine are high. The deviation function between rabbit muscle aldolase and human heart aldolase is 4.17 (Dedman, 1972).

**Tryptic Fingerprints**

Peptide maps were made using human heart aldolase that had been radioactively labeled at the active site by $^{14}$C-FDP. Rabbit muscle aldolase, labeled in the same manner, was also run for comparison. The total number of peptides found in both enzymes was consistent with the amino acid compositions and the presence of four subunits. While minor deviations in the migration of some of the peptides was observed, there were several regions of similarity which appeared consistently and which can be observed in Figure 11. These include the group of five peptides migrating most negatively during electrophoresis, and the group of six peptides arranged above the active site peptide near the right margin. These peptides appear to have extremely conservative composition, for they are also found in tryptic fingerprints of aldolase isolated from *Ascaris lumbricoides suum* run under the same conditions described in "Methods". (Dedman, 1972).
Fig. 11. Tryptic peptide maps of rabbit muscle aldolase (A) and human heart aldolase (B). Tryptic digestion, chromatography and electrophoresis were carried out as described in "Methods". The origin is in the lower right corner of each map. The labeled active site peptides are shown by solid shading. The second, less heavily labeled peptide is indicated by stippling. The drawings are from tracings made directly from the silica gel sheets.
As might be expected, the location of the active site was identical in both enzymes. While the active site was easily identifiable by the amount of radioactivity present, a significant amount of radioactivity (usually one-third to one-half the amount found at the active site) was found in a peptide slightly removed from the active site. This peptide is shown as the stippled peptide in Figure 11. No radioactivity above background level was found in any of the other peptides.

**Kinetics**

Kinetic studies were performed on the 125-fold purified human heart aldolase. Michaelis constants were calculated from kinetic data by the method of Wilkinson (1961) as adapted for computer calculations by Cleland (1963), using a weighted, non-linear regression. The Michaelis constant for FDP was determined to be $1 \times 10^{-5}$ M. As shown in Table III, this compares very well with the $K_m$ determined for other muscle aldolases (Gracy et al., 1970, Marquardt, 1969a, Kawabe et al., 1969, Rutter et al., 1963). The Michaelis constant for F1P was determined to be $2.8 \times 10^{-3}$ M. While this varies from the reported value for many muscle aldolases (Marquardt, 1969a, Rutter et al., 1963, Wolf and Leuthardt, 1957, Gracy et al., 1970, Kawabe et al., 1969), it agrees with the published value for human erythrocyte aldolase (Foranini et al., 1964).
### TABLE III

A COMPARISON OF THE KINETIC PROPERTIES OF ALDOLASE FROM DIFFERENT TISSUES

<table>
<thead>
<tr>
<th>Property</th>
<th>Human Heart</th>
<th>Rabbit Muscle</th>
<th>Rabbit Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$ (FDP)</td>
<td>$1.00 \times 10^{-5}$ M</td>
<td>$6.00 \times 10^{-5}$ M</td>
<td>$1.0 \times 10^{-6}$ M</td>
</tr>
<tr>
<td>$K_m$ (F1P)</td>
<td>$2.80 \times 10^{-3}$ M</td>
<td>$1.00 \times 10^{-2}$ M</td>
<td>$9.0 \times 10^{-4}$ M</td>
</tr>
<tr>
<td>$V_{max}$ (FDP)</td>
<td>$3.73 \times 10^{-3}$</td>
<td>$5.30 \times 10^3$</td>
<td>$2.5 \times 10^2$</td>
</tr>
<tr>
<td>$V_{max}$ (F1P)</td>
<td>$1.09 \times 10^{-3}$</td>
<td>$1.06 \times 10^2$</td>
<td>$2.5 \times 10^2$</td>
</tr>
<tr>
<td>FDP/F1P</td>
<td>3</td>
<td>50</td>
<td>1</td>
</tr>
</tbody>
</table>
An FDP/F1P ratio of 3 was obtained from this data. Plots of rate against substrate concentration yielded rectangular hyperbolas and had the usual characteristics of Michaelis Menten kinetics for both substrates.
<table>
<thead>
<tr>
<th>Physical Property</th>
<th>Method</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native enzyme:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular weight</td>
<td>Sedimentation equilibrium</td>
<td>158,000</td>
</tr>
<tr>
<td></td>
<td>Sedimentation velocity</td>
<td>158,000</td>
</tr>
<tr>
<td></td>
<td>Gel filtration</td>
<td>160,000</td>
</tr>
<tr>
<td>Sedimentation coefficient ((s_{20,w}^0))</td>
<td>Sedimentation velocity</td>
<td>(7.46 \times 10^{-13}) sec</td>
</tr>
<tr>
<td>Diffusion coefficient ((D_{20,w}))</td>
<td>Gel filtration</td>
<td>(4.43 \times 10^{-7}) cm²/sec</td>
</tr>
<tr>
<td>Partial specific volume</td>
<td>Amino acid composition</td>
<td>0.73 ml/g</td>
</tr>
<tr>
<td>Stokes' radius</td>
<td>Gel filtration</td>
<td>46 Å</td>
</tr>
<tr>
<td>Subunit:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular weight in guanidinium chloride</td>
<td>Sedimentation equilibrium</td>
<td>40,000</td>
</tr>
<tr>
<td></td>
<td>Sodium dodecyl sulfate disc gel</td>
<td>42,500</td>
</tr>
<tr>
<td></td>
<td>electrophoresis</td>
<td></td>
</tr>
</tbody>
</table>
Aldolase has been purified from human heart and has been shown to be homogeneous by a number of criteria. The initial specific activity of the crude extract from heart, 0.09 units/mg, compares with 0.11 units/mg from human skeletal muscle (Ikehara et al., 1969), and 0.085 units/mg from human brain (Dikow et al., 1971). The specific activity of the pure human heart aldolase was comparable to that of other mammalian muscle aldolases (Ikehara et al., 1969, 1970). However, it was substantially higher than values reported for other purified human aldolases (Ikehara et al., 1969, Dikow et al., 1971). The specific activity of the original muscle extract and of the purified extract indicate that about 0.8% of the extract is aldolase. The percentage aldolase present in muscle extract from other species has been estimated, and some of these values are: snake (Boa constrictor), 7% (Schwartz and Horecker, 1966), tuna (Neothunnus macropterus), 3% (Kwon and Olcott, 1965), chicken, 22% (Marquardt, 1969a), and rabbit, 8-10% (Czok and Bucher, 1960). In contrast, Agress and Estrin (1963) have found the highest concentrations of aldolase in human tissues to be in skeletal muscle, myocardium, and liver, with small amounts present in blood serum. Although the
concentration in heart muscle is not as high as that of skeletal muscle (Olsen, 1962), it is, nevertheless, present in high enough quantities to create an overall rise of enzyme in serum following ischemia or infarction in the myocardium (Van Rymenant and Tagnon, 1959). This phenomena was first demonstrated by Warburg and Christian in 1943. The explanation of the low value for aldolase reported in this paper appears to be the conditions affecting the tissue before it arrives in the laboratory. After death, the tissue is subjected to an indefinite waiting period before autopsy. This time varies with the particular circumstances of each individual case, and it can range from minutes to several days. The heart contains a large number of lysosomal acid hydrolases, and their release into the soluble phase in heart muscle following ischemia is well documented (Clendenon et al., 1971, Leighty et al., 1967, Brachfeld, 1969). It is probable that lysosomal destruction and/or modification could account for the low levels of aldolase present in the tissue used in this study.

Three different determinations of molecular weight all yielded a value of 158,000 for the native enzyme. These values compare with 160,000 for human skeletal muscle aldolase (Dikow et al., 1971), 158,000 for rabbit muscle aldolase (Kawahara and Tanford, 1966), and a range
of 155,000 to 165,000 for the majority of animal aldolases studied (Rutter et al., 1968).

Human heart muscle aldolase was composed of four subunits, each subunit exhibiting a molecular weight of 40,000. This is in agreement with ultracentrifugation studies by Kawahara and Tanford (1966) and Sia and Horecker (1968a) who have observed that rabbit muscle aldolase dissociated into four subunits with molecular weights of 40,000. The four subunit model of aldolase has recently been substantiated by X-ray crystallography (Heidner et al., 1971).

Polyacrylamide disc gel electrophoresis has also indicated that the preparation of human heart aldolase is pure and that it is composed of only one species, since other isoenzymes do not appear. Based on molecular weight, electrophoretic mobility (Penhoet et al., 1966), and catalytic properties, as well as its reduction with sodium borohydride, human heart aldolase is assumed to be a Class I, type A, muscle aldolase. This agrees with the data of Lebherz and Rutter (1969) who showed that embryonic human heart tissue contained hybrids of muscle and brain (A and C), but as development progressed, the C species gradually disappeared and could not be demonstrated in adult heart.

The amino acid composition of human heart aldolase compared to that of rabbit muscle aldolase shows an overall
similarity, particularly in amounts of arginine, aspartate, proline, methionine, leucine, tyrosine, and tryptophan. However, the deviation function between human heart aldolase and human skeletal muscle aldolase, two tissues that would be assumed to be more closely related than tissues from another species, is 5.73, indicating only a limited similarity. All aldolases have certain molecular features in common. One of these is a relatively constant high proportion of amino acids with aliphatic side chains. For example, the sum of proline, alanine, valine, isoleucine and leucine residues for the three forms of chicken aldolase is 534 ±12, and the additive value of these residues is thought to correlate as well between species as among aldolases of the same species (Marquardt, 1970). A similar observation has been reported for glyceraldehyde-3-phosphate dehydrogenase (Allison and Kaplan, 1964). When this test is applied to human heart muscle aldolase, a value of 514 is obtained. While this is slightly lower than the average value for chicken aldolase, it is, nevertheless, reasonably close, and compares favorably to other mammalian aldolases, for example, 524 for ox, 520 for pig, and 531 for rabbit (Anderson et al., 1969). In contrast the value determined by Dikow et al., (1971) for human skeletal muscle is 612. Therefore, the values reported for human skeletal muscle should be regarded as somewhat tenuous.
While human heart aldolase is similar to other aldolases in many aspects, it varies markedly with respect to some amino acids. Histidine, alanine, and isoleucine are low, in comparison to rabbit muscle aldolase, while threonine, serine, and phenylalanine are high. Four of these six amino acids (histidine, isoleucine, serine, and phenylalanine) show a great degree of variance from species to species (Marquardt, 1969b). Wilson and Kaplan observed that the degree of variability in amino acid composition for several species of animals was similar in magnitude to the anatomical differences that have been used with the classical taxonomical methods. They also observed that in a given species, certain proteins tend to evolve at a similar rate. By analogy with other proteins, a difference in amino acid sequence in rabbit and human aldolases would be hypothesized (Wilson, 1964, Allison, 1964). However, these changes may occur in areas of the molecule that are not necessary for maintaining a structure that will permit catalytic activity to occur. There are very few differences between the peptide maps of tryptic digests of the carboxymethylated human heart aldolase and rabbit muscle aldolase. This again confirms the basic similarity of the mammalian aldolases (Anderson et al., 1969). The rabbit muscle aldolase contains three extra peptides, and this is to be expected from the amino acid analysis of both enzymes.
Despite the differences in content of some amino acids, the tryptic fingerprints revealed several regions of homology. These include the group of six peptides in the top one-third of the chromatogram, and the group of nine along the bottom edge in Figure 11. It is possible that these peptides are involved in substrate binding, or that they are important in maintaining the conformation of the protein. Thus, their conservative nature would be necessary to maintain the activity of the molecule. The active site peptides of human heart and rabbit muscle aldolase mapped at identical positions, indicating the high degree of correlation in this region of the protein. This is consistent with the findings of Lai et al., (1965) and Morse and Horecker (1968), which revealed high specificity of amino acid sequence in the active site. A recent comparative study of vertebrates and invertebrates revealed that the size of the active site peptides isolated from these different organisms was nearly identical, and the few sequence changes observed could be accounted for by single base mutations in the corresponding genes. These changes involved the substitution of amino acids with similar characteristics and would not be expected to alter the tertiary structure (Lai and Chen, 1971).

In each experiment, a second peptide showed a significant amount of radioactivity, amounting to approximately one-half the number of counts found in the active site.
This was not due to lack of separation of the peptides, for in every case, the peptide between the active site and the second radioactive peptide showed no activity above background. One explanation for this observation is that the enzyme is also binding fructose 1,6-diphosphate at a site other than the active site. The peptide containing this second binding site is radioactive, but because the active site is the site of preference, the second binding site contains less radioactivity. It has been reported that incubation of aldolase with FDP causes a loss of activity in the enzyme, and that this loss of activity is due to the binding of FDP to lysine residues other than the one found in the active site (Woodfin, 1967). However, an FDP/aldolase ratio of 1000 and more than 60 hours are required for binding with all the susceptible lysines to occur. In this experiment, however, an FDP/aldolase ratio of 600 was used, and incubation time was limited to 15 minutes. Under these circumstances only the lysine residues with the greatest affinity for binding FDP would be affected. It would not be surprising, then, to find only one spot showing significant radioactivity above background.

Another explanation of the inhibition of aldolase upon prolonged incubation with excess substrate (Lai et al., 1968) was advanced after it was observed that this inhibition is accompanied by decreased thiol group reactivity. The thiol
group reactivity was not regained by addition of the coupling enzymes and NADH, therefore, it appears that excess FDP is capable of reacting with aldolase to produce some modified form of the enzyme (Anderson and Perham, 1970). Perhaps this modified form is the additional radioactive peptide. Further characterization of the peptide is necessary to determine this. Another explanation involving the activity of the enzyme could be proposed. During incubation, the enzyme could convert the labeled FDP to labeled glyceraldehyde-3-phosphate. Lai and coworkers (1968) have proposed a covalent bonding of glyceraldehyde-3-phosphate to the active site of aldolase, causing a highly specific inactivation of the enzyme. It is possible that the modification of the charge on the active site through the formation of the covalent bond could cause the modified peptide to migrate differently during peptide mapping, thus creating a second, less-radioactive spot.

The Michaelis constant for human heart aldolase with FDP was $1 \times 10^{-5}$ M. This is in good agreement with values reported in the literature, for example, $5 \times 10^{-5}$ M for human erythrocyte aldolase (Foranini et al., 1964), and $1 \times 10^{-5}$ M for rabbit muscle (Gracy et al., 1970). In addition, human heart aldolase was not greatly affected by high concentrations of FDP.
The $K_m$ for F1P was $2.8 \times 10^{-3} \text{ M}$ for human heart aldolase. This compares with $4.5 \times 10^{-2} \text{ M}$ for human skeletal muscle (Dikow et al., 1971), $5.1 \times 10^{-3} \text{ M}$ for human erythrocyte aldolase (Foranini et al., 1964), and $9.1 \times 10^{-3} \text{ M}$ for rabbit muscle aldolase (Gracy et al., 1970). While it appears that the human aldolases have slightly lower Michaelis' constants for F1P than other aldolases, it is possible that these variations are due to the method of preparation of the tissue. The variation in aldolase activity from human tissue with time (Peterson, 1959) has already been discussed. In addition, freezing and thawing of rat liver aldolase during preparation has been found to alter the COOH-terminal, probably through autolysis (Gracy et al., 1969). It is probable that freezing and thawing, a process which could not be avoided in the present study, combined with lysosomal destruction, resulted in a modified enzyme which had slightly different catalytic properties. Studies on carboxypeptidase-treated aldolase have shown that the removal of a single tyrosine residue from the C-terminal of the protein results in an 85-90% loss of activity in muscle aldolases, lowering the FDP/F1P ratio to almost 1 (Drechler et al., 1959, Tung et al., 1954, Rutter et al., 1961). The reported Michaelis constant for FDP after carboxypeptidase degredation is $2.0 \times 10^{-5}$, while that
for FlP is $2.3 \times 10^{-3}$ (Drechler et al., 1959), or $3.4 \times 10^{-3}$ (Tung et al., 1954). These values are in excellent agreement with those for human heart aldolase, and in view of the probability of proteolysis, the removal of a tyrosine residue is proposed to explain the low FDP/FlP ratio.

This study has dealt with the physical properties of human heart aldolase. The work is intended to be a foundation for further studies on the effect of myocardial infarction on aldolase and other enzymes of human heart tissue. There are numerous approaches that could be taken to explore the problem further. For example, the effect of lysosomal destruction and binding of aldolase to muscle membranes (Arnold and Pette, 1968) could be studied. Also the leakage of aldolase through the membrane upon destruction of tissue should be further studied, for the mechanism of movement through the membrane is not understood (Sibley and Fleisher, 1954). While it has been established that aldolase is probably not the controlling enzyme in myocardial infarction (Sobel, 1972), it is nevertheless an important enzyme in the biochemistry of the heart, and one that merits further study.
SUMMARY

1. A procedure has been developed to purify aldolase from human heart 128-fold by phosphocellulose column chromatography.

2. The protein was shown to be homogeneous by the criteria of zone electrophoresis, sedimentation velocity ultracentrifugation, and rechromatography. The purified enzyme exhibited a specific activity of 11.52 units per mg protein.

3. The molecular weight of the enzyme, determined by ultracentrifugation and gel filtration, was 158,000. The subunit molecular weight was determined by ultracentrifugation after dissociation in guanidinium chloride and by sodium dodecyl sulfate disc gel electrophoresis. Both methods yielded a subunit molecular weight of 40,000.

4. Sedimentation velocity ultracentrifugation yielded a value for $s_20,w^0$ of $7.79 \times 10^{-13}$ sec. A value for $D_{20,w}$ of $4.58 \times 10^{-7}$ cm$^2$ sec$^{-1}$ was obtained by gel filtration. The partial specific volume of the enzyme calculated from the amino acid composition was 0.73 ml/g. Stokes' radius, determined by gel filtration, was 46 Å.
5. A comparison of the amino acid analysis of human heart aldolase and other mammalian muscle aldolases yielded minor differences in the content of some residues, however, a basic overall similarity was demonstrated.

6. Tryptic fingerprints of human heart aldolase and rabbit muscle aldolase indicated that one-third to one-half of the peptides showed almost identical migration. The active site peptides also migrated identically in both enzymes.

7. Kinetic studies yielded Michaelis constants which were similar to those determined for other muscle aldolases. However, the FDP/F1P ratio was significantly lower than the reported value for most muscle aldolases. This is proposed to be the result of the proteolytic removal of tyrosine from the C-terminal of the enzyme.
REFERENCES


