Purification and Properties of Human Liver $\beta$-Glucuronidase*

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$\beta$-Glucuronidase is an enzyme which catalyzes in vitro the hydrolysis of the $\beta$-$\alpha$-glycosidic bond of certain glucosiduronic acids. Its function in the cell is unknown. The purpose of this article is to describe a method of achieving a 2900-fold purification of $\beta$-glucuronidase from human liver and to describe some of the properties of this human enzyme.

Isolation of human liver $\beta$-glucuronidase has not previously been reported. Previous purifications from other mammals have been described. Whenever examined electrophoretically they have not been proved to be pure. Criteria for purity have generally been based upon specific activity, that is, the activity of enzyme per mg of protein. Bernfeld, Nisselbaum, and Fishman purified $\beta$-glucuronidase from bovine liver and attained a specific activity of 60,000 to 107,000 Fishman units per mg of protein (1). (A Fishman unit is that amount of enzyme liberating 1 $\mu$g of phenolphthalein per hour at 37$^\circ$.) Based on electrophoretic data showing 15 to 20% impurity, they calculated that pure $\beta$-glucuronidase would have a specific activity of 120,000 Fishman units per mg of protein (2). Levy, McAllan, and Marsh, utilizing female rat preputial glands which in crude tissue have a specific activity of 18,000, achieved a 25-fold purification and a preparation with specific activity of 455,000 (3). Most purification methods have utilized ammonium sulfate or organic solvent fractionation. Ion exchange resins and gel filtration have not been successfully applied.

MATERIALS

Human liver was obtained at autopsy. No special effort was made to select the materials other than rejection of patients dying with infections or severe liver disease. The livers were dissected as free as possible from blood vessels and connective tissue. If not processed immediately, they were frozen and stored at -20$^\circ$.

Reagents—Special water was distilled, deionized, and further purified by passage over a diethylaminoethyl cellulose column as previously described by Seal and Doe (4). This was important since dialysis against merely distilled water resulted in significant loss of enzyme activity.

Visking tubing for dialysis was prepared by twice heating it for 30 min at 80$^\circ$ in a solution of 0.5 g of ascorbic acid, 1.0 g of disodium ethylenediaminetetraacetate per liter, and rinsing it four times with special water. It was then stored in buffer at 4$^\circ$.

DEAE-Sephadex A-50 and carboxymethyl Sephadex C-50 were obtained from Pharmacia Inc., New Market, New Jersey, and prepared according to the instructions supplied.

Hydroxyapatite gel was prepared from reagent grade chemicals by the method described by Tiselius, Hjerten, and Levin (5). All other chemicals used were reagent grade. Acrylamide and $m$-bis-acrylamide for preparation of electrophoresis gel were obtained from Eastman Chemical Company. The cinchonidine salt of phenolphthalein glucuronide was obtained from Sigma Chemical Company.

METHODS

Enzyme Assay

The determination of $\beta$-glucuronidase was a modification of the method of Plaice utilizing hydrolysis of 0.001 $\mu$m phenolphthalEin glucuronide (6).

A solution of 0.01 $\mu$m phenolphthalein was prepared from the cinchonidine salt as described by Fishman (7). The solution was stable for months at 4$^\circ$.

A 1% solution of bovine serum albumin was also used.

Sodium acetate-acetic acid buffer, pH 4.3, 0.1 $m$, was used.

$\text{NaOH-NaHCO}_3$ buffer, pH 10.5; 80 g of $\text{NaHCO}_3$ and 150 ml of 20% $\text{NaOH}$ was diluted to 1000 ml. Final pH was adjusted to 10.5 with 20% $\text{NaOH}$ with a pH meter.

Phenolphthalein standard solution was 100 $\mu$g of phenolphthalein in 100 ml of absolute ethanol.

The enzyme was appropriately diluted to allow for release of 8 to 38 $\mu$g of phenolphthalein. This was to allow for an absorbance of 0.100 to 0.600. Above 0.600, phenolphthalein does not give a straight line with concentration.

Enzyme solution, 0.1 ml, was added to a preincubated 12-ml centrifuge tube containing 0.1 ml of substrate, 0.1 ml of 1% bovine albumin, and 0.7 ml of 0.1 $m$ acetate buffer. This gave final substrate concentration of 0.001 $m$. The tube was shaken gently to mix the ingredients, stoppered, and incubated at 37$^\circ$ for 1 hour in a Gyrotary water bath. A blank containing all ingredients except enzyme was similarly treated.

At 1 hour, 3.5 ml of $\text{NaOH-NaHCO}_3$ buffer were added to the blank and to the unknown. It was not necessary to precipitate the protein. (Serum or crude tissue assays were immersed in boiling water for 5 min; alkali was then added, and the precipitate was removed by centrifugation at 1600 rpm for 30 min.) Addition of the alkaline $\text{NaOH-NaHCO}_3$ buffer stopped the reaction and developed the pink phenolphthalein color. The unhydrolyzed glucuronide turned pale yellow which did not interfere with the reading. The mixture was transferred to Coleman colorimeter tubes and read against the blank at 550 $\mu$m in the Coleman...
In this instance, it was more difficult to tell when the gel was overloaded. However, careful scrutiny disclosed a pale yellow band of adsorbed protein descending the gel. When Solution II was adsorbed, the gel was washed with several volumes of 0.01 m phosphate buffer, pH 5.8. All eluates were discarded. In this case the enzyme remained on the gel. The enzyme was first eluted with 100 ml of 0.2 m phosphate buffer, pH 7.3. Then 0.5 g of NaCl was dissolved into the remaining gel, and suction was applied. The dark, brownish red material settled through the gel and collected in the suction flask. Only when all of Solution I had been adsorbed was the gel discarded. The sediment was thoroughly mixed, centrifuged at 20,000 rpm for 20 min. The residue was washed twice with 400 ml of 0.1 m acetate buffer, pH 5.0, and recentrifuged. All supernatant fluids were pooled and the residue discarded. This solution was labeled crude extract (2100 ml).

Step 2—To 2100 ml of the crude extract, were added 700 g of ammonium sulfate. The mixture was gently stirred on a rotating table for 30 min, allowed to stand for 24 hr at 4°, then centrifuged at 20,000 rpm for 20 min at 1600 rpm. The supernatant solution was discarded. The sediment was thoroughly washed, then centrifuged three times with 500 ml of 23% (w/v) ammonium sulfate at 20°. Again all supernatant solutions were discarded. The residue was dissolved in 1000 ml of 0.02 m sodium phosphate buffer, pH 7.3. The solution was centrifuged and the residue discarded. The solution was dialyzed against 20 liters of 0.02 m sodium phosphate buffer, pH 7.3, for 2 days in a large Waring Blendor. The homogenate was transferred to a glass container and incubated overnight at 37°.

Protein Assay

Protein was determined by ultraviolet absorption at 215 and 225 nm according to Waddell (8).

Purification

All purification steps were done at room temperature unless stated otherwise.

Step 1—Human liver, 1488 g, was diced and homogenized in 1000 ml of 0.1 m acetate buffer, pH 5.0, for 5 min at top speed in a large Waring Blendor. The homogenate was transferred to a glass container and incubated overnight at 37°. On the following morning, the homogenate was centrifuged at 2000 rpm for 20 min. The residue was washed twice with 400 ml of 0.1 m acetate buffer, pH 5.0, and recentrifuged. All supernatant fluids were pooled and the residue discarded. This solution was labeled Solution I (1100 ml).

Step 2—Into 1100 ml of Solution I was added 390 g of ammonium sulfate. When the gel had reached its adsorbing capacity, before the border reached 1 inch from the bottom of the gel, more gel was added to the funnel. This prevented exceeding the gel capacity and thus not adsorbing the maximal amount of extraneous protein. When all of Solution I had been adsorbed, 002 m phosphate buffer, pH 7.3, was poured over the gel until the eluate had an ultraviolet absorption at 280 nm of less than 0.075. This was Solution II (4120 ml). The gel was discarded.

Step 3—Solution II was diluted to twice its volume with special water and its pH was adjusted to 5.8 with phosphoric acid. A suction funnel was again prepared as in Step 3. In this instance, a 5-cm funnel was used. The cationic exchange resin, CM-Sephadex C-50, was prepared and equilibrated with 0.01 m phosphate buffer, pH 5.8, and poured into the funnel as described in Step 3. The diluted Solution II was then poured over the gel. In this instance, it was more difficult to tell when the gel was overloaded. However, careful scrutiny disclosed a pale yellow band of adsorbed protein descending the gel. When Solution II was adsorbed, the gel was washed with several volumes of 0.01 m phosphate buffer, pH 5.8. All eluates were discarded. In this case the enzyme remained on the gel. The enzyme was first eluted with 100 ml of 0.2 m phosphate buffer, pH 7.3. Then 0.5 g of NaCl was dissolved into the remaining gel, and suction was applied. The dark, brownish red material settled through the gel and collected in the suction flask. Only when all of Solution I had been adsorbed was the gel discarded. The sediment was thoroughly mixed, centrifuged at 20,000 rpm for 20 min. The supernatant solution was discarded. The sediment was thoroughly washed, then centrifuged three times with 500 ml of 23% (w/v) ammonium sulfate at 20°. Again all supernatant solutions were discarded. The residue was dissolved in 1000 ml of 0.02 m sodium phosphate buffer, pH 7.3. The solution was centrifuged and the residue discarded. The solution was dialyzed against 20 liters of 0.02 m sodium phosphate buffer, pH 7.3, for 2 days in a large Waring Blendor. The homogenate was transferred to a glass container and incubated overnight at 37°.

Step 4—The anion exchange resin, DEAE-Sephadex A-50, was equilibrated with 0.02 m sodium phosphate buffer, pH 7.3, and poured into a 12-cm glass suction funnel with filter paper on the base. The packed volume of the gel was about 400 ml. Care was needed not to allow the gel to dry or become cracked. Solution I was then gradually poured onto the gel (a filter paper lying on top of the gel will prevent stirring of the gel) and allowed to settle through the gel and collect in the suction flask. Only gentle suction was applied. The dark, brownish red material was absorbed on the gel, and a pale yellow fluid was eluted. A gradually descending brownish red boundary was useful to indicate when the gel had reached its adsorbing capacity. Before the border reached 1 inch from the bottom of the gel, more gel was added to the funnel. This prevented exceeding the gel capacity and thus not adsorbing the maximal amount of extraneous protein. When all of Solution I had been adsorbed, 002 m phosphate buffer, pH 7.3, was poured over the gel until the eluate had an ultraviolet absorption at 280 nm of less than 0.075. This was Solution II (4120 ml). The gel was discarded.

Step 5—Solution II was diluted to twice its volume with special water and its pH was adjusted to 5.8 with phosphoric acid. A suction funnel was again prepared as in Step 3. In this instance, a 5-cm funnel was used. The cationic exchange resin, CM-Sephadex C-50, was prepared and equilibrated with 0.01 m phosphate buffer, pH 5.8, and poured into the funnel as described in Step 3. The diluted Solution II was then poured over the gel. In this instance, it was more difficult to tell when the gel was overloaded. However, careful scrutiny disclosed a pale yellow band of adsorbed protein descending the gel. When Solution II was adsorbed, the gel was washed with several volumes of 0.01 m phosphate buffer, pH 5.8. All eluates were discarded. In this case the enzyme remained on the gel. The enzyme was first eluted with 100 ml of 0.2 m phosphate buffer, pH 7.3. Then 0.5 g of NaCl was dissolved into the remaining gel, and suction was applied. The dark, brownish red material settled through the gel and collected in the suction flask. Only when all of Solution I had been adsorbed, 002 m phosphate buffer, pH 7.3, was poured over the gel until the eluate had an ultraviolet absorption at 280 nm of less than 0.075. This was Solution II (4120 ml). The gel was discarded.

Step 6—Solution II was diluted to twice its volume with special water and its pH was adjusted to 5.8 with phosphoric acid. A suction funnel was again prepared as in Step 3. (In this instance, a 5-cm funnel was used.) The cationic exchange resin, CM-Sephadex C-50, was prepared and equilibrated with 0.01 m phosphate buffer, pH 5.8, and poured into the funnel as described in Step 3. The diluted Solution II was then poured over the gel. In this instance, it was more difficult to tell when the gel was overloaded. However, careful scrutiny disclosed a pale yellow band of adsorbed protein descending the gel. When Solution II was adsorbed, the gel was washed with several volumes of 0.01 m phosphate buffer, pH 5.8. All eluates were discarded. In this case the enzyme remained on the gel. The enzyme was first eluted with 100 ml of 0.2 m phosphate buffer, pH 7.3. Then 0.5 g of NaCl was dissolved into the remaining gel, and suction was applied. The dark, brownish red material settled through the gel and collected in the suction flask. Only when all of Solution I had been adsorbed, 002 m phosphate buffer, pH 7.3, was poured over the gel until the eluate had an ultraviolet absorption at 280 nm of less than 0.075. This was Solution II (4120 ml). The gel was discarded.

Step 7—Solution II was diluted to twice its volume with special water and its pH was adjusted to 5.8 with phosphoric acid. A suction funnel was again prepared as in Step 3. In this instance, a 5-cm funnel was used. The cationic exchange resin, CM-Sephadex C-50, was prepared and equilibrated with 0.01 m phosphate buffer, pH 5.8, and poured into the funnel as described in Step 3. The diluted Solution II was then poured over the gel. In this instance, it was more difficult to tell when the gel was overloaded. However, careful scrutiny disclosed a pale yellow band of adsorbed protein descending the gel. When Solution II was adsorbed, the gel was washed with several volumes of 0.01 m phosphate buffer, pH 5.8. All eluates were discarded. In this case the enzyme remained on the gel. The enzyme was first eluted with 100 ml of 0.2 m phosphate buffer, pH 7.3. Then 0.5 g of NaCl was dissolved into the remaining gel, and suction was applied. The dark, brownish red material settled through the gel and collected in the suction flask. Only when all of Solution I had been adsorbed, 002 m phosphate buffer, pH 7.3, was poured over the gel until the eluate had an ultraviolet absorption at 280 nm of less than 0.075. This was Solution II (4120 ml). The gel was discarded.

Summary of purification steps of β-glucuronidase

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
<th>Concentration</th>
<th>Specific activity</th>
<th>Total enzyme</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>2100 ml</td>
<td>7,650 mg/ml</td>
<td>24.0</td>
<td>318 Fishman units</td>
<td>15.9</td>
</tr>
<tr>
<td>Solution I</td>
<td>1,100 ml</td>
<td>7,975 mg/ml</td>
<td>4.6</td>
<td>1,735 Fishman units</td>
<td>8.8</td>
</tr>
<tr>
<td>Solution II</td>
<td>4,210 ml</td>
<td>890 mg/ml</td>
<td>0.3</td>
<td>2,960 Fishman units</td>
<td>3.8</td>
</tr>
<tr>
<td>Solution III</td>
<td>380 ml</td>
<td>7,700 mg/ml</td>
<td>1.2</td>
<td>6,410 Fishman units</td>
<td>3.0</td>
</tr>
<tr>
<td>Solution IV</td>
<td>820 ml</td>
<td>2,300 mg/ml</td>
<td>0.04</td>
<td>57,500 Fishman units</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* For liver homogenate, enzyme concentration is expressed as Fishman units per g, wet tissue, and specific activity is calculated by assuming 16% of wet tissue weight is protein.
Fig. 2. Schlieren patterns in the analytical ultracentrifuge: a, Solution III in different protein concentrations as indicated by upper and lower lines, and b, Solution IV. The solutions were concentrated by ultrafiltration prior to ultracentrifugation.

again applied. Two further washings with 100 ml each of 0.2 \text{m} phosphate, pH 7.3, were done. In each of these washings, the gel was thoroughly stirred with buffer; then suction was applied and the solution filtered through. These eluates were pooled to form Solution III (390 ml).

Step 5—Solution III was added to an equal volume of hydroxylapatite gel which had been equilibrated against 0.2 \text{m} sodium phosphate buffer, pH 6.8. (It is more effective to carry out this procedure in 100-ml aliquots in separate 250-ml centrifuge bottles.) The mixture was thoroughly shaken, then centrifuged at 1000 rpm for 10 min. The supernatant was decanted and the gel washed with an equal volume of 0.2 \text{m} phosphate buffer, pH 6.8, and recentrifuged. The supernatants were discarded and the enzyme remaining was adsorbed to the gel. The enzyme was eluted from the gel by thoroughly mixing the gel with 390 ml of 0.1 \text{m} phosphate, pH 6.8, in 2 \text{m} NaCl. This mixture was centrifuged, the supernatant was decanted, and the process was repeated. The gel was finally washed with 100 ml of 0.1 \text{m} NaHPO_{4} in 0.4 \text{m} NaCl. All supernatant fluids, which represented pure enzyme, were pooled to form Solution IV (820 ml).

**RESULTS**

The results of the purification procedure are shown in Table I. There is less total activity in liver homogenate than in the crude extract. This may reflect the intramicellar location of the enzyme or perhaps the presence of inhibitors. The subcellular particles are lysed upon overnight incubation.

The analytical ultracentrifuge and polyacrylamide gel electrophoresis were used as tests for protein homogeneity. By cutting an unstained gel into small segments, crushing it in a test tube, and incubating it in 0.2 \text{m} acetate buffer, pH 5.0, the enzyme activity could be localized in the gel. It consistently occurred 2 cm from the origin.

Electrophoresis was done at pH 9.0 in 0.1 \text{m} Tris-borate-EDTA buffer at 400 volts, 300 ma for 90 to 120 min. Fig. 1 shows the results of successive steps in purification. The final material shows a single band which migrates 1.5 cm from the origin. Contrary to reports by Plapp, Hopkins, and Cole (2), with bovine liver, pH 9.0 did not inactivate the enzyme. Electrophoresis at pH 4.5 gave poorer resolution than at pH 9.0. Paper electrophoresis was also used but was markedly inferior to polyacrylamide gel in resolving power.

The analytical ultracentrifuge proved inferior to acrylamide gel as a means of testing homogeneity. Fig. 2 is a photograph of the schlieren pattern of a concentrate of Solutions III and IV. In this figure, Solution III appears to be a homogenous peak but is actually quite impure when observed in gel electrophoresis (Fig. 1). The single peak of Solution III is identical with the peak in Solution IV as examined in the ultracentrifuge.
FIG. 4. Effect of temperature upon the activity of purified β-glucuronidase.

FIG. 5. Change in β-glucuronidase activity with pH. Solid lines represent enzyme without added albumin. Dotted lines represent enzyme assayed in presence of 0.1% bovine albumin. X—X, 0.1 M glycine-HCl buffer; ••, 0.1 M sodium acetate-acetic acid buffer; ○—○, 0.1 M NaH₂PO₄-Na₂HPO₄ buffer; and - - - - , 0.1 M citrate phosphate buffer.

Properties of β-Glucuronidase

Effect of Time of Hydrolysis—Under the stated assay conditions, the rate of liberation of phenolphthalein was linear for 1 hour, and 71 μg of phenolphthalein were liberated (Fig. 3). At substrate concentrations less than 0.5 mM, the rate of hydrolysis decreased with time.

Effect of Temperature—The enzyme was preincubated at various temperatures for 1 hour and then assayed at the stated temperature. The enzyme showed an increase in activity of about 8% per degree C. Above 70° the enzyme was inactivated (Fig. 4).

Effect of pH—The enzyme exhibited an optimum pH of 4.3 in 0.1 M acetate. The activity was inhibited by citrate below pH 4.5. The pH optimum was unchanged in the presence of albumin (Fig. 5). Contrary to results observed with bovine preparations (9), in which pH optima at 3.4, 4.5, and 5.2 have been shown, only one peak was observed.

Effect of Dilution of Enzyme—Various dilutions of the enzyme were prepared and incubated long enough to liberate 10 to 20 μg of phenolphthalein. The enzyme was preincubated for 2 hours with or without albumin and at zero time substrate was added. The results are shown in Fig. 6. In accordance with results published by Bernfeld et al. (10), the relative activity of the enzyme fell progressively with dilution. The activity was restored upon addition of 0.1% bovine albumin. This restoration

FIG. 6. Effect of dilution of β-glucuronidase upon specific activity. ••, purified enzyme without addition of albumin; and X—X, purified enzyme assayed in presence of 0.1% bovine albumin.

FIG. 7. Effect of substrate concentration on enzyme activity. X—X, enzyme incubated in presence of 0.1% bovine albumin; ○—○, enzyme incubated without albumin; and ▲—▲, enzyme in half of the previous concentration incubated with 0.1% bovine albumin.
was to 100% only above enzyme concentrations of 0.04 µg per ml. Below concentrations of 0.04 µg per ml, relative activity also began to decline, even in the presence of albumin. This decline paralleled the enzyme activity decline without albumin. However, albumin maintained a distinct activating effect at even the lowest concentrations.

**Effect of Substrate**—In order to study reaction kinetics, the enzyme assay was performed while varying the concentration of substrate. This was done with albumin at two different enzyme concentrations and without albumin at one concentration. The results are illustrated in Fig. 7. As noted in previous studies (11), an excess of phenolphthalein glucuronide inhibited the reaction. For this reason and since the reaction is not linear with time at low substrate concentrations, Lineweaver-Burk graphs of substrate concentration against velocity did not give straight lines and therefore, the apparent $K_m$ could not be accurately calculated. The value of $K_m$ was approximated from the data as that concentration of substrate giving half of the observed maximum velocity. There was some objection to this on the basis that maximum velocity will err on the low side because of substrate inhibition. The apparent $K_m$ thus obtained for phenolphthalein glucuronide as substrate was $4 \times 10^{-4} M$.

**Sedimentation Velocity and Molecular Weight** Purified β-glucuronidase was concentrated by ultrafiltration and various dilutions were prepared in 0.2 M phosphate buffer, pH 9.1. The solutions were studied in the Spinco model E ultracentrifuge at 59,780 rpm. Photographs of the schlieren pattern were taken at appropriate time intervals and measured in the microcomparator. Average $s$ values were calculated for each concentration and corrected for solvent density error due to temperature and buffer concentration ($s_0$) (12). The results are shown in Fig. 8. Protein concentration was determined from the change in refractive increment with interference optics. The sedimentation coefficient was found to be 5.728.

The molecular weight of β-glucuronidase was determined by sedimentation equilibrium according to the equation

$$M = \frac{dc}{dz} \times \frac{RT}{\omega^2(1 - \frac{V}{V_p})}$$

where $c_0$ is the concentration, $z$ is the distance from the center of rotation, $\omega$ is the angular velocity, $V$ is the partial specific volume, $\rho$ is the density of the solution, $R$ is the gas constant, $T$ is the absolute temperature, and $M$ is the molecular weight (13).

The partial specific volume was calculated from the amino acid composition (14) and found to be 0.722 ml per g. The molecular weight was calculated for four concentrations and the results extrapolated to zero concentration (Fig. 8). The molecular weight was found to be 218,000.

**Amino Acid Composition of β-Glucuronidase**—The enzyme was hydrolyzed for 22 hours in 6 N HCl at 110°C. The hydrolysate was then analyzed for amino acid composition. Tryptophan was measured in the unhydrolyzed protein by ultraviolet spectrophotometry. The amino acid composition is listed in Table II.

**Sulfhydryl Groups and S—S Bonds**—No sulfhydryl groups could be demonstrated spectrophotometrically upon addition of $p$-hydroxymercuribenzoate (15). The enzyme was not inhibited by 0.1 M mercaptoethanol.

### DISCUSSION

The purification of an enzyme is an important step in the systematic study of its properties. The purification of human liver β-glucuronidase has not been previously reported. This procedure for purification is somewhat simpler and gentler than procedures requiring solvent precipitation. The Sephadex ion exchange agents are particularly useful because of their great absorptive capacity. Previous investigators who tried using ion exchange found that its application was difficult because of enzyme loss upon dialysis against weak buffers (16). The use of
specially prepared water and dialysis tubing eliminated these losses and allowed 100% recovery following dialysis. The final step, adsorption on hydroxylapatite, may need to be repeated if optimal specific activity is not attained. This delay could be eliminated if only small (100 ml) amounts are adsorbed in any one container.

The evidence for homogeneity was strong. Gel electrophoresis is a powerful method in resolving proteins. However, we cannot say that further purification is not possible or even that the majority of protein in the single band is enzyme. Thus far, it has not been possible to separate more inactive protein.

Properties of the enzyme are, in general, similar to those of enzyme from other mammalian sources. These properties include the activation of purified dilute enzyme by albumin and the drop in specific activity of enzyme upon dilution. The single pH optimum of 4.3 did not change in the presence of albumin. Other investigators have noted two (17, 18) or even three (9) pH optima from various mammalian sources. The explanation for more than one optimum is obscure, but impurities such as heavy metals or DNA are known to alter the pH optima (19).

Physical characteristics have not been previously reported. The sedimentation constant was found to be 5.72S. Molecular weight was 218,000. The amino acid composition was remarkable because of the small amount of sulfur-containing amino acids. This correlates with our preliminary observations that the enzyme is not inhibited by p-hydroxymercuribenzoate or mercaptoethanol. No sulfhydryl groups were demonstrated by titration with p-hydroxymercuribenzoate.

SUMMARY

1. A 2900-fold purification of β-glucuronidase from human liver was achieved utilizing ammonium sulfate precipitation, ion exchange, and hydroxylapatite gel adsorption. The preparation exhibited homogeneity by acrylamide gel electrophoresis and analytical ultracentrifuge.

2. The protein had a sedimentation constant of 5.72S; the molecular weight was 218,000. Amino acid composition showed paucity of sulfur-containing amino acids. No sulfhydryl groups could be demonstrated. Disulfide bonds did not appear to be essential for enzyme activity.

3. A study of enzymic properties showed a single pH optimum at 4.3. There was an 8% increase in activity for every degree C rise in temperature, and inactivation of the enzyme above 70°. Enzyme activity decreased upon dilution, but was restored by albumin. At very low concentrations, albumin did not restore full activity. The enzyme was inhibited by the substrate phenolphthalein glucuronide.

Acknowledgments—We are grateful to the following people for their assistance: Dr. Ronald Roberts for assisting and teaching the use of the ultracentrifuge; Miss Janet Johnson for running the amino acid analyses; Miss Mary Miller for preparation of the manuscript; Mr. Lawrence Besner, Mr. Walter Shaw, and Mr. Merrill Bauman for preparing the illustrations; and Miss Mary Lewis for technical assistance.

REFERENCES

CORRECTIONS

We have learned, subsequent to the publication of our recent paper in the Journal (Vol. 240, No. 6, June 1965, PC2764), that F. Karush (J. Am. Chem. Soc., 78, 5519 (1956)) used a competitive dialysis procedure to test the binding of antibody-antigen complexes. Although the purposes of his study were somewhat different from ours, the procedures were similar, and we regret our failure to mention this important reference in the original text.

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In the paper by Byron U. Musa, R. P. Doe, and Ulysses S. Seal (Vol. 240, No. 7, July 1965, page 2811), page 2812, left-hand column, line 16 of the paragraph headed "Step 3," "002. M phosphate" should read "0.02 M phosphate."

In the paper by Stuart Tauber and Leonard L. Madison (Vol. 240, No. 2, February 1965, page 645), the following changes should be made in Table IV. The column headed "Found" should read "Grams of amino acid residue per 100 g of gastrin," and the column headed "Calculated" should read "Calculated residues per molecule of gastrin." The italic heading, "residues/molecule," should be deleted.

In the Preliminary Communication by Koji Toji, Elward Bynum, Elaine Norris, and Harvey A. Itano (Vol. 240, No. 8, August 1965, PC3455), Figs. 3 and 4, but not their respective legends, should be transposed.
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