Chromatography of Rat Liver Soluble Proteins and Localization of Enzyme Activities*

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(Received for publication, November 20, 1959)

Substituted cellulose ion exchange columns have been used for the fractionation of serum proteins and for the purification of a number of enzymes from various sources (1, 2). Furthermore, the distribution of proteins among fractions of the soluble portion of liver has been studied extensively by electrophoretic and ultracentrifugal techniques (3-6). Such methods are valuable for broad surveys and for the comparison of enzymes, or proteins in general, in normal and in altered tissues, such as cancerous and precancerous tissues. Evidence has been obtained recently that the same enzyme may exist in several separable forms within the same tissue (7-11). These methods could also be used to investigate multiple forms of the same enzyme, and the states of enzymes as they exist in the supernatant fractions of tissue homogenates.

This paper will show that the study of soluble proteins of liver homogenates may be approached by use of substituted cellulose anion exchange chromatography. Enzyme activities can be localized in the various protein fractions and their characteristic and reproducible patterns of distribution demonstrated.

METHODS

Preparation of Extracts—Normal Sprague-Dawley rats, 200 to 250 g, were decapitated and the livers perfused with 0.25 M sucrose by way of the aorta. All further operations were carried out at 2-4°C unless otherwise stated. The livers were weighed, homogenized in 2 volumes of 0.25 M sucrose with a Teflon pestle homogenizer, and the homogenate was centrifuged at 20,000 × g for 3 to 4 hours. The clear supernatant fluid was dialyzed for 20 hours against 2 liters of 0.005 M Tris-phosphate, pH 8.0, and immediately applied to the chromatography column. There was evidence that freezing and thawing of the extract resulted in slight alterations in chromatographic patterns and, therefore, the supernatant fluids were used unfrozen.

Preparation of Columns—The anion exchanger, DEAE-cellulose (12) (Distillation Products Industries) required further purification to remove yellow ultraviolet-absorbing material. Twenty grams of the DEAE-cellulose were washed with 1 liter of 1 N NaOH, followed by water to neutrality, then ethanol, and finally ether. The product was dried at room temperature and suspended in the starting buffer to give a 2% suspension, which was adjusted to pH 8.0. The DEAE-cellulose had an exchange capacity of 1.00 ± 0.05 meq per g.

The columns were packed at 10 pounds per square inch, as described by Peterson and Sober (1), to a depth of 30 cm in 1.1 cm inside diameter tubes with fritted glass bottoms. They were then washed at least 20 hours with 0.005 M Tris-phosphate, pH 8.0, at a flow rate of 12 to 15 ml per hour, before they were used.

Elution of Columns Two hundred to 250 mg of dialyzed soluble rat liver proteins in a volume of 20 ml, which represented one rat liver, were applied to the column and allowed to soak in by gravity. Fractions were collected when the protein was first applied to the column. After all the extract had entered, 5 to 10 ml of 0.005 M Tris-phosphate, pH 8.0, were used to wash down the column, which then was eluted with 120 to 150 ml of the same buffer in order to remove unadsorbed and loosely adsorbed material. The remaining material was then eluted by a parabolic chloride gradient developed by a modification of the methods described by Peterson and Sober (13). Three open 250 ml Erlenmeyer flasks were connected in series by means of tubes at their bottoms as shown in Fig. 1. Flasks 1 and 2, which were magnetically stirred, each contained 200 ml of the starting buffer (0.005 M Tris-phosphate, pH 8.00 ± 0.03), and Flask 3, 200 ml of the limit buffer (1.0 M sodium chloride, 0.05 M sodium phosphate, pH 6.50 ± 0.03). Values of pH were determined at room temperature.

It was verified by chloride titration that the chloride concentration of the effluent from the reservoirs (C) bears the following relationship to the limit concentration (C0), the volume of effluent (V), and the total volume of the flasks (V0) (13):

C = C0(V/V0)3

Fractions were collected at 20-minute intervals and the volumes varied from approximately 3 ml at the beginning to approximately 5 ml at the end of elution. Volumes of fractions were measured by comparing the levels in the tubes with a calibrated tube; when these volumes were summed over the entire chromatogram the result always agreed within 2% of the actual volume used to elute the column.

Treatment of Fractions Protein content in each fraction was measured by the method of Lowry et al. (14), with serum as a protein standard. Absorbancy at 260 and 280 mμ was measured and in addition certain regions containing heme proteins were measured at 413 mμ. The pH of each fraction was also taken. Within 8 hours after each fraction appeared, an aliquot was pipetted into a small tube containing enough 1 M 2-mercaptoethanol to give a final concentration of 0.01 M. This was found necessary in order to protect certain enzyme activities from rapid inactivation. The 2-mercaptoethanol was added after the chromatography since it interferes in the protein determination.

Enzyme Activity Measurements—Preliminary columns were run and the fractions were screened for all the enzyme activities...
in order to obtain an approximate localization. In subsequent columns, only the necessary fractions were assayed for each activity. All enzyme activity determinations were performed in total incubation volumes of 50 to 200 μl. Volumes were measured in Lang-Levy constriction pipettes (15); optical densities were measured in microcells (16) (Pyrocell) in a Beckman DU spectrophotometer equipped with a photomultiplier, and in some cases oxidized or reduced pyridine nucleotides were measured by fluorometry (17) in a Farrand fluorometer. Incubations were at 38°C and in most cases for a time of 30 minutes. Bovine plasma albumin was generally present in the incubation medium in order to stabilize the enzymes. Table 1 shows the conditions employed for each enzyme activity determination. All enzyme activities were shown to be linear with respect to concentration of enzyme under the conditions used with the exception of glycer-aldehyde-3-P dehydrogenase. However, recoveries were not calculated for this enzyme, since its activity could not be measured in the extract due to the presence of α-glycerophosphate dehydrogenase.

Sources for substrates and other reagents were as follows: DPN+, TPN+, DPNH, TPNH, disodium glucose-6-P, sodium 6-phosphogluconate, L-alanine, α-ketoglutaric acid, L-aspartic acid, phenolphthalein β-glucuronide, and Tris, from the Sigma Chemical Company; ni-isocitric acid lactone, d-glyceraldehyde, L-malic acid, cyclohexylamine salt of dihydroxyacetone phosphate dimethylketal, dioxane complex of d-glyceraldehyde-1-bromide 3 phosphoric acid, from California Corporation for Biochemical Research; fructose 1,6-diphosphate, from Schwarz Laboratories; sodium pyruvate, from General Biochemicals; 2-amino-2-methyl-1-propanol and 2-amino-2-methyl-1,3-propanediol from Distillation Products Industries (the 2-amino-2-methyl-1,3-propanediol was recrystallized from ethyl acetate). Other chemicals were reagent grade, commercial products.

Rechromatography Experiments—Appropriate fractions selected for rechromatography were pooled (after P-mercaptoethanol had been added). The combined material was dialyzed against 2

**Table 1**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Buffer</th>
<th>pH</th>
<th>Measured</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Glucose-6-P dehydrogenase</td>
<td>glucose-6-P</td>
<td>2-amino-2-methyl-1,3-propanediol</td>
<td>9.5</td>
<td>TPNH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18</td>
</tr>
<tr>
<td>(2) 6-Phosphogluconate dehydrogenase</td>
<td>6-phosphogluconate</td>
<td>Tris phosphate</td>
<td>8.2</td>
<td>TPNH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19</td>
</tr>
<tr>
<td>(3) Isocitrate dehydrogenase</td>
<td>D-isocitrate</td>
<td>Tris phosphate</td>
<td>8.1</td>
<td>TPNH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20</td>
</tr>
<tr>
<td>(4) Glutamic-pyruvic transaminase</td>
<td>L-alanine + α-ketoglutaric acid</td>
<td>DPNHe</td>
<td>7.4</td>
<td>DPNH&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21</td>
</tr>
<tr>
<td>(5) Glutamic-oxaloacetic transaminase</td>
<td>L-aspartate + α-ketoglutarate</td>
<td>DPNHe</td>
<td>7.4</td>
<td>DPNH&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22</td>
</tr>
<tr>
<td>(6) α-Glucuronidase</td>
<td>phenolphthalein β-glucuronide</td>
<td>Acetate</td>
<td>5.0</td>
<td>phenolphthalein&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23</td>
</tr>
<tr>
<td>(7) Glycerol dehydrogenase (TPN&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>glycerol or d-glyceraldehyde</td>
<td>triethanolamine</td>
<td>9.5</td>
<td>TPNH&lt;sup&gt;f&lt;/sup&gt;</td>
<td>24</td>
</tr>
<tr>
<td>(8) Malic dehydrogenase</td>
<td>L-malate</td>
<td>triethanolamine</td>
<td>7.0</td>
<td>TPNH&lt;sup&gt;g&lt;/sup&gt;</td>
<td>24</td>
</tr>
<tr>
<td>(9) Aldehyde dehydrogenase</td>
<td>acetaldehyde</td>
<td>pyrophosphate</td>
<td>9.3</td>
<td>DPNH&lt;sup&gt;h&lt;/sup&gt;</td>
<td>25</td>
</tr>
<tr>
<td>(10) Phosphohexoisomerase</td>
<td>glucose-6-P</td>
<td>pyrophosphate</td>
<td>9.3</td>
<td>DPNH&lt;sup&gt;i&lt;/sup&gt;</td>
<td>25</td>
</tr>
<tr>
<td>(11) Lactic dehydrogenase</td>
<td>pyruvate</td>
<td>Tris phosphate</td>
<td>7.5</td>
<td>fructose (color rgt.)</td>
<td>18</td>
</tr>
<tr>
<td>(12) Aldolase</td>
<td>fructose-1,6-di-P</td>
<td>Hydrazine</td>
<td>8.2</td>
<td>color with dinitrophenylhydrazine&lt;sup&gt;j&lt;/sup&gt;</td>
<td>18</td>
</tr>
<tr>
<td>(13) Alcohol dehydrogenase</td>
<td>ethanol</td>
<td>Glycine</td>
<td>9.6</td>
<td>DPNH&lt;sup&gt;k&lt;/sup&gt;</td>
<td>27</td>
</tr>
<tr>
<td>(14) Glutamic dehydrogenase</td>
<td>L-glutamate</td>
<td>Phosphate</td>
<td>7.4</td>
<td>DPNH&lt;sup&gt;k&lt;/sup&gt;</td>
<td>28</td>
</tr>
<tr>
<td>(15) α-Glycerophosphate dehydrogenase</td>
<td>dihydroxyacetone phosphate</td>
<td>Triethanolamine</td>
<td>7.5</td>
<td>DPNH&lt;sup&gt;k&lt;/sup&gt;</td>
<td>29</td>
</tr>
<tr>
<td>(16) Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>glyceraldehyde-3-phosphate</td>
<td>Pyrophosphate + arsenate</td>
<td>8.4</td>
<td>DPNH&lt;sup&gt;k&lt;/sup&gt;</td>
<td>30</td>
</tr>
</tbody>
</table>

<sup>a</sup> Spectrophotometric.
<sup>b</sup> Fluorometric.
<sup>c</sup> B. W. Moore, unpublished results.
liters of 0.005 M Tris-phosphate, pH 8.0 in 0.01 M 2-mercaptoethanol, and then applied to a 1.1 X 30 cm DEAE-cellulose column. The chloride gradient elution was carried out as described above except both the starting and limit buffers were in 0.01 M 2-mercaptoethanol.

Thiol interferes in the Lowry protein determination and therefore a modification was made as follows. The protein in 100 to 500 μl of each fraction was precipitated by adding half that volume of 30% trichloroacetic acid and centrifuged. The precipitate was washed once with 0.5 ml of 10% trichloroacetic acid and dissolved in 100 μl of 1 N NaOH. Protein determinations were carried out as previously described. Enzyme activity measurements were made on the rechromatographed fractions exactly as described before.

RESULTS

Protein and Nucleic Acid Distribution—In 10 chromatograms of separate rat liver preparations proteins always separated into a number of peaks (see Figs. 2, 3, 4, and 5). The numbered peaks are those which appeared consistently in nearly all the chromatograms at the same effluent chloride concentrations. The pH of the effluent dropped smoothly from 8.0 to 6.5 with no sudden changes (as shown in Fig. 2). The chloride concentrations shown are calculated values for the effluent from the column, taking into consideration the hold-up volume of the column and the tubing leading to the column from the reservoir. The protein eluted from the column by 0.4 M chloride was followed by a sharp peak of 260 μm-absorbing material, assumed to be RNA, eluted at 0.5 M chloride. The RNA consistently showed evidence of a second small peak at 0.6 M chloride (Fig. 2). A large amount (20 to 30%) of the protein appeared in a basic fraction (Peak 1) which was not adsorbed at pH 8.0. The protein in Peaks 1 to 19 showed a typical protein ultraviolet absorption with 280 to 260 ratios of 1.6 to 1.7, and the RNA peak showed typical absorption with 280 to 260 ratios of 0.55.

Two lots of DEAE-cellulose, both with an exchange capacity near 1.0 meq per g were used in the fractionations, and some minor differences between the batches were noted in the protein and enzyme patterns. These differences were confined to the region before the chloride gradient was applied. With the first lot (Fig. 2, as well as 5 other chromatograms) a sharp protein peak appeared immediately after Peak 1. This contained the second peak of glutamic-oxaloacetic transaminase and the peak of TPN+ glycerol dehydrogenase (see "Enzyme Activity Distribution" below). With the second lot, the sharp peak was spread out and the two enzymes appeared later (Figs. 3, 4, and 5). No differences were seen in the protein or enzyme patterns with the two lots after the chloride gradient was applied. Apparently the loosely bound proteins were bound more tightly to the second lot than to the first.

Recoveries of protein (Lowry method) (14) and of optical density were 65 to 78% when the elution was carried out as described above. In one experiment, after the gradient elution to 1 M chloride had been carried out, an additional linear gradient elution to 2 M chloride, 0.04 M NaOH with a total volume of 200 ml, resulted in an additional 10% recovery of protein. Some nucleic acid was also present in this material since the 260 to 280 ratios were approximately 1.0. No enzyme activities were measured, and work is in progress to attempt to further fractionate and characterize this material.

Enzyme Activity Distribution—In Fig. 3 are shown the distributions of six of the enzymes measured. Other chromatograms gave essentially the same patterns for these enzymes, with minor variations in relative peak heights. The greatest variation was

![Fig. 2. Chromatogram on 0.9 X 30 cm column of DEAE-cellulose of 292 mg of liver soluble proteins.](http://www.jbc.org/)

- Protein
- Absorbancy at 260 μm
- M chloride
- pH

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in relative heights of the glucose-6-P dehydrogenase peaks in the region 0.20 to 0.28 M chloride and 0.32 to 0.37 M chloride. The glucose-6-P dehydrogenase activity between 0.20 to 0.23 M chloride was labile in the absence of 2-mercaptoethanol and lost much of its activity in 1 to 2 days. The peak marked Gly-DH is a TPN+ specific glycerol dehydrogenase not described previously. A more detailed report on its properties will be published elsewhere (31). In addition to the six enzymes shown in Fig. 3, other activities, lactic dehydrogenase, aldolase, isomerase, alcohol dehydrogenase, glutamic dehydrogenase, and aldehyde dehydrogenase, were found to be localized exclusively in the basic fraction, Peak 1.

In Fig. 4 are shown the distributions in another column of several additional enzymes as well as some of the same ones shown in Fig. 3. Malic dehydrogenase and glyceraldehyde-3-P dehydrogenase appeared not only in the basic peak but also in later fractions. α-Glucuronidase dehydrogenase showed one sharp peak at 0.13 M chloride.

In Table II are shown the positions at which the various enzyme activities consistently appeared in a number of chromatograms, along with the total activity of the enzyme recovered from the column, and the apparent percentage recovery. The percentage recoveries were calculated by dividing the total activity of the enzyme eluted from the column by the total amount measured in the extract added, and therefore may be in error if there were activators or inhibitors in the extract, or if there were other sources of error inherent in use of crude extracts.

Proteins containing heme groups, evidenced by absorption at 413 μm, were distributed characteristically as shown in Table II. There was no evidence of other pigmented proteins even when pooled fractions were concentrated 15 to 20 times by lyophilization.

A number of the enzyme activities separated into two or more peaks (Figs. 3 and 4, Table II). Glucose-6-P dehydrogenase always showed three major fractions, the middle one of which is composed of several closely spaced peaks. Glutamic-oxaloacetic transaminase appeared not only in the basic fraction but also somewhat later as a sharp peak; the same held true for malic dehydrogenase. β-Glucuronidase and glutamic-pyruvic transaminase each consisted of a number of closely spaced peaks spread over a considerable region. When the basic fraction was rechromatographed on carboxymethyl cellulose cation exchanger, some of the enzymes in this fraction again split into 2 or more peaks each. The chromatograph of the basic peak on carboxymethyl cellulose will be reported in detail later.

Rechromatography Three regions from one column were rechromatographed with the use of the same volume and elution schedule as described above. These results along with the original chromatogram are shown in Fig. 5. The percentage recoveries of protein and enzyme activity paralleled each other as shown in Table III.

Both protein and enzyme activity appeared as single peaks at the anticipated positions. The second peak of glutamic-oxaloacetic transaminase was not present in the second chromatogram, and neither were the second and third peaks of glucose-6-P dehydrogenase. In Fig. 5B, the un asymmetrical portion of the protein curve at its leading edge probably represents contamination of Peak 11 by some of the trailing edge of Peaks 8, 9, or 10. Peaks 15 and 16, from another column (not shown here) containing the middle peak glucose-6-P dehydrogenase, were also re-

Fig. 3. Localization of enzyme activities. GO-T and GP-T are glutamic-oxaloacetic and glutamic-pyruvic transaminase; GlyDH is TPN+ specific glycerol dehydrogenase; ICDH, isocitric dehydrogenase; GPDH, glucose-6-P dehydrogenase; PGDH, 6-phosphogluconic dehydrogenase. Activities are in μmoles per ml per hr.

Fig. 4. Localization of enzyme activities. MDH is malic dehydrogenase; GAP DH, glyceraldehyde-3-P dehydrogenase; α-GP DH, α-glycerol-P dehydrogenase; G-6-PDH, glucose-6-P dehydrogenase; β Gluc, β glucuronidase. Activities are in μmoles per ml per hr.
Table II
Summary of elution patterns and apparent recoveries of liver enzymes from DEAE-cellulose

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Position eluted, M Cl−</th>
<th>Total activity recovered from 20 ml extract</th>
<th>Apparent recovery</th>
<th>No. of columns</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Lactic dehydrogenase</td>
<td>0°</td>
<td>113,000-222,000</td>
<td>83-116</td>
<td>4</td>
</tr>
<tr>
<td>(2) Isomerase</td>
<td>0°</td>
<td>6,400</td>
<td>96</td>
<td>1</td>
</tr>
<tr>
<td>(3) Aldolase</td>
<td>0°</td>
<td>0,090</td>
<td>90</td>
<td>1</td>
</tr>
<tr>
<td>(4) Glutamic dehydrogenase</td>
<td>0°</td>
<td>40.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(5) Aldehyde dehydrogenase</td>
<td>0°</td>
<td>26-36</td>
<td>86-90</td>
<td>1</td>
</tr>
<tr>
<td>(6) Alcohol dehydrogenase</td>
<td>0°</td>
<td>442-636</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>(7) Glutamic-oxaloacetic transaminase</td>
<td>I 0°</td>
<td>8,800-15,700</td>
<td>64-113</td>
<td></td>
</tr>
<tr>
<td>(8) Glyceraldehyde-3-P dehydrogenase</td>
<td>I 0.01</td>
<td>42-52</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>(9) Malic dehydrogenase</td>
<td>I 0°</td>
<td>817-2,110</td>
<td>38-42</td>
<td>3</td>
</tr>
<tr>
<td>(10) Glycerol dehydrogenase (TPN+)</td>
<td>I 0.06-0.08 (3 fused peaks)</td>
<td>161-179</td>
<td>60-100</td>
<td>3</td>
</tr>
<tr>
<td>(11) 6-Phosphogluconic dehydrogenase</td>
<td>I 0.08 (sharp)</td>
<td>951-1,904</td>
<td>56-85</td>
<td>4</td>
</tr>
<tr>
<td>(12) Isocitric dehydrogenase</td>
<td>I 0.09 (sharp)</td>
<td>498-797</td>
<td>35-53</td>
<td>2</td>
</tr>
<tr>
<td>(13) α-Glycerophosphate dehydrogenase</td>
<td>I 0.13</td>
<td>15,200-20,000</td>
<td>82-98</td>
<td>5</td>
</tr>
<tr>
<td>(14) β-Glucuronidase</td>
<td>I 0.07 (small peak)</td>
<td>14.8-44.6</td>
<td>52-105</td>
<td>5</td>
</tr>
<tr>
<td>(15) Glucose-6-P dehydrogenase</td>
<td>I 0.11 (sharp)</td>
<td>14.8-44.6</td>
<td>52-105</td>
<td>5</td>
</tr>
<tr>
<td>(16) Glutamate-pyruvic transaminase</td>
<td>I 0.12-0.20 (fused peaks)</td>
<td>4,370-6,130</td>
<td>52-94</td>
<td>2</td>
</tr>
<tr>
<td>(17) 413-mg material</td>
<td>I 0° (small)</td>
<td>169-211mg</td>
<td>65-78</td>
<td>7</td>
</tr>
<tr>
<td>(18) Protein</td>
<td>I 0.48-0.52</td>
<td>65-78</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

* Appeared in protein Peak 1 and not adsorbed by DEAE-cellulose (Figs. 2, 3, and 4).
* In arbitrary optical density units.
* See text under "Enzyme Activity Measurements."
* Appeared before chloride gradient was begun and was eluted with 130 ml of the starting buffer.

The results show that the soluble proteins of liver homogenate can be resolved on a cellulose anion exchanger to give a characteristic pattern of protein and nucleic acid distribution and a consistent pattern of distribution of a number of enzyme activities among the fractions. With the exception of those found in the basic fraction, no two enzyme activities exactly overlapped. The apparent recoveries of activities were high except in the region where 6-phosphogluconic, malic, and isocitric dehydrogenases appeared.

The high resolution of the chromatographic method is demonstrated by the fact that some of the activities appeared as sharp, approximately symmetrical peaks, such as those for TPN+ specific glycerol dehydrogenase, 6-phosphogluconic dehydrogenase at 0.08 M chloride, α-glycerophosphate dehydrogenase at 0.13 M, the glucose 6-phosphate dehydrogenase peaks at 0.11 M and 0.24 M, and the second peak of glutamic-oxaloacetic transaminase at 0.01 M. Also, the nucleic acid in the extract, presumably RNA, was well separated from the protein.

The patterns also show that some information can be obtained about the ionic properties and other characteristics of enzymes in the tissue extract. Most of the glycolytic enzymes assayed for appeared in the basic fraction (Peak 1 in Figs. 2, 3, 4). The only exception was the second peak of glyceraldehyde-3-P dehydrogenase which represents approximately 17% of the total activity of this enzyme. This probably reflects the relative basicity of the proteins in Peak 1 at the low ionic strength of the starting buffer. For example, glyceraldehyde-3-P dehydrogenase has isoelectric pH values of 7.2, 6.6, and 5.9 at ionic strengths of 0.05, 0.1, and 0.2, respectively, in phosphate buffers, and, when extrapolated to zero ionic strength the value is above 8.0 (32). Similarly, muscle aldolase has an extrapolated isoelectric pH of 9 at zero ionic strength (33), and bovine liver aldolase has an isoelectric pH of 6.6 to 6.7 (34, 35). The isoelectric pH of liver lactic dehydrogenase at ionic strength 0.1 is 6.3 (36), and of liver alcohol dehydrogenase is 7.0 (37). Crystalline bovine serum albumin which has an isoelectric pH about 4.8 at ionic strength 0.1, when chromatographed alone under the same conditions used for liver supernatant, appeared as a sharp peak at 0.11 M chloride.

Many of the enzyme activities appeared as multiple peaks, the present data does not rule out any of several explanations. For example, in addition to the possibility that there exist structurally different proteins with the same enzyme activity, alternatively there may be binding between the enzyme and large or
small molecules giving rise to several separately migrating species. The bound molecules could be proteins, nucleic acids, carbohydrates, substrates, or lipids. The rechromatography experiments show that, in the case of glucose-6-P dehydrogenase and glutamic-oxaloacetic transaminase, the differences between the several forms are stable during chromatography, and therefore must not be loosely bound complexes.

**TABLE III**

<table>
<thead>
<tr>
<th>Peak rechromatographed</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>99.2</td>
</tr>
<tr>
<td>7</td>
<td>84.9</td>
</tr>
<tr>
<td>11</td>
<td>103.3</td>
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</table>

**SUMMARY**

A method was developed for chromatography of rat liver soluble proteins on diethylaminoethyl cellulose, with a parabolic chloride gradient for elution. Protein, nucleic acid and 16 enzyme activities were determined in the fractions, and each gave a consistent pattern of distribution in a number of preparations.

Some of the enzyme activities, for example, lactic dehydrogenase, isomerase, and aldolase, were not adsorbed by the diethylaminoethyl cellulose at pH 8.0 and low ionic strength. Several of the enzymes gave multiple peaks of activity. With two of the multiple peak enzymes, glucose 6-phosphate dehydrogenase and glutamic-oxaloacetic transaminase, rechromatography of one of the peaks, under the same conditions, gave a single peak at the anticipated position.

**REFERENCES**

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