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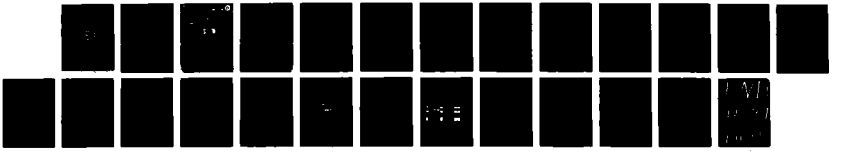

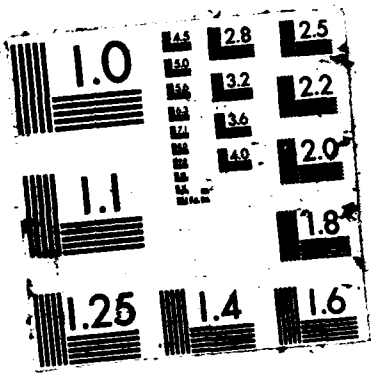
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A purification scheme for human plasma dopamine-B-hydroxylase was developed incorporating affinity chromatography on CON A-Sepharose, and Red Sepharose CL-6B, ion exchange chromatography and gel filtration. This procedure yielded a purified enzyme preparation with an apparent molecular weight of about 450,000 using gradient gel electrophoresis. The specific activity of the purified enzyme was 7.8 IU/mg of protein and represented a more than 11,000 fold purification from the crude plasma fraction.

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

Commanding Officer
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INTRODUCTION

Dopamine-β-hydroxylase (E.C. 1.14.17.1) (DBH) catalyzes the biosynthesis of norepinephrine from dopamine in the biosynthetic pathway for catecholamines. The enzyme is localized within the synaptic vesicles of the sympathetic nerve terminals, the storage vesicles of adrenal medulla chromaffin cells (1), and peripheral and central sympathetic nerve terminals (2). The most frequently used source of the enzyme is the bovine adrenal medulla, but it has been isolated from sheep adrenals (3), rat adrenals (4), human pheochromocytoma (5), and human serum or plasma (6).

The release of DBH accompanies the secretion of neurotransmitters by exocytosis from the vesicles of sympathetic nerve terminals and from the storage vesicles of adrenal medulla chromaffin cells (7). Consequently, the enzyme has been considered a potential marker for the study of noradrenergic nerve and chromaffin cell function. There have been many attempts to use serum DBH activity measurements as an index of sympathetic nerve activity or associated adrenergic dysfunction (8). Levels of the enzyme are elevated in patients with pheochromocytoma and decline after removal of the tumor. However, plasma levels vary widely among individuals. The variations may be related more to genetic factors than to sympathetic nerve activity (9). → over (pg 2

Bovine and human DBH have structural similarities. Both exist in soluble and membrane bound forms (10), and are composed of four major subunits with a total molecular weight of about 300,000. Under various reducing or denaturing conditions species of molecular weights between 130,000 to 160,000 and species between 75,000 to 77,000 are obtained (11, 12, 13). Only the tetrameric form of the bovine enzyme is active while both the tetrameric and a dimeric form of the human enzyme seem to be catalytically active species. There does not seem to be any interconversion of the two forms of the human enzyme (13).

There is ample evidence that cold stress can have a profound effect on the ability of troops to adequately wage a military campaign. The recent experiences of the Royal Marines in the Falklands have been documented by the British correspondents, Hastings and Jenkins, in their book "The Battle for the Falklands" (14). They describe how cold

induced problems of exhaustion, diarrhea, and trench foot severely hampered the Marines' mission. There is substantial evidence that norepinephrine (NE) plays an important role in initiating cold induced nonshivering thermogenesis (NST) in rats and other animals after acclimation to cold (15). The levels of the norepinephrine synthesizing enzyme, DBH, in serum may prove useful as an indicator of the degree of cold acclimation in man. Although the plasma levels of DBH activity do not seem to be a useful indication of cold stress, the significance of the active dimeric and tetrameric forms of the human enzyme is not clear nor has the presence of isozymes been tested. Perhaps the levels of these forms vary with the degree of cold acclimation. This report describes a purification procedure for human plasma DBH as a preliminary step in the development of an analytical tool for studying the various forms of the human enzyme in crude preparations, including plasma. This may serve as the basis for continuing studies on the levels of DBH in cold stress or acclimation and on ways to ameliorate the adverse effects of cold stress.

EXPERIMENTAL PROCEDURES

MATERIALS

Normal fresh frozen human plasma was obtained from a local blood bank. Dextran sulfate, Red Sepharose CL-6B, and Concanavilin A-Sepharose were purchased from Pharmacia Fine Chemicals. Trisacryl M-DEAE was obtained from LKB and Bio-Gel A-0.5 M, electrophoresis grade acrylamide, N,N-methylenebisacrylamide, SDS and TEMED were obtained from Bio Rad Laboratories. Other chemicals were obtained as reagent grades and used without further purification.

METHODS

Plasma fractionation units of fresh frozen human plasma was thawed overnight at 4°C. A 1300 to 1800 ml volume of the pooled plasma was adjusted to pH 7.0 by the addition of 500 ml of 0.08 M sodium phosphate, 2 M NaCl, pH 7.0. All subsequent steps were performed at 4°C. Ten milliliters of 50% (w/v) dextran sulfate in water followed by the addition of 200 ml of 50% (w/v) polyethylene glycol were slowly added over a period of 20-30 minutes with stirring to precipitate plasma lipoproteins, globulins, and fibrinogen. The suspension was stirred for at least 60 minutes or in some cases overnight, and the precipitate collected by centrifugation at 5000xg in a Sorvall GSA rotor for 30 minutes.

Glycoprotein Affinity Chromatography: A 5 cm x 18 cm column of Con A-Sepharose was equilibrated with 20 mM sodium phosphate, 500 mM NaCl, 0.5 mM $MnCl_2$, 0.5 mM $CaCl_2$, pH 7.0. The supernatant from the previous step was applied to the column at a flow rate of 30 ml/h and the column was washed with 2 to 3 L of 20 mM sodium phosphate, 500 mM NaCl, pH 7.0 until the absorbance of the effluent at 280 mM was less than 0.1. The bound DBH activity was eluted with a 1500 ml solution of 10% (w/v) α-methyl-D-mannopyranoside in 20 mM sodium phosphate, 500 mM NaCl, pH 7.0 at a flow rate of 60 ml/h.

Ion exchange chromatography: The enzyme fractions from the Con A-Sepharose chromatography were pooled, concentrated and diafiltered using an Amicon DC2 with a H2P 10-20 cartridge; 5 mM phosphate, pH 6.5 was used as the replacement buffer. Alternatively, the pooled fractions were concentrated using an Amicon ultrafiltration device with a PM-30

membrane. The preparation was dialyzed against several changes of 5 mM phosphate buffer, pH 6.5 and then applied at 30 ml/h to a 5 cm x 15 cm column of Trisacryl M-DEAE previously equilibrated with 5 mM phosphate buffer, pH 6.5. The column was washed with the above buffer until the absorbance of the effluent measured at 280 nm was less than 0.05. DBH activity was eluted from the column with a 500 ml linear gradient from 0 - 200 mM NaCl in 5 mM phosphate, pH 6.5. Five milliliter fractions were collected at a flow rate of 30 ml/h.

Red Sepharose Affinity Chromatography: The active fractions of DBH activity from the Trisacryl M-DEAE column were pooled and applied without dialysis to a 2.5 cm x 15 cm. Red Sepharose Cl-6B column equilibrated with 5 mM phosphate buffer, pH 6.5. The column was washed with the same buffer and DBH was eluted with a 500 ml linear gradient from 0 - 3.0 M NaCl in 5 mM phosphate, pH 6.5.

Gel Filtration: The active fractions from Red Sepharose Cl-6B were pooled and concentrated to a volume of 6 ml using an Amicon ultrafiltration device with a PM-30 membrane. The sample was applied to a 2.5 cm x 90 cm Bio-Gel A-0.5 M column equilibrated with 5mM phosphate, pH 6.5 and eluted with this buffer at 15 ml/h. The eluted enzyme was again concentrated and rechromatographed on the column. The pooled enzyme fractions were stored at 4°C in the same buffer.

ANALYTICAL METHODS

Enzyme Assay: Dopamine-β-hydroxylase activity was measured using the method of Nagatsu and Udenfriend (16). The standard reaction mixture (total volume 1.0 ml) contained 200 µmoles sodium acetate, pH 5.0, 10 µmoles sodium fumarate, 10 µmoles freshly prepared ascorbic acid, 50 µg catalase, 1 µmole pargyline, 30 µmoles N-ethylmaleimide, 20 µmoles tyramine, and 10-200 µl of human plasma as enzyme. Reaction mixtures containing no enzyme or enzyme plus 1 µmole fusaric acid, a potent inhibitor of DBH activity, were run as blanks. The addition of enzyme initiated the reaction. The reaction mixture was exposed to air and incubated at 37°C in a water bath for 60 min with continual shaking. The addition of 0.2 ml of 3 M trichloroacetic acid terminated the reaction and the mixture was centrifuged at 2000 x g for 10 min. The supernatant fluid was transferred to a small Dowex-50 (H^+, 200-400 mesh)

column prepared using a disposable Pasteur pipet (0.5 cm x 10 cm) and containing 0.20 ml packed volume of resin. The reaction tube and precipitate were washed with 1 ml of water, and the washings transferred to the Dowex column. After two additional 2.0 ml water washes the absorbed amines were eluted with 2.0 ml of 4 M NH_4OH. The octopamine in the eluate was converted to p-hydroxybenzaldehyde by adding 0.20 ml of 2% (w/v) $NaIO_4$ solution. Excess periodate was reduced by adding 0.20 ml of 10% (w/v) $Na_2S_2O_5$ solution.

The absorbance was measured against 4 M NH_4OH at 330 nm in a microcuvet with a 1 cm light path using a Varian DMS 90 spectrophotometer. Various amounts of octopamine were carried through the isolation and oxidation procedure to prepare standard curves. Absorbance was linear with octopamine concentrations from 20-160 mM.

Protein was measured using the method of Bradford (17) with bovine serum albumin as a standard. Activities were expressed in international units and specific activities were stated as international units per mg of protein.

Polyacrylamide Gel Electrophoresis: Seven percent PAGE in a *discontinuous Tris-glycine buffer system* were performed according to the method of King and Laemmli (18). Gradient gel electrophoresis on Pharmacia PAA 4/30 gels were run at pH 8.4 using a Tris-boric acid EDTA buffer system according to the method described in the Pharmacia product literature. Protein samples for SDS gel electrophoresis were prepared by heating for 5 min at 95°C in 1% SDS with 1% β-mercaptoethanol. The protein bands were stained with Coomassie Blue R250.

RESULTS

Enzyme Purification: Purification of human plasma dopamine-β-hydroxylase through the CON A-Sepharose stage is similar to the method of Frigon and Stone (6). Dextran sulfate precipitated lipoproteins and β-globulins, while polyethylene glycol removed fibrinogen and increased the capacity for chromatography on CON A-Sepharose.

The chromatography of DBH on CON A-Sepharose is shown in Figure 1. The enzyme was released in a single peak after washing with a 10% (w/v) α-methyl-D-glycopyranoside solution in 20 mM phosphate, 0.5 M NaCl, pH 7.0. CON A-Sepharose generally afforded more than a 40 fold purification as shown in Table 1.

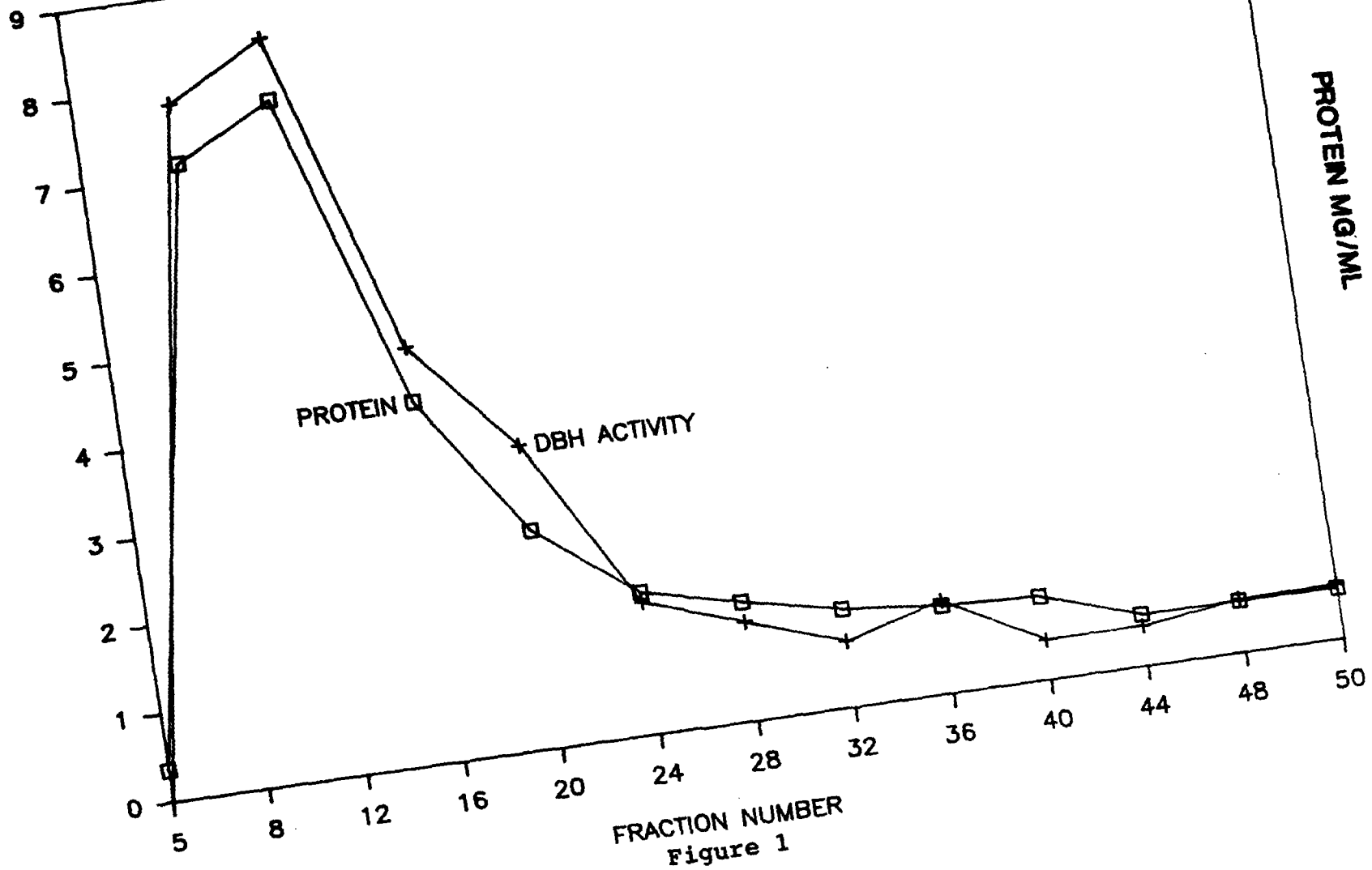
Attempts to reproduce the purification step using Octyl-Sepharose (7) were unsuccessful. When the pooled fractions from the CON A-Sepharose column containing DBH were applied to a 2.6 cm x 25 cm Octyl-Sepharose column that had been equilibrated with 20 mM phosphate, 500 mM NaCl, pH7.0, about half the DBH activity and half the total protein passed through the column, indicating that the column was overloaded. A 0-50% linear gradient of ethylene glycol in the above buffer was used to elute the bound DBH activity but no activity could be recovered. Either the enzyme binds too tightly to this resin, or it is very unstable in the presence of ethylene glycol. Figure 2 shows the chromatography of DBH on Trisacryl M-DEAE. Prior to applying the enzyme to the column it was dialyzed against 5 mM phosphate, pH 6.5. Dialysis afforded an additional benefit since the removal of NaCl caused about half of the non-enzyme protein to precipitate. This protein was removed by centrifugation prior to application of DBH to the Trisacryl M-DEAE column.

A previous report (19) described the interaction of bovine DBH with the dyes, Cibacron Blue and Procion Red. Both these dyes covalently coupled to Sepharose Cl-6B were tested as possible affinity columns. Blue Sepharose Cl-6B was equilibrated with 20 mM phosphate, pH 7.0 and partially purified human plasma DBH was applied. The enzyme bound to this column and more than 80% of the applied activity could be recovered with a 0-0.5 M NaCl linear gradient in the above buffer but there was no significant increase in the specific activity. Variations of the linear gradient did not increase the specific activity of the recovered DBH activity. Red Sepharose CL-6B was more successful and it was incorporated into the purification scheme for DBH yielding about a 50-fold purification as seen in Table 1. It was not necessary to dialyze the pooled fractions from the Trisacryl M-DEAE column since the low salt (about 80 mM NaCl) did not inhibit the binding of DBH to the Red Sepharose CL-6B. The enzyme bound very tightly to this resin but could be eluted at high concentrations of NaCl ahead of most of the non-enzyme protein as shown in Figure 3.

Attempts were made to prepare an affinity column for human DBH using its substrate tyramine. Using the method described in the product literature, Tyramine was coupled to Bio Rad Affi-Gel 10 using 0.1 M $NaHCO_3$, pH 8.1. This column failed to provide any purification since

essentially all the applied protein and most of the applied DBH activity passed through the column and was recovered with no increase in specific activity.

Chromatography of the Red Sepharose Cl-6B purified enzyme on a Bio-Gel A-0.5 M column produced the elution profile as shown in Figure 4. The major activity peak corresponded to a molecular weight of about 500K daltons by gel filtration and yielded a major sharp band at 455K daltons and a major diffuse band at 280K daltons on 4-30% gradient gel electrophoresis (Figure 5). Rechromatography of the major activity peak on the Bio-Gel A-0.5 M column (Figure 6) yielded only the sharp band at 455K daltons on gradient gel electrophoresis. Gel filtration chromatography of the Red Sepharose Cl-6B fraction always produced a trailing peak of enzyme activity. With many of the preparations a small peak appeared containing DBH activity corresponding to a molecular weight by gel filtration of about 45,000. In all cases described above the Bio-Gel A-0.5 column had been equilibrated with 5 mM phosphate, pH 6.5. In one preparation the column was equilibrated in the same buffer but also containing 100 mM NaCl. None of the smaller molecular weight peaks appeared, but when the major peak of activity was rechromatographed on the same Bio-Gel A-0.5 column (but equilibrated in 5 mM PI, pH 6.5 without NaCl) a small peak of DBH activity appeared corresponding to a molecular weight of about 45,000. When the 45K molecular weight peak was electrophoresed on a 4-30% gel, again two protein bands appeared, a sharp band at 455K daltons and a diffuse band at 280K daltons. The appearance of this 45K lower molecular weight peak containing DBH activity was not consistent and in some cases would not appear with the first chromatography on Bio-Gel A-0.5 but would then appear when the major peak of DBH activity was rechromatographed under identical conditions. In one preparation both the major peak of DBH activity and the 45K molecular weight peak of DBH activity were concentrated and incubated in the presence of SDS and β-mercaptoethanol. After electrophoresis on a 7% SDS - polyacrylamide gel (Figure 7) the protein bands were scanned using a laser densitometer. Both fractions yielded nearly identical electrophoretic patterns.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PROTEIN
DBH ACTIVITY
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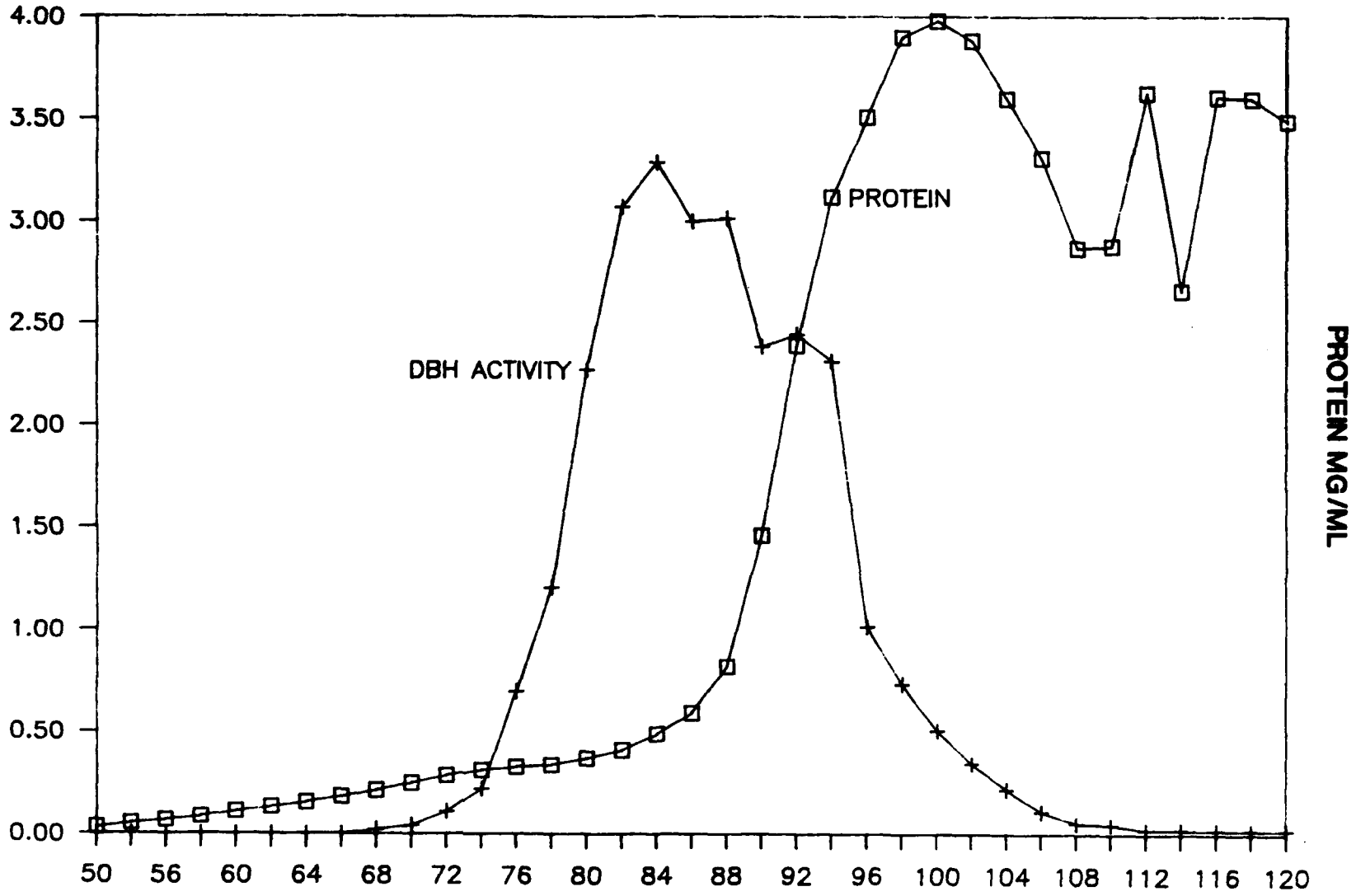
12
16
20
24
28
32
36
40
44
48
50
FRACTION NUMBER

Figure 1

Table 1. Purification of Human Plasma Dopamine-Beta-Hydroxylase

Fraction	Volume	Total Protein	Total Activity	Specific Activity	Recovery	Purification
	ml	mg	IU	IU/mg	%	-Fold
Plasma	2,000	110,000	75	6.82×10^{-4}	100	1
Dextran Sulfate-Peg	2,200	101,000	69	6.83×10^{-4}	92	1
Con-A-Sepharose	650	1,000	34	3.40×10^{-2}	45	50
Trisacryl M-DEAE	103	236	15	6.36×10^{-2}	20	93
Red Sepharose CL-6B	43	2.4	7.8	3.25	10	4,760
Gel Filtration 1 (all fractions)	11.9	0.98	5.1	5.20	6.8	7,625
Gel Filtration 2 (major fraction)	4.4	0.25	1.95	7.8	2.6	11,400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PROTEIN MG/ML
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FRACTION NUMBER

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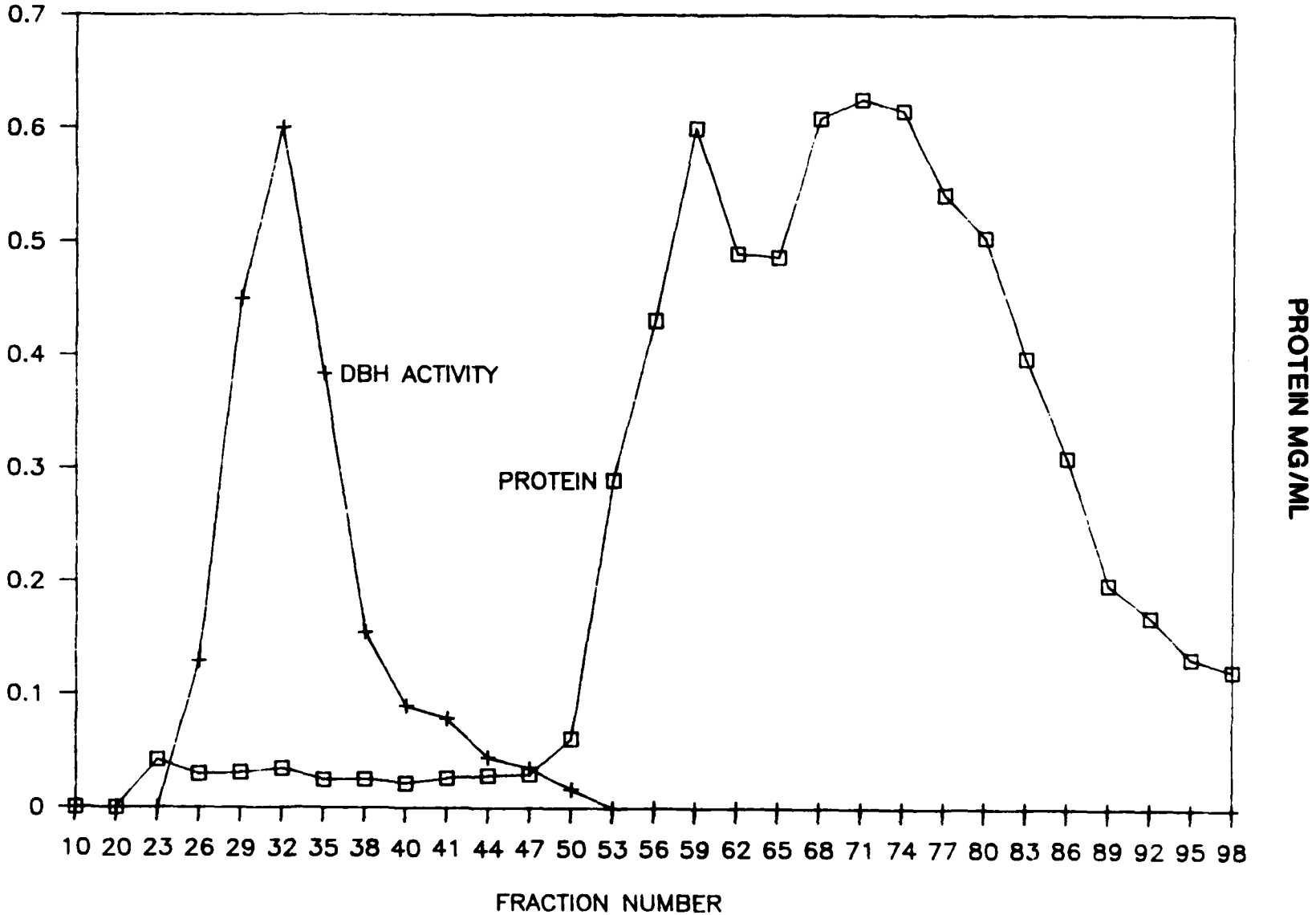
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DBH ACTIVITY
PROTEIN
PROTEIN MG/ML
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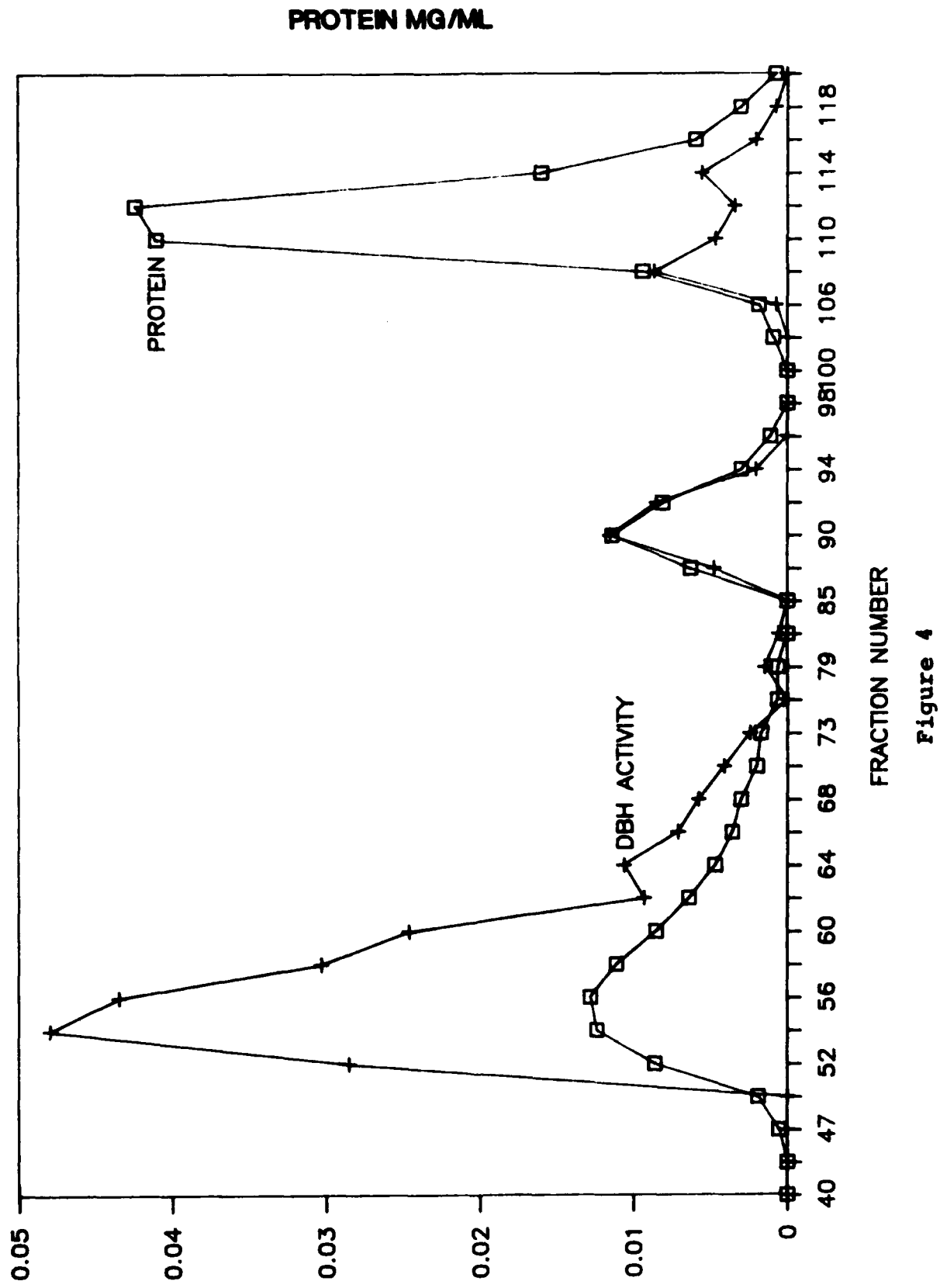
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DBH ACTIVITY
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0.02
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0
40
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56
60
64
68
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79
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90
94
98
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106
110
114
118
FRACTION NUMBER

Figure 4

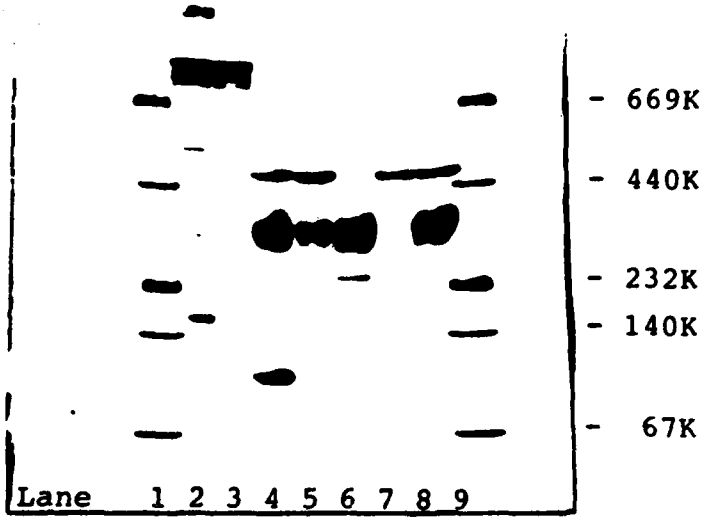
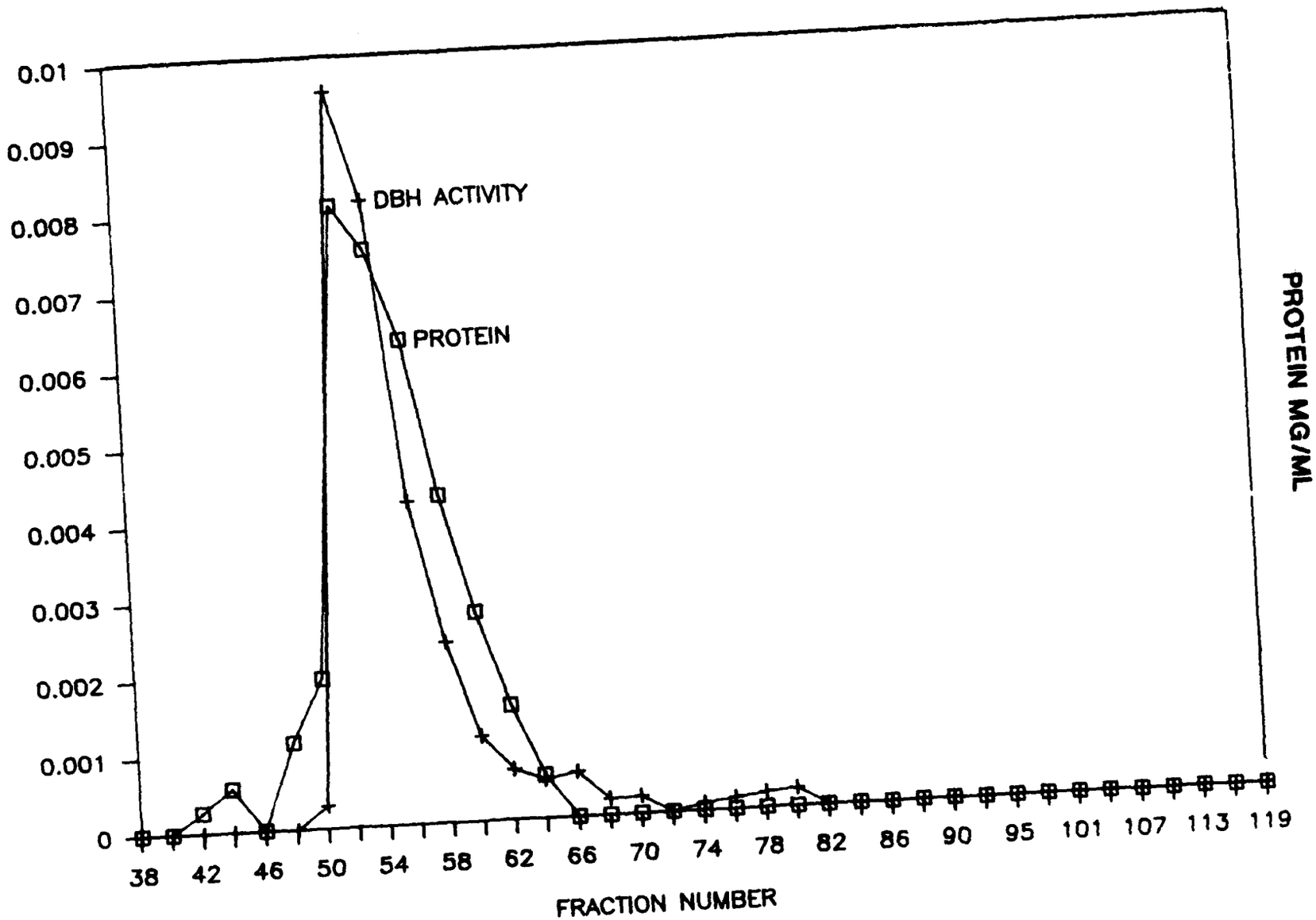


Figure 5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PROTEIN MG/ML
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FRACTION NUMBER

Figure 6

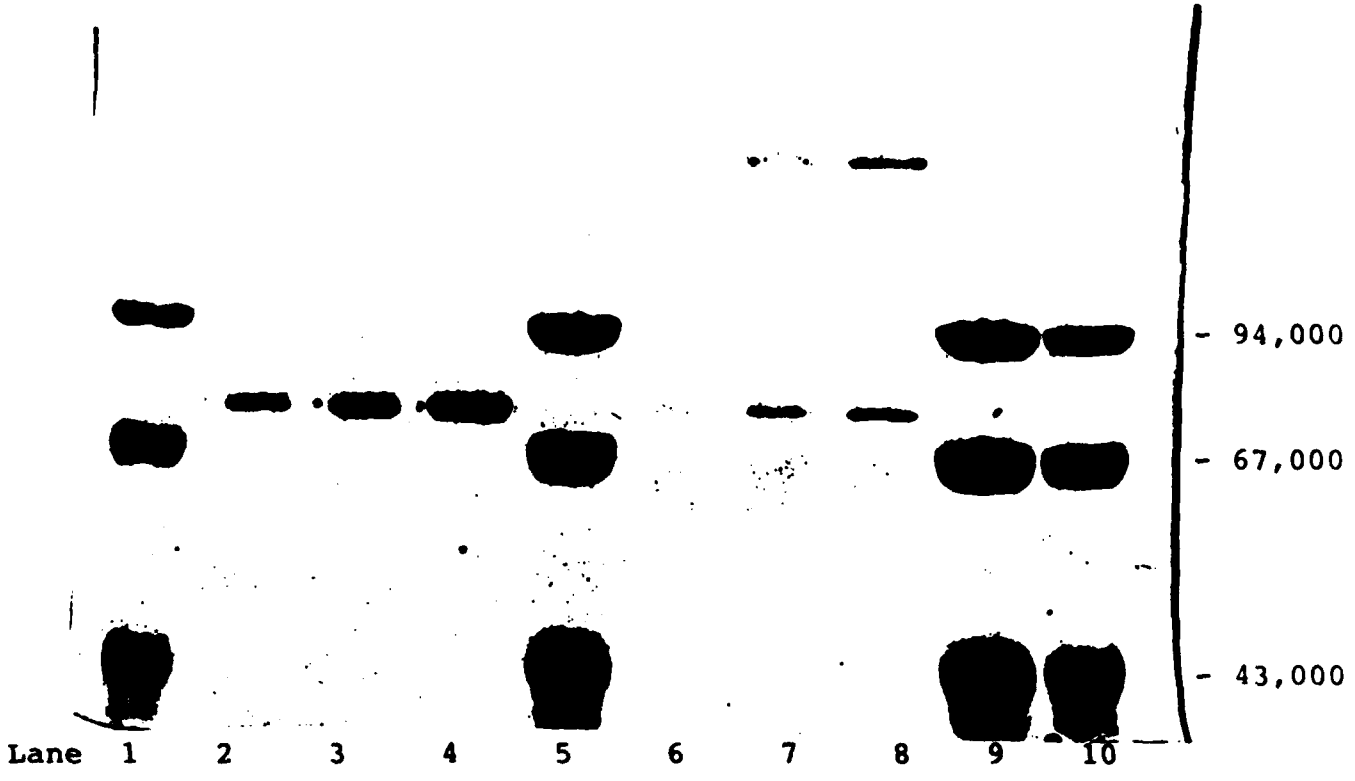

Figure 7

Table 2. Specific Activity of Human Plasma DBH Preparations

Preparation	Specific Activity (μmole/min/mg of protein)
Ikeno, et. al. (26)	0.019
Frigon, et. al. (29)	0.22
Frigon and Stone (7)	1.8
Miras-Portugal, et. al. (28)	1.2
Von Tersch and Kalk	7.8

REFERENCES

1. Kleaher, J. J Biol Chem, 1957, 226:821.
2. Stjarne, L., R. H. Roth, and F. Lishyko. Biochem Pharmacol, 1967, 6:1729.
3. Rush, R. A. and L. B. Geffen. Circ Res, 1972, 31:444.
4. Grzanna, R. and J. T. Coyle. J Neurochem, 1976, 27:1091.
5. Stone, R. A., N. Kirshner, J. Reynolds, and T. C. Vanaman. Mol Pharmacol, 1974, 10:1009.
6. Frigon, R. P., and R. A. Stone. J Biol Chem, 1978, 253:6780-6786.
7. Viveros, O. H., L. Arqueros, and N. Kirshner. Life Sci, 1968, 7:609.
8. Stone, R. A. West J Med, 1975, 85:211-223.
9. Kopin, I. J. Ann Int Med, 1976, 85:211-223.
10. Winkler, H., H. Hortnagl, and A. D. Smith. Biochem J, 1970, 118:303-310.
11. Craine, E., G. H. Daniels, and S. Kaufman. J Biol Chem, 1973, 248:7838-7844.
12. Ljones, T., T. Skotland, and T. Flatmask. Eur J Bioch, 1976, 61:525.
13. Rosenberg, R. C. and W. Lovenberg. Mol Pharmacol, 1977, 13:652-661.
14. Hastings, M. and S. Jenkins. "The Battle for the Falklands," 1983, W. W. Norton and Co., Inc., NY, p. 420.
15. Cannon, B., and J. Nederguard. J Therm Biol, 1983, 8:85-90.
16. Nagatsu, T., and S. Udenfriend. Clin Chem, 1972, 18:980-983.
17. Bradford, M. Annal Biochem, 1976, 72:248.
18. King, J. and U. K. Laemmli. J Mol Biol, 1971, 62:465-473.
19. Skotland, T. Biochem Biophys Acta, 1981, 659:312-325.
20. Miras-Portugal, M. T., P. Mandel, and D. Annis. Neurochem Res 1976, 1:403.
21. Goldstein, I. J., C. E. Hallerman, and E. E. Smith. Biochemistry, 1965, 44:876-883.
22. O'Connor, D. T., R. P. Frigon, and R. A. Stone. Mol Pharmacol, 1979, 16:529-538.
23. Watson, D. H., M. J. Harvey, and P. D. G. Dean. Biochem J, 1978, 173:591-596.

24. Lowe, C. R., D. A. P. Small, and A. Atkinson. Int J Biochem, 1981, 13:33-40.

25. Lau, E. P. and R. R. Fall. J Chromato, 1981, 205:213-217.

26. Ikeno, T., S. Hashimoto, H. Kuzuza, and T. Nagatsu. Mol Cell Biochem, 1977 18:117-123.

27. Rosenberg, R.C. and W. Lovenberg. Essays in Neurochemistry and Neuropharmacology, 1980 John Wiley and Sons, Inc., New York, pp 163-209.

28. Miras-Portugal, M., D. Annis, and P. Mandel. Biochimie 1975 57:669-675.

29. Frigon, R. P., D. T. O'Connor, and G. L. Levine. Mol Pharmacol 1981, 19:444-450.

FIGURE LEGEND

Figure 1. α-methyl-D-mannopyranoside elution of human plasma DBH from CON A-Sepharose.

Figure 2. Elution of human plasma DBH from Trisacryl M-DEAE.

Figure 3. Chromatography of human plasma DBH on Red Sepharose CL-6B.

Figure 4. Chromatography of human plasma DBH on Bio-Gel A-0.5.

Figure 5. Four-thirty percent polyacrylamide gel electrophoresis of human plasma dopamine-β-hydroxylase at different stages of purification. Lane 1, 9-molecular weight standards. Lane 2 - Con A-Sepharose eluate, Lane 3 - Trisacryl M-DEAE eluate, Lane 4 - Red Sepharose CL-6B eluate, Lane 5 - first gel filtration (500K dalton peak), Lane 6 - first gel filtration (trailing of major DBH activity peak), Lane 7 - second gel filtration (500K dalton peak), Lane 8 - second gel filtration (45K dalton peak)

Figure 6. Rechromatography of the major DBH activity peak on Bio-Gel A-0.5.

Figure 7. Seven percent SDS-polyacrylamide gel electrophoresis of human plasma dopamine-β-hydroxylase after second gel filtration. Lanes 1, 5, 9, 10 - molecular weight standards. Lanes 2, 3, 4 - 500K dalton peak. Lanes 6, 7, 8 - 45K dalton peak.

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