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BINDING OF BIOLOGICALLY ACTIVE AMINES TO PLASMA PROTEIN FRACTIONS*

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Abstract—Studies have been initiated to provide information on biochemical relationships between biogenic amines and plasma proteins. The binding *in vitro* of several biogenic amines, i.e. 3,4-dimethoxyphenylethylamine, mescaline, tyramine, and normetanephrine, labeled with ^{14}C or ^{3}H to human plasma protein fractions have been studied. Considerable binding of 3,4-dimethoxyphenylethylamine to components in Cohn fraction III was found, but there was negligible binding of this compound to other Cohn fractions. When Cohn fraction III was subjected to chromatography on DEAE-cellulose and Sephadex G-200, a number of the fractions, especially those having properties of an α_2-macroglobulin fraction, bound the compound to a greater extent than the other fractions. It is suggested that a specific plasma protein, possibly an α_2-macroglobulin, may be involved in the transport of some biogenic amines. No appreciable binding to any of the plasma protein fractions was observed with mescaline or normetanephrine.

MANY compounds including metals and hormones, are carried in the blood stream complexed with specific proteins whose function appears to be that of a specific transport protein or vehicle. In some cases at least, these transport proteins appear to regulate the biological activity of the compound and to be involved in the control of the passage of the compound into tissues or its excretion into the urine. It is entirely possible that certain of these transport proteins may be involved in the transport of psychotoxic compounds, and alterations in their concentration may be associated with various types of mental disease. It is also possible that certain drugs may act by blocking such compounds from the binding sites of specific transport proteins.

Various aspects of the extensive literature on the metabolism of monoamines and their possible relationship to behavior have been well reviewed by Schildkraut.[1]

Studies of biochemical abnormalities in plasma proteins in schizophrenic patients have yielded conflicting results in our laboratories and in others.[2–8] It is clear that schizophrenia is a complex entity, and that firmer and more precise diagnostic criteria are necessary. It is one of the aims of this study to investigate whether or not there are

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specific transport proteins in blood for a series of biologically active compounds of potential interest in mental disease.

In these studies the binding of certain of the more interesting ^{14}C- or ^{3}H-labeled compounds to plasma protein fractions of normal individuals and a smaller group of selected patients with mental disease was compared. Isolation of plasma proteins was carried out initially by ethanol fractionation followed by DEAE-cellulose and Sephadex columns. Each protein fraction was characterized by various electrophoretic procedures.

EXPERIMENTAL PROCEDURE

1. *Selection of compounds.* Recently, 3,4-dimethoxyphenylethylamine (DMPEA), a compound that bears a striking resemblance to mescaline, has been found to be a constituent of urine obtained from schizophrenic patients,[9–14] although there is disagreement whether it is excreted only in this condition.[15–17] Pertinent to the consideration of the potential importance of DMPEA is the report by Ernst[18] that gives evidence for the necessity of O-methylation in the para-position of the molecule in order to produce profound functional changes in the central nervous system. He has found that only *p*-O-methylated derivatives of the phenylethylamine will induce the catatonic condition in cats referred to as the hypokinetic rigid syndrome. Barbeau *et al.*[19–20] reported on the effect of DMPEA injections on the concentration of catecholamines in the rat brain. We have observed that injection of DMPEA into rats causes changes in the rat-climbing test developed by Winter and Flataker[4] similar to those obtained with an active Cohn fraction III obtained from schizophrenic patients.[7] Bergen[21] found that i.p. injections of ^{14}C-labeled DMPEA into rats led to the appearance of appreciable radioactivity in plasma within 5 min. Total counts in brain and plasma reach a maximum at 20–25 min, the time when behavioral effects as determined by the rope-climbing test may be observed. Using the labeled drug in experiments *in vitro* with human plasma, he found that there was appreciable binding with plasma proteins. We have therefore examined the capacity of different fractions of plasma from normal and schizophrenic individuals to bind DMPEA, mescaline, and some compounds related to DMPEA.

The binding activity is shown as cpm/mg of protein. The cpm/mg of protein can be converted to μmoles/mg of protein for the ^{14}C-labeled compounds by multiplying cpm/mg protein $\times$ $1{\cdot}167 \times 10^{-7}$ for DMPEA, $2{\cdot}03 \times 10^{-7}$ for tyramine, and $2{\cdot}51 \times 10^{-7}$ for mescaline.

These factors (F) were obtained from the equation:

$$F = \frac{\text{cpm/mg protein}}{2{\cdot}2 \times 10^{6}\ \text{dpm}/\mu\text{c}} \times \frac{1}{0{\cdot}667\ \text{cpm/dpm}} \times \frac{1}{\text{sp. act. } (\mu\text{c}/\mu\text{mole})}.$$

In these experiments, 50 μg of 0·05% DMPEA·HCl solution, 0·1142 μmole (approximately 1 million cpm in the counter), was added to 5 ml of 0·2% protein solution (0·05 μmole protein estimated at mol. wt. — 200,000). The concentrations of 50 μl of solution for mescaline, normetanephrine, and tyramine were respectively: 0·1000, 0·0027, and 0·1152 μM. More than 90 per cent of total radioactivity was dialyzed out.

2. *Subjects.* Donors 293, 301, 302, 318, 328, and 331 were examined physically, medically, and psychiatrically and found to be normal. Other donors included in the

normal group were selected volunteers from various walks of life. One subject had been on a tetracycline antibiotic a few days for minor skin irritation. The rest of the normal donors were not under medication.

Patients diagnosed as schizophrenic were 293, 297, 303, 306 and 329. Other donors included in this group were psychiatric patients presenting some features of the schizophrenic syndrome, though final diagnosis would not include them within this specific category. All the schizophrenic patients were on phenothiazone medication for brief periods at least.

3. *Preparation of plasma fractions.* The Cohn ethanol fractions were prepared from 200–250 ml plasma by modifications described be Lever *et al.*[22] and by Sanders *et al.*[8] The fractions obtained were dissolved in 0·01 M sodium phosphate buffer, bH 7·8, one fourth the original plasma volume, and dialyzed overnight against the same buffer to remove alcohol and traces of insoluble protein.

4. *Subfractionation and purification.* All procedures used in DEAE-cellulose chromatographic subfractionation of the Cohn fractions have been described in previous reports.[23–25]

The gel filtration experiments (Sephadex G-200) were performed essentially as described by Flodin and Killander.[26] The buffer used for elution was 0·01 M sodium phosphate, pH 7·8. The columns were 4 × 75 cm without extension tube. The volume of the gel bed was 980 ml for 4 × 65 cm. The flow rate was maintained at 27 ml/hr. and the effluent was collected in 9-ml portions. Optimum sample size was 100–150 mg protein in 3–15 ml volume.

5. *Determination of protein concentration.* The protein concentration of each fraction was estimated by absorbance at 280 mμ ($E^{1\%}_{1\,cm} = 10$) with a Beckman DU-2 spectrophotometer and also by the method described by Lowry *et al.*[27]

9. *Electrophoretic characterization.* The methods used for characterization of the protein fractions, microzone electrophoresis on cellulose acetate, disk electrophoresis. and immunoelectroyhoresis have been described previously.[23]

7. *Binding studies.* The DMPEA-8^{14}C (specific activity, 5·85 mc/m-mole), the mescaline (sp. act. 2·72 mc/m-mole), and the tyramine (sp. act. 4·71 mc/m-mole) were obtained from New England Nuclear Corp., Boston, Mass. Each of the labeled compounds was made 0·05% solution with 0.01 M sodium phosphate pH 7·8. The normetanephrine-^{3}H (sp. act. 450 mc/m-mole), obtained from Volk Radiochemical Co., Chicago, Ill., was made 0·001% solution with the same phosphate buffer.

The protein fractions (5 ml of 0·2% protein solution) were incubated at 37° in 0·01 M sodium phosphate buffer, pH 7·8, and 50 μg of the prepared solution of labeled compound for 1-hr. Incubation was carried out in a Dubnoff metabolic shaking bath at atmospheric conditions. Optimum conditions for these experiments, such as the period of incubation, temperature, pH, protein concentration, and addition of labeled compound, were previously determined.

Each incubated sample was dialyzed against the same buffer, 500-ml aliquots with four changes, for 48 hr at 4°; this time had previously been found sufficient to obtain a dialysate with no radioactivity.

Each sample (0·5 ml) was dissolved in 10 ml of a liquid scintillation solvent and counted in a Nuclear-Chicago liquid scintillation counter. The solvent was 1:1, v/v, respectively, of toluene and 2-ethoxyethanol containing 0·66% diphenyloxazol (PPO) and 0·022% 1,4-di [2-(phenyloxazolyl)]-benzene (POPOP).

RESULTS

In previous work,[24] the Cohn fraction III from individual human plasma has been successfully separated into thirteen globulin subfractions by employing a simple and rapid elution procedure on a DEAE-cellulose column. A typical separation of Cohn fraction III from normal plasma is shown in Fig. 1. Table 1 shows relative amounts of protein in the reproducible thirteen characteristic absorbance peaks (A.P.), presented as each mean of 17 normal and 9 schizophrenic subjects.

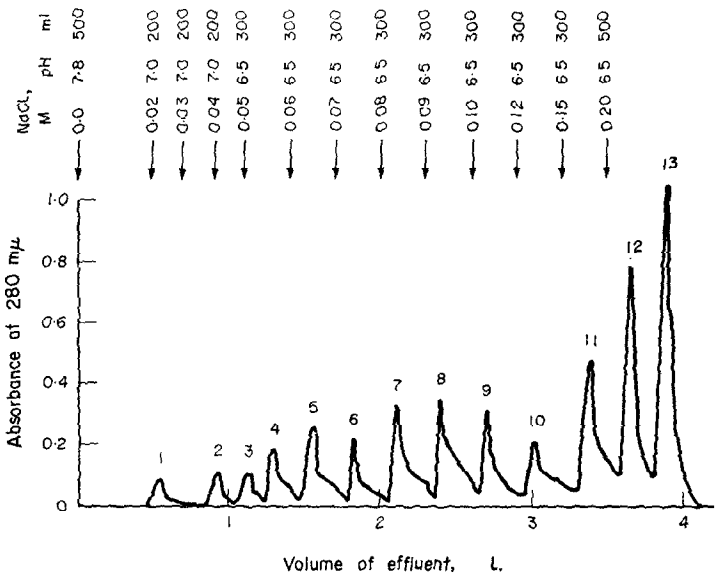

FIG. 1. Effluent diagram of Cohn fraction III from normal human plasma. Approximately 760 mg of fraction III was applied to a column of DEAE-cellulose (2·7 × 72 cm). Flow rate, 250 ml/hr; temperature, 4°. Other conditions given in the previous paper.[23, 24]

TABLE 1. RELATIVE AMOUNTS* OF PROTEIN IN EACH ABSORBANCE PEAK ON DEAE-CELLULOSE FROM COHN FRACTION III

Absorbance peak	Normal (17)		Patients (9)	
	Mean (%)	Standard deviation	Mean (%)	Standard deviation
1–3	6·8	2·0	6·0	1·5
4	4·6	1·8	5·8	2·0
5	6·6	1·9	7·9	1·0
6	5·8	2·1	7·1	1·4
7	7·9	2·1	9·3	2·7
8	7·9	3·1	7·4	2·9
9	7·3	4·2	5·1	2·0
10	5·1	1·2	6·5	0·5
11	10·1	3·1	10·9	1·5
12	13·4	8·3	13·9	4·3
13	24·5	7.6	20·0	6·7

* The amount of protein in each absorbance peak is expressed as a percentage of the total amount recovered from the column and the mean of seventeen normal individuals and nine schizophrenic subjects. The range of protein introduced on column was 600–1200 mg.

The original thirteen DEAE-cellulose peaks (Fig. 1) were individually concentrated, mixed with DMPEA as described above, and dialyzed to remove unbound DMPEA. Since many of these peaks contained little protein, it was usually found necessary to pool the samples from about three column runs. These pooled fractions (containing between 100 and 150 mg protein) were dialyzed after incubation with DMPEA and chromatographed on Sephadex G-200 columns. In these experiments, binding was confined to the 19/S peak (Fig. 2). When an excess of DMPEA was added to the undialyzed sample, the unbound DMPEA was recovered after approximately 1000 ml of the buffer had been passed through the Sephadex G-200 column.

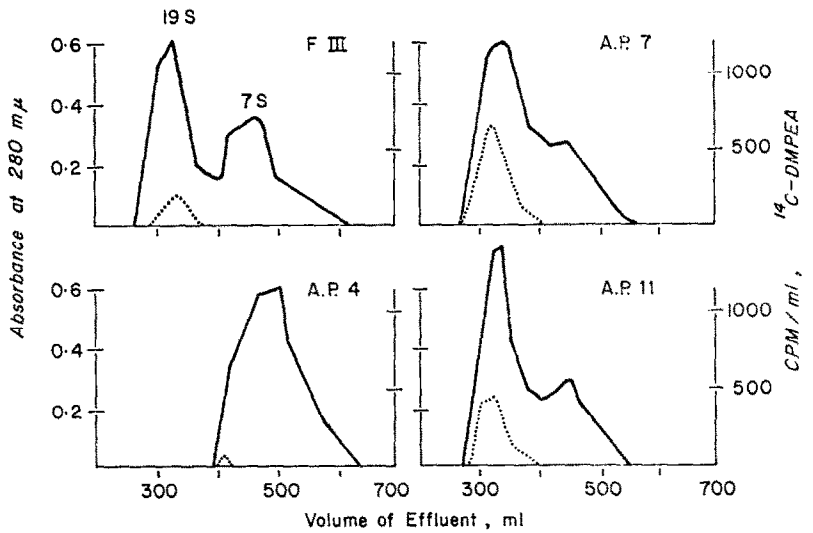

FIG. 2. Effluent diagrams on Sephadex G-200 columns (4 × 65 cm) of Cohn fraction III and absorbance peaks 4, 7, and 11 with ^{14}C-labeled DMPEA obtained from DEAE-cellulose chromatogram in Fig. 1. Approximately 100–150 mg of each protein with DMPEA was applied to a column of Sephadex G-200. Flow rate, 27 ml/hr; temperature, 4°. Other conditions as given in the text. Solid line represents absorbance at 280 mμ, dotted line shows cpm/ml effluent in radioactivity. Pooled original plasmas used for these experiments were from donors 331, 332, and 337.

In an attempt to separate and purify the DMPEA binding protein or proteins it was decided to recycle the DEAE-column peaks through the Sephadex G-200 in the same buffer system. The experiments were carried out in the following manner: (1) pooled samples were split into two aliquots (A and B); (2) aliquot A was incubated with 50 μg of 0·05% DMPEA, dialyzed 48 hr, and the specific activity determined; (3) aliquot B was put on the G-200 Sephadex column and four cuts were made on the resultant effluent; (4) aliquot B cuts were incubated with 50 μg of 0·05% DMPEA, dialyzed 48 hr, and the specific activity determined.

The results of these experiments indicate that the binding protein has been purified severalfold (Fig. 3 and Table 2).

Identification of components in each protein fraction isolated from the chromatographic columns was made by immunoelectrophoresis of whole antisera and specific antisera produced in goats and obtained from the Hyland Laboratory, Los Angeles,

Calif. Complete disk and immunoelectrophoretic patterns of the various protein fractions obtained from the Sephadex G-200 separation (Fig. 4) will be reported elsewhere.[28] The more important disk and immunoelectrophoresis patterns pertinent to this study are shown in Figs. 5 and 6.

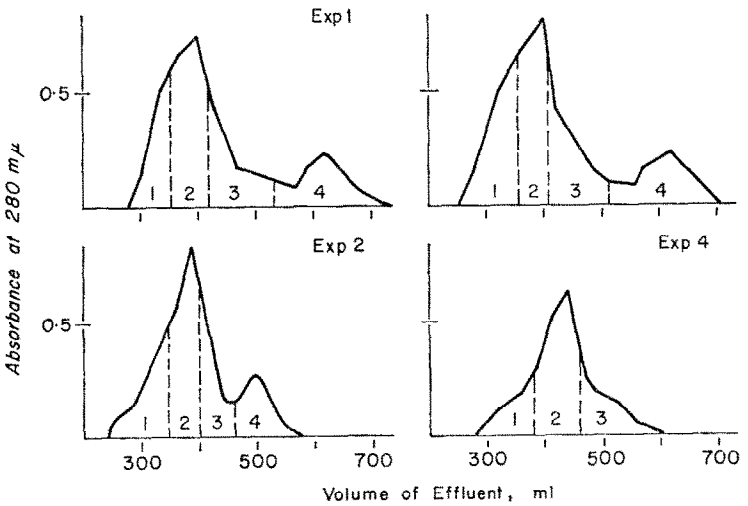

Fig. 3. Effluent diagrams on Sephadex G-200 column (4 × 65 cm) of protein samples as described in the text, Approximately 100–150 mg of each protein sample was applied to a column of Sephadex G-200. Flow rate, 27 ml/hr; temperature, 4°.

Table 2. The DMPEA binding activities of Sephadex G-200 column cuts from DEAE-cellulose column peaks

Expt.	Sample used	Specific activity aliquot A (cpm/mg protein)	Sephadex G-200 cuts; specific activity (cpm/mg protein)			
			1	2	3	4
I	Pooled DEAE-cellulose A.P. 6, 7, 8 Donor 301	477	1012	894	804	275
II	A.P.11 Donor 301	806	1206	1922	2122	Sample lost
III	Pooled DEAE-cellulose A.P. 9, 10, 11 derived from combined Donors 301 and 302	1358	4089	2078	3329	390
IV	Pooled cut 3 from Expts. II and III	2726	2010	7493	1359	

Table 3 shows mean values and standard deviation for DMPEA binding in the chromatographic fractions from twenty normal and nine schizophrenic subjects. Tables 4 and 5 show the DMPEA binding activities of the fractions in comparisons with mescaline and tyramine. We were unable to see appreciable binding with normetanephrine. The binding characteristics with DMPEA obtained from several individual

donors, both with total fraction III and in the summation of the individual chromatographic fractions are given in Table 6. Binding activity was observed in chromatographic fractions, A.P. 4–13. Absorbance peaks 1–3 on DEAE-cellulose, which correspond to the γ^G-globulins, contained comparatively small amounts of protein and

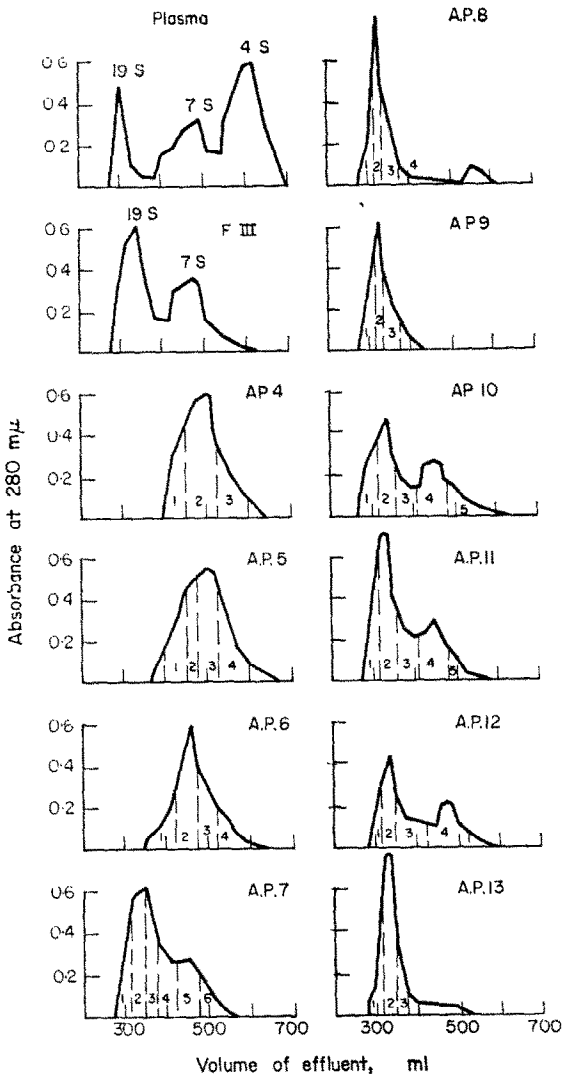

FIG. 4. Effluent diagrams on Sephadex G-200 columns (4 × 65 cm) of plasma, Cohn fraction III, and absorbance peaks 4–13 obtained from DEAE-cellulose chromatogram in Fig. 1. Approximately 100–150 mg of each protein sample was applied to a column of Sephadex G-200. Flow rate, 27 ml/hr; temperature, 4°. Other conditions as given in the text.

exhibited practically no binding; they are not considered in the present communication. In order to present more clearly the peak-to-peak variation of twenty donors, Table 7 is included.

Table 3. The DMPEA binding activities of the chromatographic absorbance peaks of Cohn fraction III

Absorbance peak	Normal		Patients	
	No.	Mean ± mean dev.	No.	Mean ± mean dev.
4	13	459 ± 187	4	380 ± 87
5	18	533 ± 250	6	393 ± 162
6	19	731 ± 591	9	235 ± 86
7	19	1019 ± 985	9	515 ± 366
8	20	999 ± 1008	9	305 ± 138
9	16	691 ± 528	6	384 ± 207
10	16	734 ± 504	8	345 ± 164
11	20	864 ± 744	9	325 ± 150
12	20	528 ± 276	9	417 ± 168
13	30	514 ± 254	7	426 ± 136
FIII	20	248 ± 53	9	240 ± 92

Binding activity is calculated as cpm/mg protein.

Table 4. The DMPEA and mescaline binding activities of the chromatographic absorbance peaks of Cohn fraction III from several individuals

Donor number	Normal				Patients			
	307		315		343		344	
	DMPEA	Mes.	DMPEA	Mes.	DMPEA	Mes.	DMPEA	Mes.
Absorbance peak								
4		83	885				356	26
5	181	30	811		594		358	18
6	345	43	991	65	232		404	22
7	632	18	1111	89	1196		102	42
8	451	59	1093	88	228			
9	175	81	920	142				
10	376	52	789	76	278	68		
11	320	46	565	77	192	46		
12	312	35	377	70	704	226		
13	321	51	516	54	626	226		

Binding activity is calculated as cpm/mg of protein.
No data are available for black spaces. Specific activity of DMPEA: 5·85 μc/μmole; of mescaline: 2·72 μc/μmole.

Table 5. The DMPEA and tyramine binding activities of the chromatographic absorbance peaks of Cohn fraction III from several individuals

Donor number	Normal						Patient	
	318		340		341		335	
	DMPEA	Tyr.	DMPEA	Tyr.	DMPEA	Tyr.	DMPEA	Tyr.
Absorbance peak								
4					318	116		
5			286		526	144		
6	353	388	452		500	142		
7	314	211	160	124	430	152		
8	424	172	172	148	408	146		
9			190	114	218	44	180	199
10	532	219			182	62	274	246
11	554	218	146	112	266	78	680	314
12	315	128	514	434	282	96	464	332
13	434	358	434	296	380	118	350	330

Conditions as in Table 4. Specific activity of DMPEA: 5·85 μc/μmole, of tyramine: 4·71 μc/μmole.

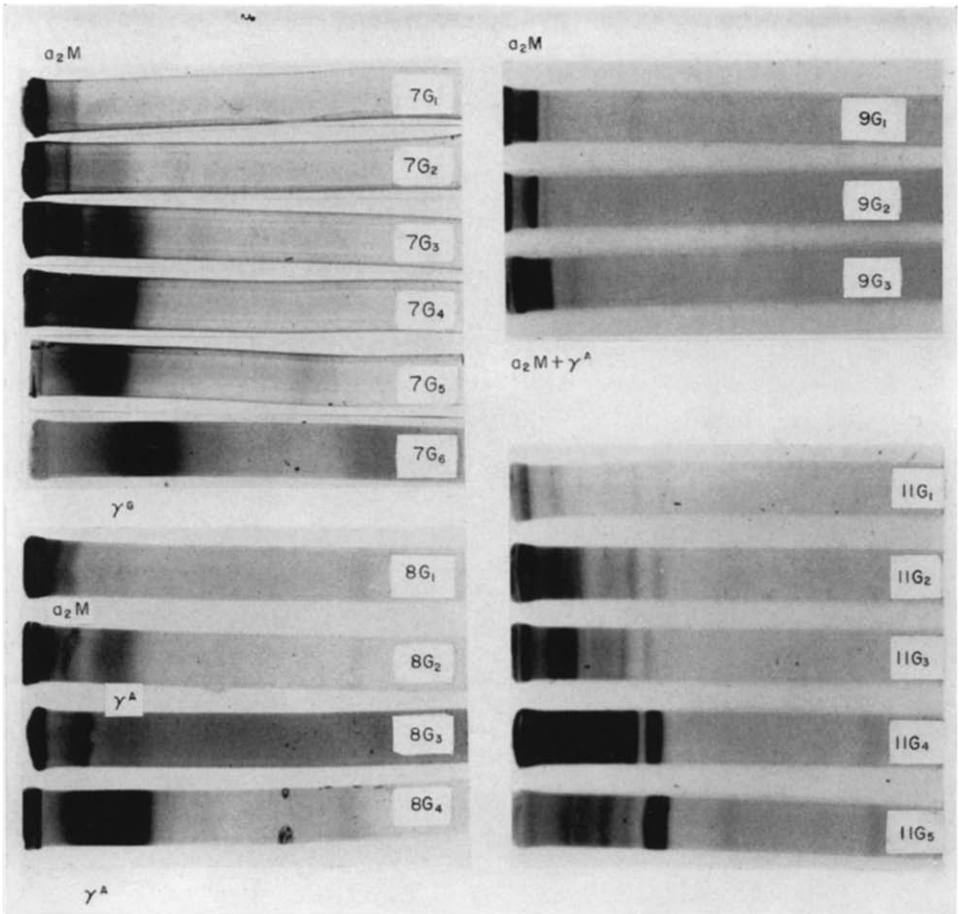

FIG. 5. Disk electrophoretic patterns of the Sephadex G-200 chromatogram in Fig. 4. Each protein sample is designated by its fraction number on DEAE-cellulose and Sephadex G-200 chromatography. For instance, $7G_1$ designates that protein sample which was the first fraction of Sephadex G-200 column (Fig. 4) from the A.P. 7 of DEAE-cellulose chromatographic fractions (Fig. 1).

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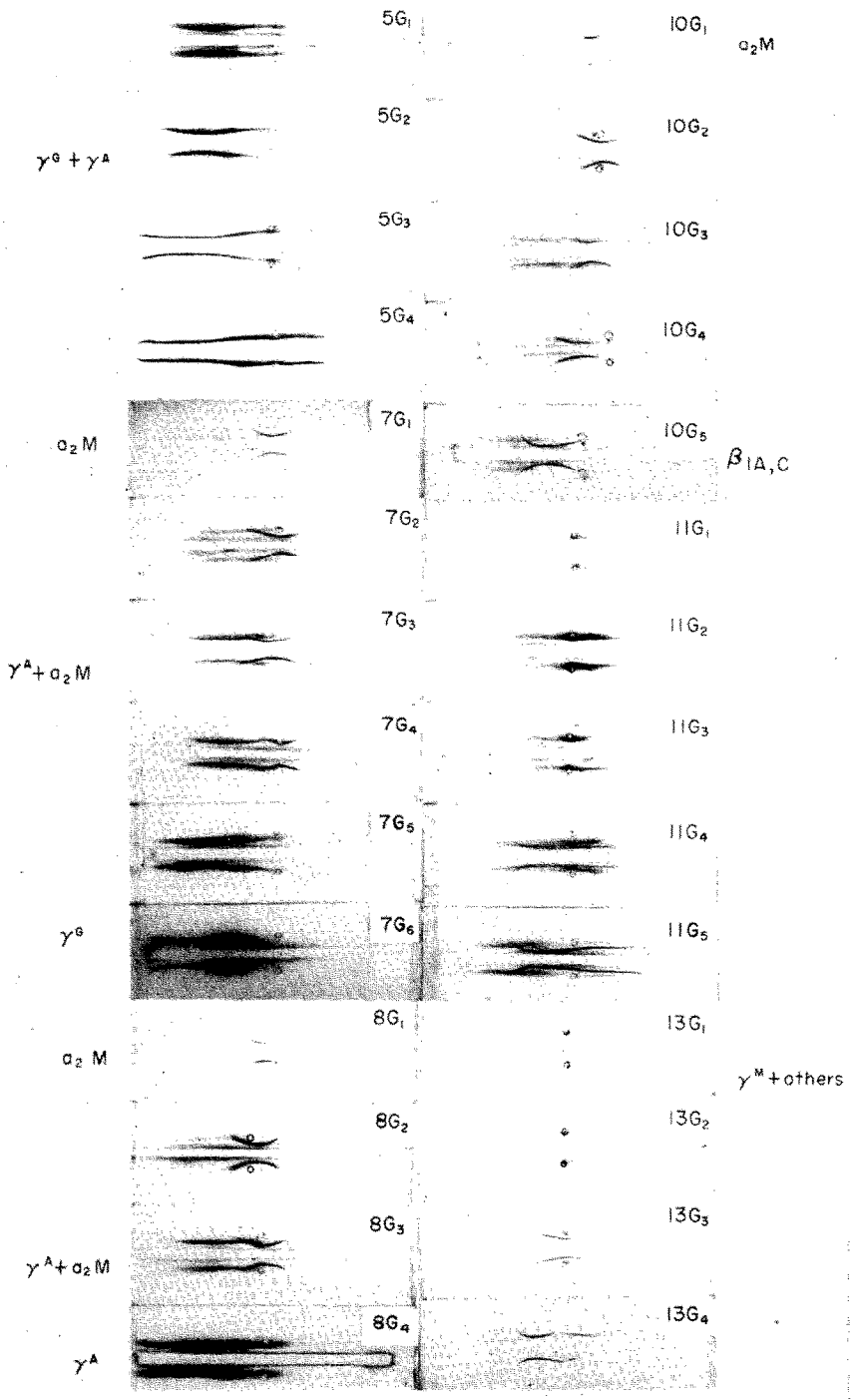

FIG. 6. Immunoelectrophoretic patterns of absorbance peaks from DEAE-cellulose chromatogram (Fig. 1) and protein samples obtained from the Sephadex G-200 chromatograms (Fig. 4) against whole antisera produced in goat (Hyland Lab., Calif.). Designation of protein sample as given in Fig. 5.

Further experiments were performed with Cohn fraction III to which was added ^{14}C-labeled DMPEA after a 1-hr incubation, and the sample was exhaustively dialyzed against 0·01 M sodium phosphate buffer, pH 7·8. This DMPEA-labeled fraction III was then placed in a DEAE-cellulose column previously equilibrated with a pH 7·8.

TABLE 6. DMPEA-BINDING ACTIVITY OF COHN FRACTION III FROM SEVERAL INDIVIDUAL DONORS

Donor number	Binding activity (cpm)/mg protein of F III	Average of total* binding capacity (cpm) of A.P.s from FIII/mg protein
Normals		
290	378	2962
294	217	951
299	109	210
301	228	1351
302	218	654
307	208	354
313	198	504
315	238	651
316	315	593
318	265	416
320	243	466
322	216	397
324	201	475
326	259	392
328	244	501
331	206	371
332	242	232
337	396	286
340	304	382
341	276	336
346	308	274
351	269	1118
Mean ± S.D.	252 ± 61	631 ± 580
Patients		
293	94	146
297	165	581
303	149	351
306	260	519
329	186	525
335	332	370
338	368	236
343	350	532
344	254	210
Mean ± S.D.	240 ± 92	386 ± 152

$$* \frac{\text{A.P.}\Sigma\ 4\text{–}13\ (\text{Specific activity, cpm/mg protein}) \times (\text{mg protein in peak})}{\text{A.P.}\Sigma\ 4\text{–}13\ (\text{mg protein})}$$

phosphate buffer at the same molarity. In these experiments all the radioactivity was recovered prior to the first protein peak during the washing step with 0·01 M sodium phosphate buffer at pH 7·8. The DMPEA-protein complex was therefore shown to be labile in ion-exchange chromatography.

The radioactivity did not vary markedly when the bound proteins were redialyzed against 0·01 M sodium phosphate at various pH values from 5·0 to 10·0. However,

TABLE 7. THE DMPEA-BINDING ACTIVITIES OF THE CHROMATOGRAPHIC ABSORBANCE PEAKS OF COHN FRACTION III FROM NORMAL DONORS

Donors	290	294	299	301	302	307	313	315	316	318	320	322	324	326	328	331	332	337	340	241	Avg.
Absorbance peak																					
4	120		142	1023	619		459	885					496	267	391	486	424	332		318	459
5	510		102	1997	736	181	426	811	642		302	384	350	410	463	509	366	388	286	526	533
6	4800	600	344	2022		345	501	991	380	353	444	324	383	413	342	433	92	178	452	500	731
7	8400	1010		2051	450	632	362	111	874	314	286	214	865	299	352	498	636	396	160	430	1018
8	4000	810	224	7917	595	451	431	1093	1068	424	424	308	391	252	369	324	184	134	172	408	999
9	2300		289	2791	800	175	712	920	849				272	389	372	407	144	222	190	218	691
10	3050	520	330	2061	1077	376	501	789	559	532	560	313			396	361	144			182	734
11	5150	6020	270	806	614	320	634	565	517	554	544	456	309	807	553	197	86	468	146	266	864
12	2870	460	180	377	868	312	506	377	456	315	545	413	336	254	431	589	184	284	514	282	528
13	2700	100	185	325	496	321	592	516	413	434	457	499	656	375	660	284	218	244	434	380	511
FIII	378	216	109	228	218	208	198	238	315	265	243	216	201	259	244	206	242	296	304	276	248
Highest	7	11	6	8	10	7	9	7	8	11	10	13	7	11	13	12	7	11	12	5	
Second highest	11	7	10	9	12	8	11	8	7	10	12	11	13	6	11	5	4	7	6	6	
Lowest	4	13	5	13	7	9	7	12	6	12	7	7	9	8	6	11	11	8	11	10	

Binding activity is calculated as cpm/mg protein. No data available for blank spaces.

a significant dissociation of DMPEA from A.P. 7 fraction occurred with dialyzed at pH 4·0. No appreciable difference was found in binding activity of the stored protein fractions at 4° for 2 or 3 weeks. Also, no difference in binding activities was observed when the buffer system was increased in ionic strength with NaCl up to 0·1 M by addition of NaCl to 0·01 M sodium phosphate buffer at pH 7·8.

Attempts were made to perform binding studies with 3,4-dihydroxyphenylethylamine (Dopamine) and 3,4-dihydroxyphenylalanine (Dopa); however, experiments with both compounds were unsuccessful because of their polymerization to form melanin derivatives in various buffer systems investigated.

Utilizing information from these studies we found either γ^A- or α_2-type macroglobulins present whenever good binding was demonstrated. Our most highly purified protein demonstrating DMPEA binding has the properties of α_2-macroglobulins. However, further purification of these protein fractions will be necessary before the binding molecule or protein can be identified. Other characterization data of all Sephadex G-200 chromatographic fractions are also reported elsewhere.[28]

DISCUSSION

Preliminary studies of the binding of several biogenic amines such as DMPEA, mescaline, tyramine, and normetanephrine to various Cohn fractions derived from normal human plasma indicated appreciable binding in Cohn fraction III but negligible binding of these compounds to other Cohn fractions. We have therefore examined the capacity of Cohn fraction III of plasma from various individuals to bind these compounds. We found that the DMPEA appeared to bind more extensively to the Cohn fraction III than did other compounds tested.

It is seen that the highest activity was often found in the protein fractions which were eluted from a DEAE-cellulose column at 0·08 M NaCl (A.P. 7)–0·15 M NaCl (A.P. 11). These fractions were further separated by the use of Sephadex G-200 columns, and showed DMPEA-bound protein located in a 19 S peak, which is largely an α_2-macroglobulin, as characterized by various electrophoretic procedures. It appears that there may be more than one specific protein that will bind DMPEA if we consider the broad salt range over which the protein-bound material is eluted. There is already considerable evidence for several different α_2-macroglobulins present in human plasma.[28] At least one of these is known to bind proteins such as trypsin[29] and insulin.[30] There is thus evidence to propose that such protein–protein interaction may be what is complicating the present studies.

Accurate molecular weights and chemical compositions of proteins (from A.P.s 7–11) need to be determined after greater purification is achieved. The number of molecules of DMPEA bound per protein molecule, its binding sites, and factors affecting binding need to be determined. A specific activity of 1000 cpm/mg protein would indicate the binding of approximately 0·1 mole of DMPEA to 1 mole of a macroglobulin protein with molecular weight of 800,000. Thus with one of our best preparations of an α_2-macroglobulin about 0·8 mole DMPEA is bound per mole protein. It is interesting but not easily explained why the mole ratio of DMPEA to protein is less than 1 in even our best preparation. It is possible that these proteins were already partially saturated with structurally similar compounds. Catecholamines such as epinephrine and norepinephrine are known to be present in human plasma. Antoniades *et al.*[31] have some data on catecholamines binding to albumin

and other proteins. This same subject was considered again in an excellent review by Vendsalu.[32] More recently, O'Hanlon[33] has reported on the concentration of these two catecholamines in plasma.

It is interesting, as shown in Table 3, that the binding capacities of A.P.s 6–11 were markedly different in different donors. This phenomenon appears to be genuine, since good duplication in binding activity was observed in separate columns of the same preparation of fraction III. On the other hand, a marked difference in binding activity was observed in fractions obtained from the blood of the same donor over a period of 3 months. More data are needed on individual variations before the significance of such variations can be assessed.

As shown in Tables 3 and 6, the mean amount of binding was less for the peaks from patients than from normal subjects. However, the groups are markedly heterogeneous in variance; a *t*-test applied to the total scores in the last column of Table 6, indicates that the difference between the group means is not significant ($0{\cdot}05, < P < 0{\cdot}1$). The number of well-characterized schizophrenic patients is yet too small to permit firm conclusions.

No strong evidence is available about the nature and strength of the binding forces between DMPEA and the plasma proteins. This is an important area that merits further investigation.

The results of this study are suggestive enough to indicate that proteins in Cohn fraction III should be further studied by investigating their interactions with other phenylethylamine derivatives related to DMPEA. The important structural binding features should be evaluated, and the effect of selected drugs such as chloropromazine on the binding of DMPEA to the proteins should also be studied.

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