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# RAPID METHOD FOR THE ASSAY OF 4-AMINOBUTYRIC ACID (GABA), GLUTAMIC ACID AND ASPARTIC ACID IN BRAIN TISSUE AND SUBCELLULAR FRACTIONS

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## SUMMARY

The thin-layer electrophoretic separation at pH 4.8 of brain extracts and a procedure for fluorescent staining of the plates with fluorescamine are described for the rapid routine determination of 4-aminobutyric acid (GABA), glutamic acid and aspartic acid in brain extracts and in particulate fractions of brain tissue. Automated sample application, electrophoretic separation using two chambers, and quantitation by in situ fluorescence scanning allows the assay of 280 samples within three working days. The method is reproducible (S.D. $\leq$ 8% of the mean) within the range of 0.2—2 nmole per spot. The staining procedure can be applied to a variety of related analytical problems. The method has proved useful for the determination of the specific radioactivities of GABA, glutamic acid and aspartic acid in metabolic studies.

## INTRODUCTION

Flat bed chromatographic methods have attracted less attention since sensitive automated column chromatographic methods were developed for the routine determination of amino acids. However, the possibility of separating many samples in parallel, together with the improvement of sensitivity by using fluorescamine [1, 2] instead of ninhydrin as the staining reagent continues to make flat bed chromatographic methods attractive for application which demand numerous determinations of selected acids or related compounds.

Our study of subcellular pools of 4-aminobutyric acid (GABA) required the assay of this amino acid in many samples of subcellular fractions, which were

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obtained by density gradient centrifugation. Automated ion-exchange column chromatography was not sufficiently available for the analysis of so many samples, and this prompted us to develop the present method.

Thin-layer electrophoresis at pH 4.8 proved to be a rapid and reliable method for the separation of GABA, aspartic acid and glutamic acid from all other primary amino group-containing compounds which are present in brain extracts in comparable amounts to those of the non-essential amino acid. The staining of thin-layer plates with fluorescamine was improved. Low background fluorescence was achieved together with good reproducibility of staining of the amino acid spots. These methods are described in the present work.

## MATERIALS AND METHODS

All usual laboratory chemicals were from E. Merck, Darmstadt, G.F.R. Fluorescamine (Fluram) was purchased from Pierce. Rockford, Ill., U.S.A. Sodium diatrizoate was a gift from Winthrop Labs., Newcastle-upon-Tyne, Great Britain.

The laboratory animals (male Sprague-Dawley rats and male $CD_1$ albino mice; Charles River, St. Aubin-les-Elbeuf, France) were kept under standardised conditions, having access to standard diet and water ad libitum.

### *Sample preparation*

(a) The animals were decapitated and the heads immediately immersed in liquid nitrogen. The still-frozen brains were homogenized with 10 volumes of 0.2 *N* perchloric acid. Aliquots of the supernatants were diluted with 10 volumes of dioxane. 100-$\mu$l volumes of these solutions were directly applied to thin-layer plates in 12 mm long streaks. Alternatively, 5-$\mu$l aliquots of the extracts were applied as round spots.

(b) Synaptosomal fractions were isolated according to described methods [3—5]. The sucrose- or Ficoll-containing suspensions were mixed with one tenth of the volume of the suspension of a 40% (w/v) solution of trichloroacetic acid and the precipitates removed by centrifugation.

The supernatants were extracted four times with the same volume of water-saturated diethyl ether. The residual diethyl ether was removed from the aqueous phase in a stream of air. The samples, up to 5 ml, were then applied to a 1 ml column of Dowex 50W-X8 (200 mesh, $H^+$ form). The columns were washed with 10 ml of distilled water. The amino acids were eluted using 6 ml of a 40% (w/v) trichloroacetic acid solution. The trichloroacetic acid was removed from these eluants by extraction with diethyl ether, as described above.

Pre-separation on Dowex columns of up to 60 samples can be easily achieved in parallel using the multiple column chromatographic device described previously [6].

The samples were dried by lyophilization and the residues dissolved in 0.1 ml of water and 2.9 ml of ethanol or dioxane. Usually 100 $\mu$l of this solution were applied to a thin-layer plate for GABA determination in a synaptosomal fraction.

The synaptosomal fraction of the sodium diatrizoate gradient was diluted to 10 ml with water. 0.5-ml aliquots were mixed with 1 ml of ethanol. The precipitate was sedimented completely by centrifugation at 1000 *g* for 10 min.

100-µl volumes of the supernatant were applied on thin-layer plates for separation.

*Protein determination*

The acid precipitates of the homogenates and the sucrose and Ficoll gradients were washed twice with 0.2 *N* perchloric acid and dissolved in 4 ml of 1 *N* NaOH at 37° (2 days). The alkaline solutions were appropriately diluted with water; aliquots were used for protein determination using the method of Hartree [7].

Samples (0.5 ml) of the sodium diatrizoate gradients were precipitated with a solution of perchloric acid in methanol (0.2 *N*) instead of aqueous perchloric acid, in order to dissolve diatrizoate. The precipitates were washed twice with the methanolic perchloric acid, before they were processed further in the same manner as described for the other samples.

*Sample application and electrophoretic separation*

For the electrophoretic separation 20 × 20 cm commercial silica gel covered glass plates (silica gel 60, E. Merck; silica gel 1500, Schleicher & Schüll, Dassel, G.F.R.) were used. Ten samples (two or three standards and seven or eight tissue samples) were applied in 12-mm streaks at a distance of 8 cm from one plate edge. If a suitable thin-layer scanner for in situ fluorometry is available, samples can also be applied as spots of a diameter less than 3 mm.

The sample applicator used in this work allowed the automated application of ten spots or lines at the same time. It was the prototype of the Autodoser (Desaga, Heidelberg, G.F.R.), which was developed by one of us at the Max-Planck-Institute for Brain Research, Frankfurt/M., G.F.R. The automated sample application with this apparatus allows the concentration of volumes up to 0.8 ml on a spot of the thin-layer surface, without affecting the quality of the separations.

The plates were sprayed with about 30 ml pyridine acetic acid buffer pH 4.8 until they were evenly wetted.

Buffer composition: 100 ml pyridine; 75 ml glacial acetic acid, 30 g citric acid, 2300 ml water [8]. The cooled plate thin-layer electrophoresis equipment according to Pastuska (Camag, Muttenz, Switzerland) was used. The buffer reservoirs were filled for each separation with 35 ml of fresh buffer. The plates were cooled, with circulating methanol at 0°. At 600 V a current of about 70 mA was observed under these conditions. For the separation of GABA, glutamic acid and aspartic acid of brain extracts 60 min runs were suitable.

*Staining with fluorescamine*

After completion of the electrophoretic separation the plates were dried for 10 min at 110° in an oven with circulating air. The dried plates can be stored for two days, before they are stained with fluorescamine and evaluated by in situ fluorescence scanning.

Before staining, the plates were heated again to about 50°. Then they were dipped rapidly into a bath containing an alkaline solution, which was prepared as follows: 100 ml of saturated solution of NaOH in methanol was mixed with 100 ml of *n*-butanol. This solution was gradually diluted with 600 ml of

toluene. After 30 sec the plate was removed from the bath and dried in a horizontal position for a few minutes at room temperature and then completely for 10 min at 110°.

The staining solution contained 10 mg of fluorescamine in 100 ml of a mixture of acetone—*n*-butanol (1:1; v/v). A stainless-steel tank (22 × 20 × 0.7 cm) was used for dipping, containing approximately 200 ml of the fluorescamine solution.

The plates were cooled to room temperature. They were then dipped twice into the fluorescamine solution, with a drying period of 1—2 min. Alternatively, the plates can be sprayed with 20 ml of the same fluorescamine solution. However, it should be pointed out that even spraying requires experience in order to obtain staining as homogenous as that obtained by dipping. The plates were stored for at least 2 h at room temperature, before they were quantitatively evaluated by in situ fluorescence scanning. If the plates were stored (protected from dust and light) they could be evaluated even two days after staining. However, fluorescence staining with fluorescamine is not absolutely stable. A constant time schedule between separation, staining and in situ fluorescence measurement is advisable.

*In situ fluorescence scanning*

Any commercial scanning attachment to a spectrofluorometer should be suitable for the quantitative evaluation of the fluorescamine-stained plates. In the present work an Aminco-Bowman spectrofluorometer with the thin-film scanning attachment has been used. Activation of fluorescence was achieved at 390 nm; fluoroscence was measured at 490 nm. The recorded fluorescence intensity was evaluated by peak height measurement.

## RESULTS

*Electrophoretic separation of amino acids at pH 4.8*

At pH 4.8 neutral amino acids remain near the origin, acidic amino acids and peptides move towards the anode and basic amino acids and peptides move cathodically. As is shown in Fig. 1 none of the 25 usual amino acids co-migrate with GABA, glutamic acid or aspartic acid. Electrophoretic separation for 60 min on a silica gel thin-layer plate, using a pyridine acetate buffer (pH 4.8) allows an adequate separation of these three amino acids from all other fluorescamine-stainable constituents present in comparable amounts in brain tissue.

The 4-aminobutyrate:2-oxoglutarate aminotransferase (GABA-T) inhibitors, 4-aminohex-5-ynoic acid (RMI 71645) and 4-aminohex-5-enoic acid (RMI 71754) [9—11], are not separated from each other, but they are sufficiently separated from GABA to allow GABA determinations in their presence. β-Alanine and homocarnosine do not interfere either. Minor, but unidentified, constituents might co-migrate with GABA or at least may yield overlapping spots. The method is therefore not necessarily adequate for GABA-determinations in tissues with low GABA concentrations such as liver, muscle or kidney.

*Staining with fluorescamine*

Staining with fluorescamine of identical amounts of electrophoretically

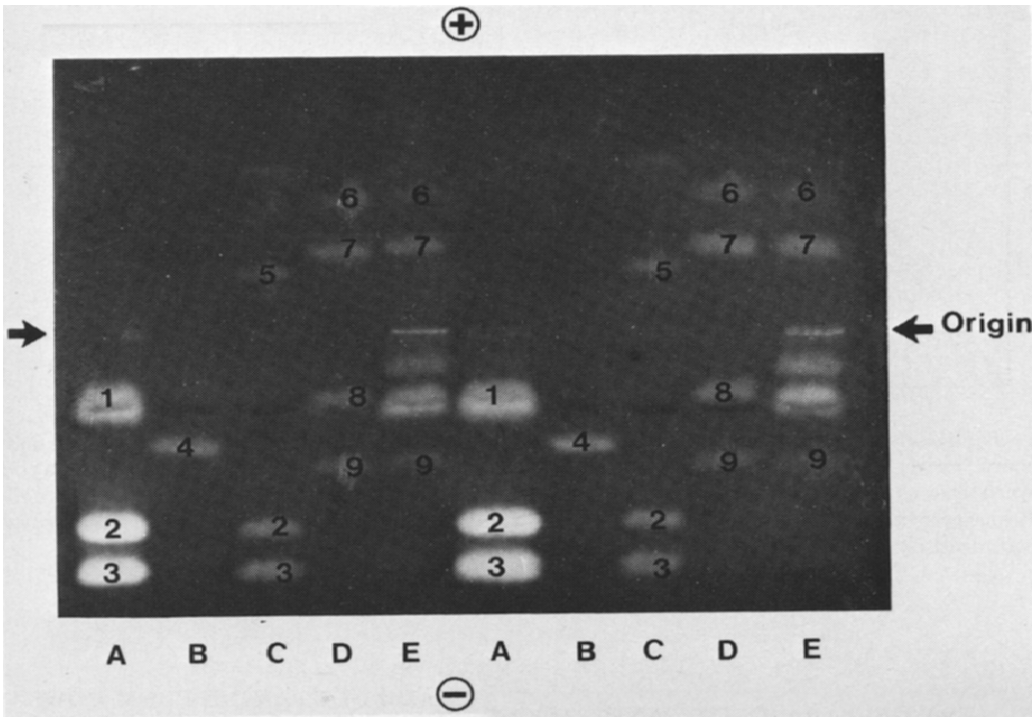


Fig. 1. Thin-layer electrophoretically-separated amino acids and brain extracts (pyridine—acetic acid buffer pH 4.8; 600 V; 60 min) stained with fluorescamine. (Activation of fluorescence at 350 nm). A = Mixture of the following L-amino acids: Ala, Arg, Cys, Gly, Ile, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, Trp, Val (about 2 nmole of each). 1 = Neutral amino acids; 2 = His + Arg; 3 = Lys. B = 4-Aminohex-5-ynoic acid plus 4-aminohex-5-enoic acid (4). C = Arg (2); Lys (3); glutathion (5). D = Asp (6); Glu (7); $GluNH_2$ (8); GABA (9). E = Rat brain extract (corresponding to 1 mg of fresh tissue); Asp (6); Glu (7); GABA (9).

separated amino acids under the conditions described in detail in the methods section results in reproducibly fluorescing spots. These show a linear relationship between the amount of amino-acid and peak height (or peak area) of the recorded fluorescence intensity in the range from 200 to 2000 pmole per spot (Fig. 2). The linear correlation coefficients for the amounts of the four amino acids and the fluorescence intensity are $\geqslant 0.996$. Amounts of an amino acid exceeding 2000 pmole per spot should be avoided, unless the amount of the standard is closely similar and only a narrow concentration range is considered for the measurement.

The reproducibility of the method, including pre-separation of the samples, was tested by applying 10-nmole amounts of each amino acid on Dowex 50W-X8 columns, and by their subsequent measurement in aliquots of the column eluates. Table I summarizes the results. It can be seen from this table that recovery was over 90% for GABA, glutamic acid, glutamine and aspartic acid. The standard deviation for the whole procedure was better than 8% of the mean value. It should be noted that under the conditions of sample preparation and sample separation glutamine is not hydrolyzed to glutamic acid.

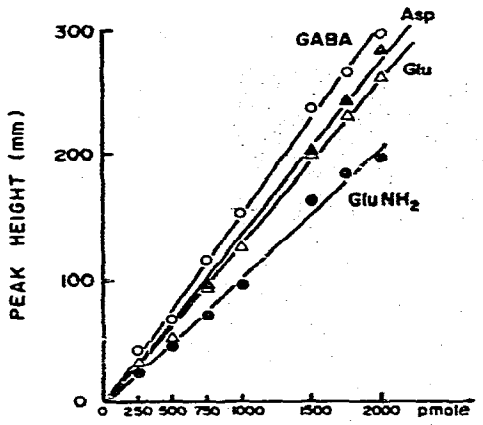


Fig. 2. Relationship between the amount of amino acid and peak height of the recorded fluorescent spots of GABA, aspartic acid, glutamic acid and glutamine. After thin-layer electrophoretic separation of standard mixtures of these amino acids, the plates were stained with fluorescamine, as described in the Materials and methods section; the fluorescent spots were evaluated by in situ scanning of fluorescence.

TABLE I

RECOVERY OF GABA, GLUTAMATE, ASPARTATE AND GLUTAMINE FROM DOWEX 50W-X8 COLUMNS AND REPRODUCIBILITY OF THE ASSAY

10-nmole amounts of each amino acid were applied to the columns. One tenth of each column eluate was separated by electrophoresis. The figures in the Table are the means of duplicate determinations.

| Sample No. | Recovery (nmole) | | | |
|---|---|---|---|---|
| | GABA | Glutamate | Glutamine | Aspartate |
| 1 | 9.0 | 9.2 | 8.7 | 10.0 |
| 2 | 9.4 | 9.3 | 8.7 | 9.7 |
| 3 | 10.0 | 9.5 | 10.0 | 10.6 |
| 4 | 10.0 | 8.2 | 8.9 | 8.3 |
| 5 | 10.3 | 8.4 | 9.7 | 9.1 |
| 6 | 8.3 | 8.1 | 9.0 | 8.9 |
| Mean ± S.D. | 9.5 ± 0.8 | 9.0 ± 0.6 | 9.2 ± 0.6 | 9.3 ± 0.7 |

*Comparison of GABA determinations in brain by electrophoresis and ion-exchange column chromatography*

In order to test the practicality of the electrophoretic method, determinations in brains of rats with varying GABA concentrations were carried out. The same tissue samples were also analyzed in duplicate using a Labotron amino-acid analyzer (Liquimat 2). Buffer: lithium citrate (23 g/l); lithium chloride (18 g/l), pH 4.6. The *o*-phthaldehyde method was utilized for detection.

The increase of GABA levels in the rat brains was achieved by pretreatment

of the animals with4-aminohex-5-enoic acid, the enzyme-activated irreversible inhibitor of GABA-T [11] mentioned previously. Table II shows the results of this comparison. From these figures it appears that the two methods give results which are in good agreement: 98 ± 13% (S.D.) of the GABA amount obtained with the ion-exchange column chromatographic method was found with the thin-layer electrophoretic method and the two sets of data were well correlated ($R^2$ = 0.94). The average standard deviation of duplicate determinations of brain GABA was for the electrophoretic method ± 9% of the means, and ± 1% for the ion-exchange column chromatographic method.

*Distribution of GABA, glutamic acid and aspartic acid in particulate fractions of mouse brain cortex*

The electrophoretic separation method combined with fluorescence staining using fluorescamine was applied to the determination of GABA, glutamic acid and aspartic acid in fractions of density gradient centrifugations. The results obtained for the distribution of these amino acids in sub-fractions of mouse brain cortex homogenates are summarized in Tables III and IV.

Table III shows the results for fractionation in sucrose solutions. It appears that the sum of GABA found in the supernatant ($S_2$) and the crude synaptosomal fraction (= crude mitochondrial fraction) ($P_2$) is somewhat larger than the GABA amount in the equivalent amount of homogenate, but this difference is not statistically significant. In the case of aspartic acid a similar relationship holds, but concentrations of free glutamic acid seem to be unchanged during tissue processing. From Table IV it appears that the concentrations of GABA and glutamic acid in synaptosomal preparations obtained by different procedures are of comparable magnitude. However, at least in our hands, the yield of synaptosomes was significantly smaller if sucrose—Ficoll gradients [4] were used instead of sucrose [3] or sodium diatrizoate [5] gradients. The GABA concentrations obtained in this work from synaptosomes of sucrose density gradient centrifugations [3] were higher, and those obtained by centrifugation into sodium diatrizoate gradients [5] were lower than those found in previous experiments [12]. These differences are most probably due to interference by sucrose and sodium diatrizoate with protein determinations by the method of Hartree [7]. In the present work these compounds have probably been removed more carefully from the protein precipitates.

The reason for the apparently high values of aspartate in the sodium diatrizoate fractions is not known. Interference of an unknown impurity with aspartate determinations cannot be ruled out. The somewhat higher yield of protein in the synaptosomal fraction from sodium diatrizoate density gradient centrifugations is in agreement with previous observations [13]. This may not reflect higher yields of synaptosomes, but rather a larger proportion of impurities (myelin fragments etc.) as was suggested by electron microscopy of the fractions [13].

## DISCUSSION

Fluorescamine as a spray reagent for the detection and quantitative analysis of primary amines, amino acids, peptides, aliphatic or aromatic amino groups

TABLE II

BRAIN GABA CONCENTRATIONS OF YOUNG RATS TREATED WITH 4-AMINOHEX-5-YNOIC ACID, AN IRREVERSIBLE GABA-T INHIBITOR

Comparison of two methods, thin-layer electrophoresis and ion-exchange column chromatography. The GABA values are the Means of duplicate determinations.

| Age of rats (days) | Drug dose (mg/kg) | Drug administration prior to sacrifice (h) | Brain GABA concentration | | |
|---|---|---|---|---|---|
| | | | Ion-exchange column chromatographic determination (nmole/mg) | Thin-layer electrophoretic determination (nmole/mg) | (Percent of value obtained by ion-exchange column chromatography) |
| 3 | — | — | 1.03 | 1.09 | 106 |
| | | | 1.19 | 1.02 | 86 |
| | | | 1.30 | 1.10 | 85 |
| | | | 1.36 | 1.57 | 115 |
| 3 | 200 | 4 | 1.27 | 1.04 | 82 |
| | | | 1.39 | 1.09 | 78 |
| | 500 | 4 | 1.49 | 1.53 | 103 |
| | 750 | 4 | 1.34 | 1.35 | 101 |
| 3 | 200 | 16 | 5.39 | 5.46 | 101 |
| | 500 | 16 | 6.07 | 6.53 | 108 |
| | 750 | 16 | 7.30 | 6.74 | 92 |
| | | | 5.68 | 6.21 | 109 |
| | 1500 | 16 | 6.29 | 6.44 | 102 |
| | | | 6.86 | 6.92 | 101 |
| 10 | — | — | 1.32 | 1.47 | 111 |
| | | | 1.21 | 1.42 | 117 |
| | | | 1.44 | 1.66 | 115 |
| 10 | 200 | 4 | 4.35 | 4.87 | 112 |
| | | | 2.95 | 2.48 | 84 |
| | 500 | 4 | 6.33 | 5.64 | 89 |
| | | | 4.40 | 4.08 | 93 |
| | | | 6.22 | 5.56 | 89 |
| | 750 | 4 | 4.79 | 4.79 | 100 |
| | | | 6.64 | 6.08 | 92 |
| | | | 6.55 | 7.84 | 120 |
| | 1500 | 4 | 6.30 | 6.52 | 103 |
| | | | 6.32 | 6.93 | 110 |
| 10 | 500 | 16 | 14.41 | 11.33 | 79 |
| | 750 | 16 | 15.29 | 13.09 | 86 |
| | | | 18.52 | 14.08 | 76 |
| | | | 18.74 | 14.13 | 75 |
| | 1500 | 16 | 12.98 | 14.97 | 115 |
| | | | | Mean ± S.D. | 98 ± 13 |

containing drugs or drug derivatives on thin-layer plates has been used for several years [1, 2]. A survey of the literature shows that several modifications of spraying techniques have been suggested from time to time [14—19] but the procedure of Feliz and Jimenez [14] is still the most widely used, mainly for qualitative mapping of peptides and related purposes. In a recent paper [20] this method was compared with ninhydrin and *o*-phthalaldehyde staining. It was stated, that the sensitivity obtained for amino acid determinations with fluorescamine was no better than that with ninhydrin. In our hands this spraying technique was not satisfactory either: high background fluorescence and low yields in fluorescent derivatives were obtained. Substitution of triethylamine by triethanolamine as base decreased background fluorescence considerably. Therefore, we used the method routinely for some time, although the fluorescent reaction products developed only slowly. It was necessary to store the plates overnight, before their evaluation by in situ fluorescence scanning was possible. The dipping method described in this work considerably improves staining of thin-layer chromatograms with fluorescamine. Its applicability should be wide and its use contraindicated only for primary amino group containing compounds of low polarity with considerable solubility in the toluene—*n*-butanol mixture.

Some practical considerations: the alkaline solution should be freshly prepared every day and not used for more than 40 plates. The fluorescamine solution must be prepared daily. About 15 ml of this solution are needed per 20 × 20 cm plate. Even though the staining procedure is highly reproducible, the differences between two plates can be significant. It is therefore advisable to run standards on each plate and to observe the precautions found to be important for quantitative evaluation of fluorescent spots by direct scanning [21]. Two or three standards are normally sufficient, so that seven or eight tissue samples can be separated on each chromatogram.

The combination of thin-layer electrophoresis and staining with fluorescamine has proved, in our hands, a practical method of reasonable sensitivity. The precision and the sensitivity of the method is influenced considerably by instrumental characteristics and was in our case obviously limited by the scanning device. Both reproducibility and sensitivity could therefore be improved with better instrumentation.

Since an equivalent of only 5—10-$\mu$l aliquots of brain extracts are normally applied for GABA determinations, sample application by manual methods is feasible. In the case where samples with low concentrations of amino acids are to be determined, the advantages of automated sample application are enormous. The cumbersome concentration of the samples to a few microliters is thereby eliminated. About 800 $\mu$l of an ethanol—water mixture (3:1) can be applied as a 12-mm streak within 30 min; 10 samples of this volume are applied simultaneously. Automated sample application therefore makes thin-layer chromatographic and thin-layer electrophoretic methods attractive for routine assay procedures in biochemistry, as was exemplified by its application to amino acid analyses in fractions from density gradient centrifugation.

An important aspect of the method described here is its rapidity. Using the automated sample applicator and two thin-layer electrophoretic chambers, it was possible to run at least 14 plates during a normal 8 h working day. Since

TABLE III

DISTRIBUTION OF GABA, GLUTAMIC ACID AND ASPARTIC ACID IN MOUSE BRAIN CORTEX PARTICULATE FRACTIONS

Brain cortex homogenates in 0.32 *M* sucrose were fractionated by differential centrifugation according to Gray and Whittaker [3]. The crude synaptosomal fraction was purified further by centrifugation into a three step sucrose density gradient [3]. The values are means of three independent experiments ± S.D.

| Fraction | GABA | | Glutamic acid | | Aspartic acid | |
|---|---|---|---|---|---|---|
| | μmole per g brain cortex | Percent of total amount of brain cortex | μmole per g brain cortex | Percent of total amount of brain cortex | μmole per g brain cortex | Percent of total amount of brain cortex |
| Homogenate | 2.3 ±0.26 | 100 | 10.2 ± 1.0 | 100 | 3.64 ±0.6 | 100 |
| Supernatant ($S_2$) | 1.9 ±0.4 | 82.6 | 8.06 ± 0.23 | 79.0 | 3.26 ±0.21 | 89.6 |
| Crude synaptosomal fraction ($P_2$) | 0.70 ±0.1 | 30.4 | 2.3 ± 0.2 | 22.5 | 1.56 ±0.07 | 42.9 |
| Purified synaptosomal fraction (B) | 0.48 ±0.08 | 20.9 | 1.40 ±0.16 | 13.7 | 0.68 ±0.21 | 18.7 |

TABLE IV

AMINO ACID CONCENTRATIONS IN SYNAPTOSOMAL FRACTIONS OF MOUSE BRAIN CORTEX. COMPARISON OF THREE METHODS.

The values are means ± S.D. of three independent experiments.

| Synaptosomal preparation | GABA | | Glutamic acid | | Aspartic acid | | Protein |
|---|---|---|---|---|---|---|---|
| | nmole per mg protein | μmole per g brain cortex | nmole per mg protein | μmole per g brain cortex | nmole per mg protein | μmole per g brain cortex | mg per g brain cortex |
| Three-step sucrose density gradient according to Gray and Whittaker [3] | 29.1 ±1.3 | 0.44 ±0.02 | 67.8 ± 6.6 | 1.03 ±0.1 | 27.7 ± 5.6 | 0.42 ±0.09 | 15.2 ± 0.4 |
| Sucrose—Ficoll density gradient according to Cotman and Matthews [4] | 25.8 ±4.2 | 0.18 ±0.03 | 77.5 ± 6.3 | 0.55 ±0.04 | 25.4 ± 7.1 | 0.18 ±0.05 | 7.1 ± 0.5 |
| Continuous sodium diatrizoate gradient according to Tamir et al. [5] | 27.7 ±7.2 | 0.54 ±0.14 | 78.3 ±13 | 1.53 ±0.26 | 78.3 ±15 | 1.53 ±0.3 | 19.5 ±0.2 |

each plate separates 10 samples, a total of 140 samples were separated during this time. Normally separations were performed on two consecutive days, and staining and quantitative evaluations were done on the third day. In other words, about 280 samples can be measured within three working days. In the case of brain extracts or particulate fractions of brain homogenates, quantitative data for three amino acids were obtained.

The fully automated amino-acid analyzer needed 60 min for one GABA determination. Its maximum sample output within 72 h was therefore 72, i.e. only 25% of that achieved with the electrophoretic method. If glutamic and aspartate were included, the appropriate separation had to be prolonged further to 90 min per sample.

Flat bed chromatographic methods allow the preparation of autoradiographs, if radioactively-labelled compounds have been separated. Moreover, radioactivity can be scanned sensitively with appropriate equipment. We are using the method described here not only for quantitative amino acid measurements, but also for the determination of their specific radioactivities in metabolic studies. In this respect the method is unsurpassed by other procedures, as far as rapidity is concerned.

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