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A SENSITIVE GAS CHROMATOGRAPHIC PROCEDURE FOR THE ESTIMATION OF NORADRENALINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE IN RAT BRAIN

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Abstract—A gas chromatographic method for the estimation of noradrenaline, dopamine and 5-hydroxytryptamine in rat brain is reported. The method utilises homogenization of the tissue in acid butanol, alumina adsorption of the catecholamines and their subsequent ion-pair extraction. This is combined with the solvent extraction of 5-hydroxytryptamine to produce a method which is sufficiently sensitive to estimate the amount of these amines in discrete areas of a single rat brain.

SEVERAL methods are available for the separation of the catecholamines and 5-hydroxytryptamine by gas chromatography. Fales and Pisano[1] successfully separated several biologically important amines, including 5-hydroxytryptamine, by gas liquid chromatography (GLC), without prior chemical modification, and amines of moderate polarity have been chromatographed by either coating the column support with potassium hydroxide[2–5] or by using high percentages of the stationary phase.[6] Most investigators have favoured the prior derivatization of the functional groups in order to both decrease the polarity and increase the vapour pressure of the compounds, thus facilitating subsequent gas chromatography.

The modifications reported for this purpose have included: Schiff's base formation,[2,5,7] silylation of the hydroxyl groups, followed by the conversion of the amino function to a Schiff 's base,[8–10] silylation of both the hydroxyl and the primary amino groups,[11,12] silylation of the hydroxyl groups followed by the acetylation of the amino function,[13] and the acetylation of both the hydroxyl and the amino groups.[14]

The need to estimate the small quantities of these amines in biological tissues led to the development of derivatives which could be used in conjunction with the electron capture detector. This detector shows considerable sensitivity towards compounds with a high electron affinity, and at the same time exhibits considerable selectivity towards them. Attempts therefore, have been made to convert biogenic amines to derivatives suitable for use with this type of detector. Conversion of these compounds to their trifluoroacetates[15] or the silylation of their hydroxyl groups followed by the formation of the heptafluorobutyryl amides[13,16] both serve this purpose.

However, while much has been published on the preparation of suitable derivatives, considerable difficulty has been experienced in their application to the analysis of biological material.[8] The difficulty lies in the preliminary separation of these relatively hydrophilic amines from the tissue and their subsequent conversion to appropriate derivatives under anhydrous conditions without excessive losses.

The extraction of these amines with organic solvents is limited by their lack of solubility in most common organic substances, though Temple and Gillespie[17] succeeded in extracting some amines of biological interest from the aqueous phase with the help of the ion-pairing compound di-(2-ethyl hexyl)phosphate. The use of this compound to recover the catecholamines from biological material in a suitable form for their derivatization and subsequent gas chromatographic analysis is described in the present paper.

The sensitivity of the method allows the estimation of as little as 5 ng of both noradrenaline and dopamine and 10 ng of 5-hydroxytryptamine in a single piece of brain tissue.

MATERIALS

AR chemicals (British Drug Houses, Poole, Dorset) were used throughout except where stated. Water was redistilled twice in an all-glass apparatus, and deionized before use.

Standard solutions. The equivalent of 20 mg of the free base of L-noradrenaline, dopamine hydrochloride (Sigma) and 5-hydroxytryptamine creatinine sulphate (Sigma) were dissolved in 20 ml of 0·01 N HCl containing 25 mg of the disodium salt of EDTA and 2 mg of potassium metabisulphite. The solutions were stored at −17° and the appropriate dilutions were made on the day of the experiment.

Alumina. About 200 g of chromatographic grade alumina (B.D.H.) was boiled in 1 litre of 1 N HCl for 30 min, washed with 20 × 100 ml of water, and then heated to dryness at 200° for 2 hr.

n-*Butanol.* Batches of *n*-butanol (butan-1-ol) were washed with 4 × 250 ml of water in a funnel fitted with a polytetrafluoroethylene stop-cock and then saturated with sodium chloride.

Acid–butanol. Washed and salt-saturated *n*-butanol (1 litre) was shaken with 0·85 ml of concentrated (s.g. 1·18 g/ml) HCl; then 1 g of potassium metabisulphite and 0·1 g of the disodium salt of EDTA were added and the mixture shaken thoroughly.

Phosphate buffer, 0·5 *M*, *pH* 8·0. The dodecahydrate of disodium hydrogen orthophosphate (17·91 g) was dissolved in 90 ml of water and the pH adjusted with sodium dihydrogen phosphate before being diluted to 100 ml with water.

Borate buffer, 0·5 *M*, *pH* 10·0. Boric acid (31·4 g) was dissolved in 900 ml of water and the pH adjusted with 10 N sodium hydroxide; this was then made up to 1 litre and saturated with sodium chloride.

20% (*v*/*v*) n-*Butanol in diethyl ether* (20 *B-E*). *n*-Butanol (20 ml) were added to 80 ml of diethyl ether and the solution shaken.

Trifluoroacetic anhydride. Trifluoroacetic anhydride (100 ml) was redistilled from phosphorus pentoxide, the fraction distilling from 39–43°, at NTP, was collected and sealed in ampoules until required. The ampoules were stored at 4°.

Methyl cyanide. This material was obtained as a specially purified reagent from the Pierce Chemical Company through Phase Separations Ltd., Deeside Industrial Estate, Connah's Quay, Flintshire.

Di-(2-ethyl hexyl)phosphate. Di-(2-ethyl hexyl)phosphate (DEHPA) was obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., and used without further purification.

General. The shaking throughout this procedure was carried out on a Griffin Flask Shaker (Griffin and George Ltd., Frederick Street, Birmingham). The evaporation of the samples to dryness was accomplished on a Buchi Rotary Evaporator, the vacuum being supplied by an Alley Vacuum Pump (Alley Compressors Ltd., Cathcart, Glasgow, type AHV 1); a solid carbon dioxide–acetone bath was inserted between the two to trap water vapour.

All centrifugation was carried out at 3000 *g* for 5 min unless otherwise stated.

Gas chromatography. The gas chromatograph used in this work was a Pye-Unicam Series 104, Model 74 instrument, fitted with an electron capture detector, the β-particles being emitted by a ^{63}Ni foil (10 mCi). The detector was operated in the pulsed mode with a pulse period of 50 μsec, pulse width of 0·75 $\pm$ 0·25 μsec and a pulse amplitude of 47–60 V. The instrument was fitted with a standard 1·5 m, 4 mm bore glass column packed with 5% SE-52 on 100–120 mesh Gas Chrom Q (Applied Science Laboratories, supplied by Field Instruments, Richmond, Surrey), the stationary phase being prepared by the standard evaporation method using ethyl acetate as the solvent. The injector heater was set at 3·0 (approx. 30° above the column temperature) and the detector at 250°. Oxygen-free nitrogen was used as the carrier gas at a flow rate of 45 ml/min, with no purge gas to the detector.

Animals. The animals used were Specific Pathogen-Free male Wistar rats, 4½ months old, obtained from the Biochemistry Department, University of Birmingham. They were killed by cervical fracture and the brains were removed as quickly as possible, at room temperature, carefully freed from the pineal and the meninges, and stored at −17° until the estimations were carried out. We have shown that the levels of the relevant amines do not change for several weeks under these conditions.

METHODS

Each tissue sample was homogenized in 7·5 vol. of acid–butanol at 4°, using a Potter–Elvejhem homogenizer fitted with a Teflon–glass pestle of clearance 0·1–0·15 mm. The smallest volume used was 5·0 ml. In each separate assay one sample volume was selected and to this was added 250 ng of each of the amines in 0·05 ml, which was then shaken for 2 min. This sample served as an internal standard for the assay and was always handled in parallel with the other brain homogenates.

Stage 1. All the homogenates were centrifuged for 10 min, 4 ml of the clear supernatant was shaken with 10 ml 2,2,4-trimethyl pentane and 5 ml water for 5 min, and the mixture centrifuged. To 4·5 ml of the lower aqueous phase was added 0·2 ml 2 M sodium acetate followed by 0·2 g of alumina, and this was also shaken for 5 min and centrifuged for 5 min. The supernatant was transferred to another tube and retained. The alumina was washed by shaking with 2 ml of water for 5 min and centrifuged; the washings were pooled with the previous alumina supernatant. This was stored at −17° overnight for the assay of the 5-hydroxytryptamine on the following day.

Stage 2. The catecholamines, noradrenaline and dopamine, were eluted from the alumina by shaking with 2 ml 0·05 M perchloric acid for 15 min; the samples were then centrifuged. The supernatant was transferred to another tube and 0·2 ml of 0·5 M phosphate buffer was added, followed by 0·2 ml of 0·5 M sodium bicarbonate, thus bringing the pH of the solution to 8·0. After shaking this solution with 2·0 ml of 2·5% v/v solution of DEHPA in chloroform for 5 min the resulting emulsion was broken by centrifuging. The lower organic layer was removed, leaving any interfacial

residue with the aqueous phase, and shaken for 5 min with 2·0 ml of 0·5 N formic acid. After centrifuging, the aqueous phase was removed and evaporated to dryness at 35° under vacuum, after which the tube was tightly capped. To each tube was added 0·5 ml of methyl cyanide and 50 μl. of trifluoroacetic anhydride. The formation of the catecholamine derivatives was complete after 5 min at room temperature, and a sample of this solution (1 μl) was then injected onto the gas chromatograph. The initial column temperature of 115° was held for 11 min and then increased at a rate of 1·5°/min until the derivatives had been eluted. The retention time of the noradrenaline derivative was 23·2 min and that of the dopamine 26·9 min. The column was cleaned for the next injection by raising the temperature to 180° for 10 min.

Stage 3. To the supernatant and the washings from the alumina was added 5 ml of the 20% v/v solution of *n*-butanol in diethyl ether, followed by 0·2 ml of 0·5 M borate buffer, pH 10. The sample was shaken for 5 min and then centrifuged. The upper organic layer was removed and the ether evaporated off at 35° under a stream of air; this extraction was repeated a further twice and the remaining butanol was evaporated to dryness at 35° under vacuum. The tubes were then capped immediately and 0·5 ml of methyl cyanide added followed by 30 μl of trifluoroacetic anhydride. The reaction was complete after 5 min at room temperature and 1 μl of this sample was injected onto the gas chromatograph with a column temperature of 180° which was held for 6 min, and then increased at a rate of 0·75°/min until the derivative had been eluted with a retention time of 13·9 min. The column was cleaned for the next injection by raising the temperature to 205° for a period of 10 min.

RESULTS AND DISCUSSION

The solvent of choice in the fluorimetric methodology was water, but the use of the electron capture detector in the present procedure has necessitated the selection of an alternative solvent. However, the change from an aqueous to a non-aqueous phase does introduce solubility problems. Not only are the amines themselves relatively insoluble, but small amounts of other materials, such as salts, are extracted along with these amines; insoluble components of the mixture can absorb amines to an unpredictable extent, a serious problem in any micro-assay. These difficulties have been overcome in the present method.

Homogenization. Initial extraction procedures involving the homogenization of brain tissue in aqueous ethanol,[18] perchloric acid[19] or trichloroacetic acid[16,20] have been reported. The use of acidified *n*-butanol as suggested by Chang[21] and developed by Ansell and Beeson,[22] was adopted in this work because of the facility with which an aqueous solution prepared from the extract can be made ready for the adsorption of the catechols onto alumina, simply by the addition of sodium acetate solution.

Alumina adsorption and elution of the catecholamines. The adsorption of the catecholamines onto alumina as originally described by Shaw,[23] and investigated in detail by Anton and Sayre,[24] was used in a form modified from that described by Ansell and Beeson.[22] The optimum amount of alumina appeared to be 200 mg though it was necessary to add only 0·2 ml of 2 M sodium acetate to obtain adsorption of the catecholamines (Table 1). The alumina was then washed with water, since there was no evidence of any considerable loss (less than 3 per cent) of the catechols on washing the alumina, as suggested by Drell.[25] The elution of the catecholamines from the alumina was carried out with 0·05 M perchloric acid as suggested by Anton and

TABLE 1. THE RECOVERY OF THE CATECHOLAMINES AFTER ADSORPTION ONTO ALUMINA

Conditions	Amines recovered (%)	
	Noradrenaline	Dopamine
Recovery with 200 mg alumina	63·4	83·5
Recovery with 400 mg alumina	59·0	85·0
Recovery after addition 1 ml 2 M sodium acetate	63·4	80·5
Recovery after addition 0·2 ml 2 M sodium acetate	63·4	83·5

To 4·5 ml of water, containing 250 ng each of noradrenaline and dopamine, was added sodium acetate solution and alumina as described under the appropriate section of Stage 1, with the variations indicated in the Table. The per cent recovery of each amine from the alumina is given using the elution conditions described in Stage 1.

Sayre.[24] This reagent was chosen to keep the molarity of the eluting solution as low as possible in order to facilitate the next stage.

Extraction of the catecholamines from the aqueous phase using DEHPA. It was shown by Temple and Gillespie[17] that certain amines could be extracted from an aqueous solution into an organic phase with the ion-pairing compound di-(2-ethyl hexyl)phosphate (DEHPA); this method has been used by Boon and Mace[26] for the assay of adrenaline in pharmaceutical products.

In the present method the catecholamines are removed from the aqueous phase by a 2·5% v/v chloroform solution of DEHPA. This compound complexes with the amino function of the catecholamines thus extracting them from the aqueous phase, provided that the concentration of the other cations in the solution is low (Table 2). When 0·05 M perchloric acid is used for the alumina elution this requirement is met, whereas if trichloroacetic acid, acetic acid or phosphate buffer is used for the elution, a considerably higher molar concentration of the reagent is required to obtain a comparable recovery from the alumina, thus giving a higher cation concentration

TABLE 2. THE EFFECTS OF THE MOLARITY OF METALLIC CATIONS AND DEHPA CONCENTRATION ON THE RECOVERY OF THE CATECHOLAMINES FROM AQUEOUS SOLUTION

Conditions of extraction	Extraction of the amine (%)	
	Noradrenaline	Dopamine
0·5 M Sodium ions; 2·5% DEHPA	21·1	84·4
0·25 M Sodium ions; 2·5% DEHPA	40·2	86·0
0·045 M Sodium ions; 2·5% DEHPA	61·4	90·1
0·01 M Sodium ions; 2·5% DEHPA	45·5	66·4
0·045 M Sodium ions; 2·5% DEHPA	61·4	90·1
0·045 M Sodium ions; 1·0% DEHPA	60·0	90·1
0·045 M Sodium ions; 0·1% DEHPA	20·9	47·6

In each case the volume of the aqueous phase was 2·2 ml and was buffered with sodium phosphate to pH 8·0, the concentration of the sodium ions in each case is indicated. The volume of DEHPA solution was 2·0 ml in each case and the per cent figure refers to its v/v concentration in chloroform. The aqueous phase contained 250 ng of each amine and there were no perchlorate ions present.

which adversely affects the subsequent ion-pair extraction of these amines from the aqueous phase with the DEHPA.

The factors affecting the efficiency of liquid ion-exchange separations have been discussed elsewhere,[27,28] and here only limited attention will be paid to the details which have guided the selection of the conditions used in this procedure.

The ion exchange takes place between the DEHPA and any available cation. Because of the low concentrations of the amines, it is important to keep the concentration of the metallic cations as low as possible, consistent with maintaining a reasonable buffering capacity (Table 2). The concentration of the DEHPA in the organic phase is also important, though the limits here appear to be wide; only at the low concentrations of DEHPA is the extraction efficiency much reduced (Table 2).

The adoption of a pH of 8·0 was a compromise, since although the data of Temple and Gillespie[17] showed increasing extraction efficiency with increasing pH, decomposition of the catecholamines becomes apparent above pH 8·5.

TABLE 3. EFFECT OF AMINE CONCENTRATIONS ON THE EFFICIENCY OF THEIR EXTRACTION WITH DEHPA

Amine (ng) in 2·4 ml	Amine extracted (%)	
Aqueous phase	Noradrenaline	Dopamine
50	27·5	28·6
150	45·1	53·9
250	55·0	91·3

Varying amounts of noradrenaline and dopamine were added to 2·0 ml of 0·05 M perchloric acid. To this solution was then added 0·2 ml of sodium phosphate buffer, pH 8·0 and 0·2 ml of 0·5 M sodium bicarbonate. This solution was then extracted with 2·0 ml of a 2·5% (v/v) solution of DEHPA in chloroform and subsequently treated as described in the text. Here the perchlorate ions were present at 0·042 M and the sodium ion concentration was 0·083 M.

The extraction efficiency of the catecholamines under these conditions is not linear, in that the extraction was more efficient with higher concentrations of the amines (Table 3). A calibration curve must therefore be constructed as shown in Fig. 1.

The catecholamines, once extracted into the DEHPA solution, can be evaporated to dryness and the resulting material reacted with trifluoroacetic anhydride using methyl cyanide as the solvent. However, the presence of DEHPA in the sample causes large solvent "tails" on gas chromatography, and it has been found advantageous, therefore, to remove the DEHPA by extracting the catecholamines back into 0·5 N formic acid. This acid can then be evaporated to dryness at 35° under vacuum without decomposition of the amines. The small amount of solid material left after the evaporation, thought to be at least in part sodium formate, is taken completely into solution by the reaction mixture. The recovery of the amines back into the acid is in excess of 96 per cent.

Extraction of 5-hydroxytryptamine from the aqueous phase. Extraction of this amine from aqueous solution after the adsorption of the catechols onto alumina, was carried

out with a 20% v/v solution of *n*-butanol in diethyl ether; the advantage of this solvent over 100% *n*-butanol is that practically no salts partition into it from the aqueous phase. Thus, on evaporation to dryness, no material which is insoluble in the reaction mixture, remains. However, the reported extraction efficiency of 98·2 per cent quoted by Maruyama[29] could not be repeated in this laboratory. After a single extraction with 5 ml of the organic solution a recovery of 26·7 ± 1·7 per cent (mean n = 3 ±S.E.M.) was obtained under the conditions given above. When this extraction procedure was repeated three times the recovery was 64·7 ± 3·9 per cent (theoretically three extractions should give a recovery of 60·7 per cent) and linear over the range 50–300 ng. It was found that the use of DEHPA solutions to extract 5-hydroxytryptamine from the aqueous phase at pH 8·0 resulted in the recovery of nearly

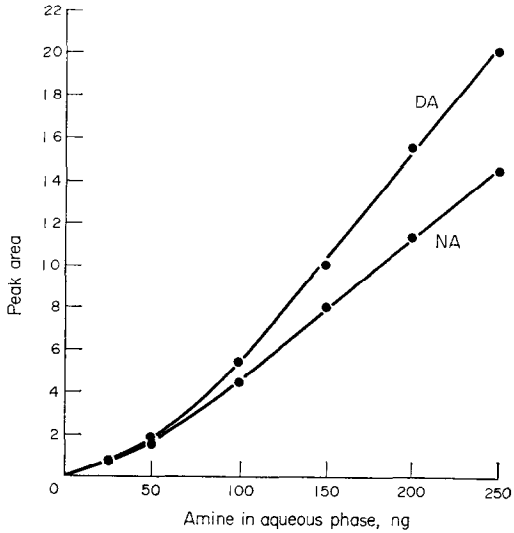

FIG. 1. Extraction curve for the catecholamines. Varying amounts of noradrenaline and dopamine were added to 2·0 ml of 0·05 M perchloric acid. To this solution was then added 0·2 ml of phosphate buffer, pH 8·0 and 0·2 ml of 0·5 M sodium bicarbonate, this solution was then extracted with 2·0 ml of a 2·5% v/v solution of DEHPA in chloroform and subsequently treated as described in the text. The vertical axis represents the peak area in arbitrary units and the horizontal axis the amount of the amine (nanogram) which was added to the aqueous solution. The upper curve is for dopamine (DA) and the lower for noradrenaline (NA).

100 per cent of the amine. However, on the evaporation of the solution to dryness and subsequent derivatization and gas chromatography, a small interference peak was found on the tail of the peak due to the 5-hydroxytryptamine derivative. It was found that this interference derived from the DEHPA and attempts to remove this by the re-extraction of the amine into formic acid and the subsequent evaporation of this solution to dryness, resulted in considerable loss of the amine, as might be expected since it is known to be unstable in strong acid. This modification was therefore abandoned.

Preparation of derivatives. Trifluoroacetyl derivatives were selected because they can be formed with great ease and are relatively stable under anhydrous conditions. For example, no decomposition of the catecholamine derivatives was found after

storing for 24 hr at room temperature, though the derivative of 5-hydroxytryptamine showed up to 10 per cent decomposition under these conditions. They are also extremely sensitive to electron capture detection and have been characterized by gas chromatography.[8]

Previously, ethyl acetate, dried by redistillation from phosphorus pentoxide, has been used as the reaction medium for trifluoroacetylation.[8,16] However, it was found that the reaction of these amines under these conditions took 30–45 min to go to completion at room temperature, but if methyl cyanide was used as the solvent, the time required was reduced to less than 5 min. Dried, purified methyl cyanide is now commercially available, and because of the faster reaction time this solvent was chosen.

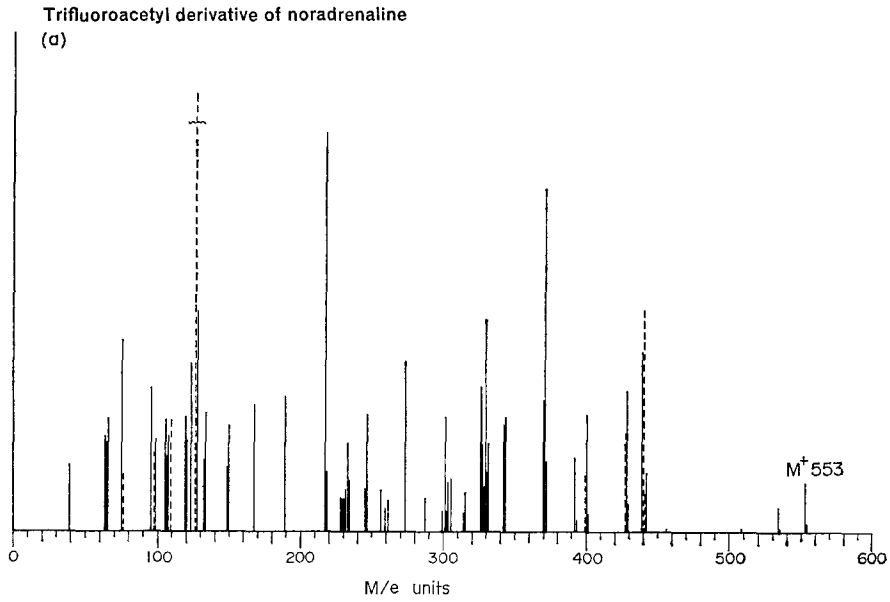

FIG. 2(a).

It is possible to evaporate the catecholamine reaction mixture to dryness in order to remove the excess trifluoroacetic anhydride but, if evaporation of the 5-hydroxytryptamine sample is attempted, decomposition of the derivative takes place. Because of the small amount of this reagent required to ensure complete reaction in this procedure, the evaporation was not found necessary in either case.

Structure of the derivatives. The derivatives formed under the conditions described were subject to gas liquid chromatography–mass spectrometry using an LKB 9000. The stationary phase used was 2% OV-1 on Gas Chrom Q, with helium as the carrier gas at a flow rate of 30 ml/min; the temperature of the column was 120° for the catecholamine derivatives and 170° for the 5-hydroxytryptamine derivative. Background traces were subtracted from the sample spectra and the resulting mass spectra of the derivatives are shown in Fig. 2 (a,b,c). The spectra are consistent with the structures shown in Fig. 3 (a,b,c).

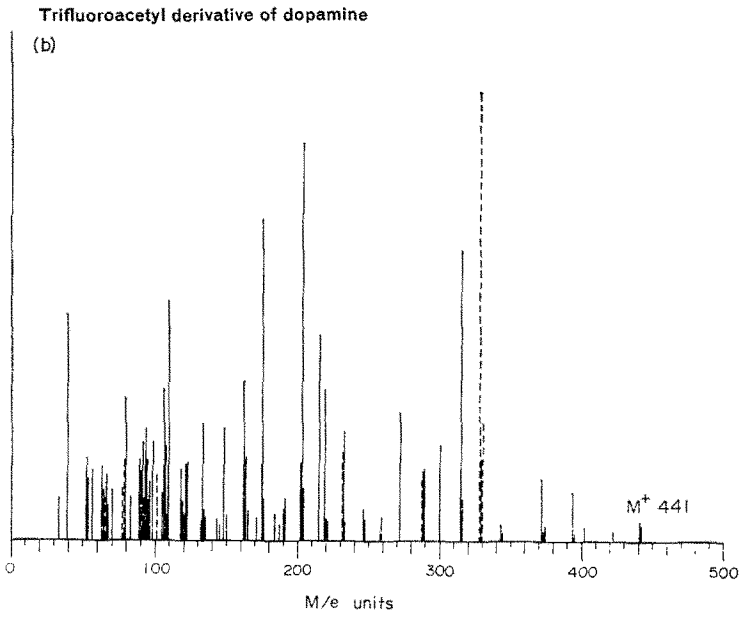

FIG. 2(b).

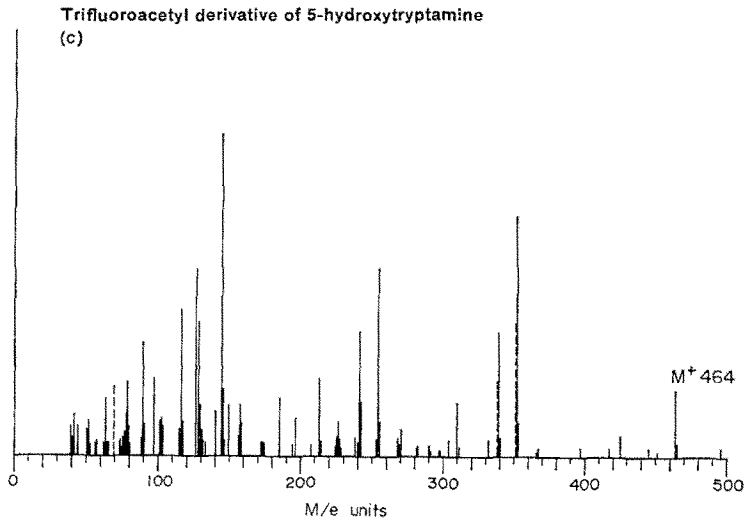

FIG. 2(c).

FIG. 2 (a,b,c). Mass spectra of the amine derivatives. The mass spectra were obtained from an LKB 9000 instrument; using a molecular separator temperature 240°, an ion source temperature 270° and an ionizing potential of 70 eV. The broken lines of the spectra are one-tenth of actual size, the vertical axis represents arbitrary response units and the horizontal axis M/e units.

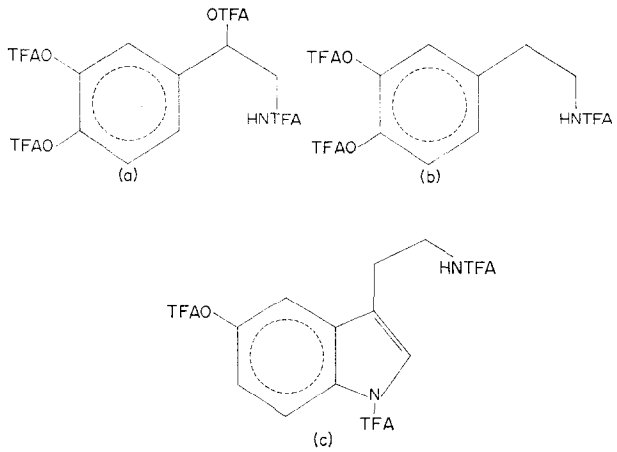

FIG. 3 (a,b,c). Structures of the amine derivatives. The structure of the derivatives formed from noradrenaline *a*, dopamine *b* and 5-hydroxytryptamine *c*.

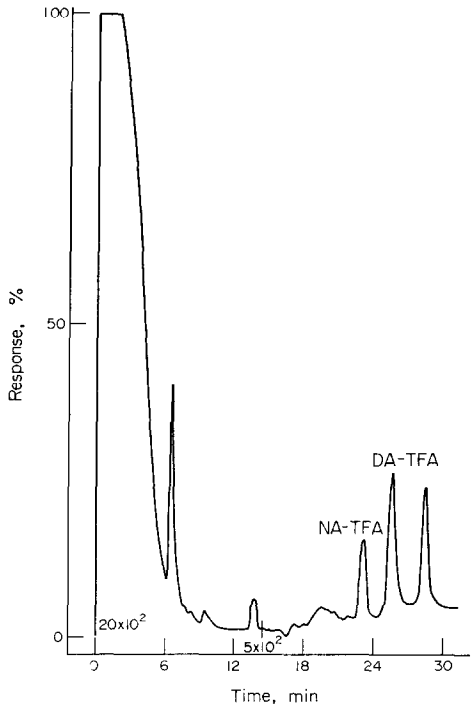

FIG. 4. Chromatogram of catecholamine derivatives from brain. A typical chromatogram obtained for the catecholamines from about 450 mg of brain tissue. The vertical axis represents the pen response and the horizontal axis the time in minutes. The amplifier attenuation settings are shown in small type; the noise level was 1% of F.S.D. at 2×10^2.

Gas chromatography. The gas chromatography was carried out on a 5% SE-52 column packed on 100–120 mesh Gas Chrom Q. The linearity of the detector response to the derivatives of the catecholamines, under the conditions used here, was up to about 125 pg on column, while that of the 5-hydroxytryptamine derivative was linear up to approximately 500 pg on column. However, calibration curves can be drawn at higher ranges as the plateau is attained relatively slowly.

The method can be used to estimate adrenaline, as well as the other two catecholamines, if a column of 5% F-60 (DC-560) on 100–120 mesh Gas Chrom Q is

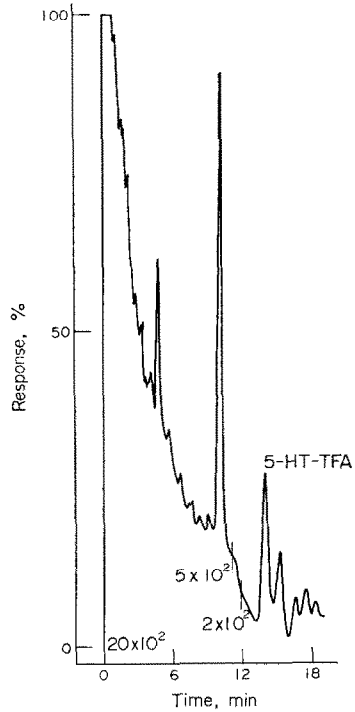

FIG. 5. Chromatogram of 5-hydroxytryptamine derivatives from brain. A typical chromatogram obtained for 5-hydroxytryptamine from about 450 mg of brain tissue. The vertical axis represents the pen response and the horizontal axis the time in minutes. The amplifier attenuation settings are shown in small type; the noise level was 1% of F.S.D. at 2×10^2.

used, a modification which is necessary if tissues containing large quantities of adrenaline are to be analysed. However, this column bleeds at the temperatures required to chromatograph these derivatives and thus regular cleaning of the detector is necessary. In extracts of brain tissue prepared as described above we have found no adrenaline[30,31] and have therefore preferred to use a polysiloxane gum as the stationary phase. This allowed the complete separation of the noradrenaline and dopamine derivatives, but only partial separation of the derivatives of noradrenaline and adrenaline. This column has excellent characteristics for the electron capture detector in that it produces no significant column bleed. Similar characteristics are provided by an OV-3 stationary phase, 5 per cent being the optimum loading in both cases. Peak areas were measured using a disc integrator.

Sensitivity. The electron capture detector system can detect about 3 pg of the catecholamines and 6 pg of 5-HT, which makes the limit of sensitivity of the present method about 5 ng for the catecholamines and 10 ng for 5-HT. However, the factor limiting the sensitivity of the method is the volume of sample that can be put onto the gas chromatograph, in order that solvent "tail effects" do not interfere with the peaks. At present less than 0·2 per cent of the total sample volume of 0·55 ml (0·53 ml in the case of 5-HT) is chromatographed, and if this fraction can be increased the overall sensitivity of the method will be improved as a result. By injecting the sample onto a short column, and allowing the volatile solvent to escape from the system through a valve, before allowing the chromatography of the derivatives to continue, it is hoped that a larger volume can be injected onto the gas chromatograph.

Concentration of the three amines in rat brain. The concentration of the amines found in whole brain tissue using this method are: noradrenaline, 0·33 $\pm$ 0·06 μg/g; dopamine 0·51 $\pm$ 0·05 μg/g and 5-hydroxytryptamine 0·54 $\pm$ 0·07 μg/g (mean $\pm$ S.D. n = 5 in all determinations). The figures are in agreement with determinations carried out previously in this laboratory with the same strain of animals.[22] There appears to be no interference from other tissue components and the standard deviation on eight duplicate determinations was 4·4 per cent. Figures 4 and 5 show the gas chromatographic records obtained from the analysis of about 450 mg of brain tissue, and it is possible to determine the concentrations of the amines in less than 200 mg of tissue.

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