

## Thin-Layer Chromatography of 1-Dimethylaminonaphthalene-5-sulphonyl Derivatives of Amino Acids Present in Superfusates of Cat Cerebral Cortex

By K. CROWSHAW, SHEILA J. JESSUP AND P. W. RAMWELL  
Worcester Foundation for Experimental Biology, Shrewsbury, Mass., U.S.A.

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1. Five new solvent systems are reported for the separation of 1-dimethylaminonaphthalene-5-sulphonylamino acids by thin-layer chromatography on silica gel. After two-dimensional chromatography with a suitable pair of these solvent systems, most of the 1-dimethylaminonaphthalene-5-sulphonyl derivatives were completely separated and could be located by their intense yellow fluorescence when viewed under u.v. light. 2. These techniques have been used to identify 21 amino acids present in superfusates of cat cerebral cortex, plasma and cerebrospinal fluid. 3. A method for the semiquantitative estimation of amino acids in biological fluids is described in which the fluorescent intensity of their separated 1-dimethylaminonaphthalene-5-sulphonyl derivatives was measured.

Studies of the release of amino acids from the cerebral cortex that are putative transmitters have been hampered by the lack of a method that provided good separation and sensitivity. The techniques currently used are based on ninhydrin, which require 1–10  $\mu$ moles of each separated amino acid, only a few of which have been unequivocally identified (Van Harreveld & Kooiman, 1965). Recently Jasper, Khan & Elliott (1965) have measured in cats the release of four amino acids, including  $\gamma$ -aminobutyric acid, after first pricking the pia-arachnoid membrane to facilitate release into cortical washings.

The present paper describes a rapid, simple and semiquantitative procedure for the separation and identification of amino acids released into superfusates of the somatosensory cortex of the anaesthetized cat. The amino acids were first treated with DNS\* chloride to form highly fluorescent derivatives (Gray & Hartley, 1963) that were readily separated by two-dimensional thin-layer chromatography.

### EXPERIMENTAL

*Reagents and chromatographic standards.* Amino acids (A grade) and DNS chloride were obtained from Calbiochem, Los Angeles, Calif., U.S.A. Each amino acid (10  $\mu$ moles) was treated with DNS chloride by the method of Boulton & Bush (1964), and the final solution was accurately made up to 10 ml. For routine chromatography, 1  $\mu$ l. (1.0  $\mu$ mole) of each DNS-amino acid solution was used.

\* Abbreviation: DNS, 1-dimethylaminonaphthalene-5-sulphonyl.

*Apparatus.* Thin-layer plates were prepared in the usual manner by using a mechanical spreader (Desaga) that produced a 0.25 mm. layer of Silica Gel G (E. Merck A.-G., Darmstadt, Germany). The fluorescence of pure DNS derivatives obtained after thin-layer chromatography was measured in an Aminco-Bowman spectrophotofluorimeter (American Instrument Co.), with fused quartz cells; the optimum activating wavelength and maximum fluorescent wavelength for most DNS-amino acids were 350 and 500  $\mu$  respectively. For subsequent routine estimations, a Turner model III fluorimeter was used equipped with Corning 7-60 and Wratten 65A primary and secondary filters respectively. Fluorescence was measured in arbitrary units.

*Cat preparation.* The somatosensory cortex of six cats was superfused at 0.1 ml./min. with a Ringer solution as described by Ramwell & Shaw (1964, 1966); samples were collected every 10 min. and frozen. Anaesthesia was induced and maintained with sodium pentobarbitone. The electrocorticogram was monitored by silver-ball electrodes applied to the cortex. The somatosensory cortex was located by identification of cinguli and gyri and by the potential evoked by bipolar stimulation of the contralateral superficial radial nerve (8–12 v, 0.01 msec. pulse width, 1 impulse/sec.).

*Preparation of samples.* Cerebrospinal fluid was removed by cisternal puncture, care being taken to avoid contamination with blood. Plasma was obtained from peripheral blood samples, and superfusates were aspirated directly from the cortex. After extraction of each sample with 75% (v/v) ethanol and evaporation of the supernatant to dryness at 40° under reduced pressure, the residue was dissolved in water and applied to a column (1 cm.  $\times$  10 cm.) of Dowex 50 (H<sup>+</sup> form), from which the amino acids were eluted with aq. NH<sub>3</sub> (2N). The dried eluate was dissolved in 0.1 ml. of NaHCO<sub>3</sub> (0.1M) to which was added an equal volume of acetone containing 0.6 mg. of the DNS chloride reagent. After 3 hr., 1–10  $\mu$ l. of the reaction mixture was applied to a

freshly activated silica gel G plate, which was developed first in an acidic solvent system (15 cm.), reactivated for 10 min. at 110° and then developed with a basic solvent system (15 cm.) in a direction at right angles to the first.

**Solvent systems for thin-layer chromatography.** The separation of DNS derivatives of amino acids was studied with the following solvents in lined and equilibrated tanks: I, di-isopropyl ether-methanol-acetic acid (100:5:1, by vol.); II, diethyl ether-methanol-acetic acid (100:5:1, by vol.); III, ethyl acetate-chloroform-methanol-acetic acid (10:10:1:1, by vol.); IV, ethyl acetate-methanol-acetic acid (20:1:1, by vol.); V, chloroform-methanol-acetic acid (15:4:1, by vol.) (Seiler & Wiechmann, 1964); VI, methyl acetate-propan-2-ol-aq. ammonia (sp.gr. 0.88) (9:7:4, by vol.) (Seiler & Wiechmann, 1964); VII, methyl acetate-aq. ammonia (sp.gr. 0.88) (19:1, v/v).

**Detection and identification of derivatives.** The positions of the highly fluorescent DNS derivatives after thin-layer chromatography were located by viewing the plates under u.v. light (360 m $\mu$ ). To facilitate identification, 'maps' showing the positions of DNS-amino acids after two-dimensional thin-layer chromatography were prepared for every pair of solvent systems used (e.g. see Figs. 1 and 2). Whenever the identification of a DNS derivative in a sample was ambiguous, a sample of the authentic amino acid derivative was co-chromatographed with the sample. The presence of a single compact spot after thin-layer chromatography in two or more pairs of solvent systems served to confirm the identification. The most commonly used solvent systems were the pairs II plus VII and IV plus VII.

**Estimation.** The intensity of each separated fluorescent spot was compared with a range of four to six DNS standards separated at the same time as the unknown samples, and a visual estimate of the concentration of each spot was obtained. Alternatively both the standards and the unknown were eluted with 2 x 1.5 ml. of chloroform-methanol-acetic acid (7:2:2, by vol.) and the fluorescence of each solution was determined in a Turner fluorimeter. From these fluorescence values the concentration of each unknown DNS derivative was calculated.

## RESULTS

**DNS derivatives of amino acid standards.** When viewed under u.v. light, the strongly acidic, polar 1-dimethylaminonaphthalene-5-sulphonic acid was blue and remained on or near the origin, whereas the DNS derivatives of amino acids and phenols were yellow and generally less polar. Some amino acids reacted to form two or more fluorescent derivatives. Thus lysine, hydroxylysine and ornithine derivatives yielded two yellow spots after chromatography and glutamic acid yielded a yellow and a weak orange spot. Tyrosine and 3,4-dihydroxyphenylalanine reacted to form two and three derivatives respectively, and were the only other amino acids giving complex spots after chromatography. The three weakly fluorescent spots of 3,4-dihydroxyphenylalanine were difficult to distinguish from neighbouring strongly fluorescent derivatives, and in consequence have not been included in Figs. 1 and

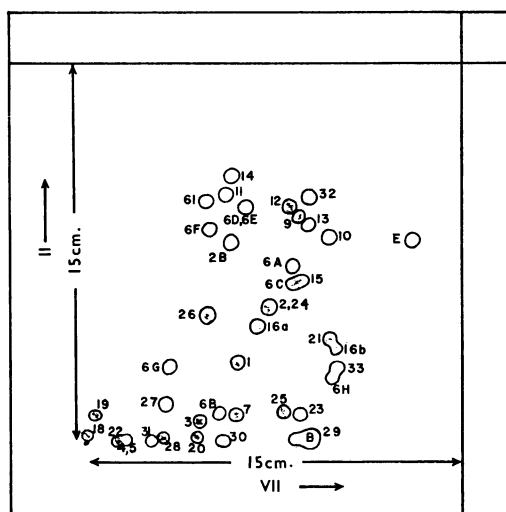


Fig. 1. Two-dimensional 'map' of DNS-amino acids separated in solvent systems II and VII. The position of each derivative (identified by a number) was plotted from the data in Table 1, and confirmed by actual separation of a number of complex mixtures of DNS standards.

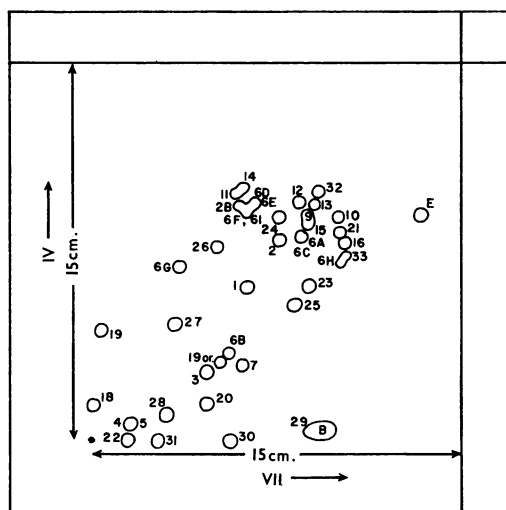


Fig. 2. Two-dimensional 'map' of DNS-amino acids separated in solvent systems IV and VII.

2. Histidine and methylhistidine both gave orange DNS derivatives.

The lowest quantity of DNS derivative that was detected on the wet plates after two-dimensional thin-layer chromatography was 0.05  $\mu$ mole, and at this concentration the fluorescent spots were never visible for more than 30 sec. Two-dimensional

Table 1. Mobilities of some DNS-amino acids after one-dimensional thin-layer chromatography in various solvents

For composition of solvents, see the text. The values refer to the distance travelled (cm.) by each derivative (1  $\mu$ mole applied) after 15 cm. development. Figures in parentheses refer to minor spots.

Code no.	DNS derivative	Solvent system ...						
		I	II	III	IV	V	VI	VII
1	Glycine	1.6	3.2	4.1	6.1	8.4	8.7	6.1
2	Alanine	2.7	5.5	5.7	8.0	10.3	9.5	7.4
3	Serine	0.4	0.8	1.4	2.7	5.5	7.6	4.6
4	Cysteine	0	0	0.4	0.6	5.0	4.2	1.5
5	Cystine	0	0	0.4	0.6	5.0	4.2	1.5
6A	$\alpha$ -Aminobutyric acid	4.4	7.0	6.3	8.2	11.2	10.1	8.3
6B	$\alpha$ -Amino- $\beta$ -hydroxybutyric acid	0.8	1.1	1.8	3.4	6.5	8.2	5.4
6C	$\alpha$ -Aminoisobutyric acid	4.3	6.4	6.5	8.4	11.3	10.1	8.4
6D	$\beta$ -Aminobutyric acid	6.1	9.4	7.1	9.4	12.0	9.0	6.5
6E	$\beta$ -Aminoisobutyric acid	6.1	9.4	7.1	9.5	12.0	9.0	6.5
6F	$\gamma$ -Aminobutyric acid	5.5	8.5	6.6	9.4	11.7	8.5	5.0
6G	$\gamma$ -Amino- $\beta$ -hydroxybutyric acid	2.4	3.0	3.8	6.9	9.2	7.8	3.4
6H	2,4-Diaminobutyric acid	1.8	2.6	4.4	7.3	10.6	11.2	10.0
6I	N-Methyl- $\gamma$ -aminobutyric acid	6.4	9.7	7.2	9.4	12.5	8.8	4.9
7	Threonine	0.5	1.1	1.8	3.0	6.5	8.8	6.0
8	Methionine	2.7	5.6	5.9	8.0	10.9	10.7	7.8
9	Valine	4.5	9.0	7.6	9.0	11.4	10.5	8.6
10	Isovaline	4.4	8.0	8.0	8.9	11.6	11.3	9.8
11	$\alpha$ -Aminovaleric acid	5.8	9.9	8.1	9.9	12.2	8.5	5.7
12	Leucine	4.6	9.4	7.8	9.5	11.4	9.7	8.2
13	Norleucine	4.6	8.7	7.8	9.4	11.4	10.5	8.8
14	$\epsilon$ -Aminohexanoic acid	6.4	10.6	8.5	10.0	12.6	8.5	5.9
15	Phenylalanine	3.1	6.4	7.1	8.7	11.1	10.3	8.6
16	Tyrosine	2.0 (1.2)	4.5 (3.8)	6.6 (4.5)	7.9	11.6 (8.9)	10.9 (8.6)	10.0 (6.9)
17	3,4-Dihydroxyphenylalanine	2.0 (1.4)	3.8 (2.6)	7.2 (5.5, 4.5)	8.2 (7.2)	12.5 (11.3, 10.5)	12.0 (8.4)	11.7 (7.7)
18	Aspartic acid	0.1	0.2	0.9	1.3	3.9	0.8	0
19	Glutamic acid	0.5	1.0	2.8 (2.4)	4.4 (3.2)	8.0 (6.5)	8.0 (1.5)	0.3 (5.1)
20	Glutamine	0	0.2	0.7	1.4	4.5	8.0	4.2
21	Lysine	1.8 (0)	4.0 (0)	6.0 (0)	8.3 (0)	11.5 (1.9, 1.0)	10.9 (5.8)	9.9 (1.4)
22	Arginine	0	0	0	0	1.3	5.6	1.3
23	$\alpha$ -Hydroxylysine	6.1 (0.8)	8.5 (1.1)	7.2 (2.6)	9.6 (6.2)	9.2	9.8	8.6
24	Tryptophan	3.2	5.3	5.9	8.9	10.0	9.3	8.6
25	Histidine	0.8	1.2	3.6	5.4	11.0	10.2	8.0
26	Proline	3.0	5.1	6.9	7.7	11.4	8.2	4.9
27	Hydroxyproline	0.9	1.5	2.3	4.6	7.7	3.2	3.2
28	Asparagine	0	0.1	0.5	1.0	3.4	6.3	3.0
29	Taurine	0	0	0.1	0.4	2.5	10.2	9.0
30	1-Methylhistidine	0	0	0	0	0.9	8.9	5.5
31	3-Methylhistidine	0	0	0	0	1.4	7.0	2.6
32	Isoleucine	4.9	8.9	8.0	9.1	11.9	10.8	9.0
33	Ornithine	1.5 (0)	2.9 (0)	5.0 (0)	7.0 (0)	11.4	11.5 (6.9)	10.1 (1.4)
2 $\beta$	$\beta$ -Alanine	4.6	8.0	7.3	9.4	11.7	8.6	5.8
E	Ethanolamine	5.6	8.0	5.9	9.0	11.7	12.2	13.1
B	1-Dimethylaminonaphthalene-5-sulphonic acid	0	0	0.1	0.5	2.3	10.0	8.6 (7.5)

chromatography of DNS mixtures containing 0.25  $\mu$ mole of each amino acid was more reliable, and the separated fluorescent spots were always well defined and easy to detect. The plates were stored overnight in the dark with little quenching of the fluorescence.

*Evaluation of the solvent systems.* The mobilities of DNS-amino acids in the different solvent systems studied are shown in Table 1. The more polar derivatives were well resolved by two-dimensional thin-layer chromatography in solvent systems V and VI (Seiler & Wiechmann, 1964). However, for the separation of less polar DNS-amino acids, propan-2-ol was unnecessary in the ammoniacal system VI, and so a new system VII was devised and used subsequently in conjunction with a number of new acidic systems I, II, III and IV. Separation of the neutral DNS-amino acids was achieved with these new systems (Fig. 1 and 2). Of the 43 fluorescent compounds studied (all those in Table 1 with the exception of 3,4-dihydroxyphenylalanine, which has not been included in the two-dimensional 'maps' because of its complex reaction with the reagent), only four pairs of inseparable derivatives were encountered: cysteine and cystine, the isomeric pairs,  $\alpha$ -amino-butyric acid and -isobutyric acid,  $\beta$ -amino-butyric acid and -isobutyric acid, and finally taurine and the blue-fluorescent 1-dimethylaminonaphthalene-5-sulphonic acid. The unambiguous separation and identification of the other derivatives was possible with a suitable pair of solvent systems, e.g. alanine and tryptophan, which were inseparable in systems II and VII (Fig. 1) and III and VII, were separated in systems IV and VII (Fig. 2). Similarly, the best separation of valine, isovaline, leucine, isoleucine, and norleucine was obtained with systems II and VII (Fig. 1), poorer but effective separations being obtained in the other systems.

*Estimation of DNS derivatives.* Equimolar quanti-

ties of various DNS-amino acid standards after thin-layer chromatography, elution and estimation fluoresced at different intensities (Table 2), possibly because DNS chloride does not react with amino acids to give 100% yields of fluorescent derivatives, the actual yields varying for each amino acid (Needle & Pollitt, 1965). However, for each DNS-amino acid there was a linear relationship between fluorescence and concentration.

A major source of error was the losses incurred in handling and treating the small quantities of amino acids in the samples. When pure amino acids (100  $\mu$ moles) such as alanine, serine, glutamine, glutamic acid and  $\gamma$ -aminobutyric acid were extracted from Ringer solution, purified on Dowex 50, treated with DNS chloride and estimated as above, there were losses of 50–68%. For this reason, only the approximate uncorrected concentrations of amino acids detected in the various samples have been recorded.

*Amino acids present in cortical superfusates.* The amino acids identified in eight samples of superfusates are shown in Table 3; for purposes of comparison, the amino acids present in both plasma and cerebrospinal fluid (five samples respectively) are also listed. The concentration of each amino acid was estimated by visual comparison of the fluorescence of each spot with that of DNS standards. However, a number of amino acids present in superfusates were estimated with a Turner fluorimeter (these values are shown in parentheses in Table 3): the two methods gave comparable results.

The rate of release of amino acids into superfusates was 2.3–2.9  $\mu$ moles/min., but because of the losses involved the actual rate is at least twice this value.

During these studies, it became clear that some of the common amino acids were released into superfusate samples at a sufficiently high concentration to be detected and estimated with an amino acid analyser. Accordingly a number of samples were pooled and 6.1 ml. of superfusion fluid was treated with Dowex 50, and the evaporated effluent was taken up in 0.5 ml. of citrate buffer, pH 2.2. Two samples (0.2 ml. each) were applied to a 0.9 cm.  $\times$  56 cm. column of Beckman-Spincro AA-15 spherical resin. The concentrations of nine of the more abundant amino acids are shown in Table 4: the results were comparable with the estimated concentrations shown in Table 3, thus providing independent evidence for the reliability of the semi-quantitative methods described in this paper. It now remains to be seen whether an accurate method of estimation of complex mixtures of amino acids can be developed that will eliminate errors due to formation of (i) multiple products including 1-dimethylaminonaphthalene-5-sulphonamide and

Table 2. Fluorescence of DNS-amino acids after two-dimensional thin-layer chromatography and elution from the plates

The  $\rho$  values refer to those amino acids compared with alanine.

DNS-amino acid (1 $\mu$ mole)	Mean $\pm$ s.e.m.	<i>n</i>	$\rho$
Glycine	9.5 $\pm$ 0.47	7	
Alanine	8.56 $\pm$ 0.60	8	
Glutamine	12.0 $\pm$ 0.68	6	< 0.01
Serine	9.28 $\pm$ 0.75	7	
Glutamic acid	6.08 $\pm$ 0.76	6	
Proline	8.9 $\pm$ 1.1	6	0.02–0.05
$\gamma$ -Aminobutyric acid	11.9 $\pm$ 0.88	8	< 0.01

Table 3. *Amino acids detected in cat cortical superfusates, plasma and cerebrospinal fluid*

The estimated values have not been corrected for losses involved in the purification and reaction procedures. Figures in parentheses were obtained with the Turner fluorimeter; the rest were estimated visually.

Amino acid	Concn. ( $\mu\text{moles/ml.}$ )			
	Superfusates	Plasma	Cerebrospinal fluid	Plasma (Tallan <i>et al.</i> 1954)
Glycine	3.0-5.0 (5.5)	150-200	150-200	310
Alanine	3.0-5.0 (4.2)	150-200	150-200	790
Serine	3.0-5.0 (3.3)	100	100	200
Glutamine	2.0	100	150-200	> 340
Proline	1.5 (2.2)	150-200	100	200
Asparagine	1.0	10-20	10-20	60
Glutamic acid	1.0	60	60	120
Lysine	1.0	60	60	190
Phenylalanine	1.0	60	10-20	55
Valine	1.0	100	100	200
Leucine	1.0 (3.8)	100	100	120
Isoleucine	0.7	60	60	60
Arginine	0.7	60	60	80
Aspartic acid	0.7	10-20	10-20	7.5
Hydroxyproline	0.7	60	60	Not detected
Threonine	0.4	60	10-20	120
Tyrosine	0.4	60	10-20	39
1-Methylhistidine	0.4	10-20	60	6.5*
Ornithine	0.2	10-20	10-20	15
$\beta$ -Alanine	0.2 (0.3)	2-4	Not detected	< 3.4
$\gamma$ -Aminobutyric acid	0.2 (0.3)	2-4	2-4	< 2.0
Histidine	Not detected	10-20	10-20	90
Tryptophan	Not detected	Not detected	Not detected	< 10
$\beta$ -Aminoisobutyric acid	Not detected	2-4	Not detected	< 3.9
$\alpha$ -Aminobutyric acid	Not detected	2-4	2-4	30*
Hydroxylysine	Not detected	2-4	2-4	Not detected

\* Detected in hydrolysed extracts.

Table 4. *Concentration of some amino acids detected in superfusates with a Spinco amino acid analyser*

These analyses were kindly performed by Dr J. Hinman and Dr A. J. Parcels of the Upjohn Co., Kalamazoo, Mich., U.S.A.

Amino acid	Concn. ( $\mu\text{moles/ml.}$ )
Glycine	6.9
Alanine	5.3
Serine	10.0
Valine	3.0
Leucine	2.0
Isoleucine	1.1
Threonine	1.6
Aspartic acid	0.9
Glutamic acid	1.7

detected directly at less than 0.1  $\mu\text{mole}$  by viewing under u.v. light. Further, by choice of suitable solvent systems, it was possible to effectively separate most of the commonly occurring amino acids. Although the solvent systems of Seiler & Wiechmann (1964) (V and VI) provided good separation of the polar DNS-amino acids, the resolution of the neutral derivatives was poor (Mesrob & Holeysovsky, 1966); but by using the new systems now reported, e.g. II and VII, excellent separation of a wide range of neutral amino acid derivatives was achieved. These thin-layer chromatographic systems are at least as effective in resolving power as the elution procedures used in conjunction with amino acid analysers.

The method possesses the additional advantage of employing volatile solvents so that the plates can be readily reactivated and developed in a short time. Reactivation of the plate did not affect the subsequent development of the DNS derivatives in the second direction. No quenching of the fluorescence was observed; indeed, the derivatives on wet plates fluoresced more strongly than those on dried plates.

(ii) derivatives with different intensities of fluorescence.

#### DISCUSSION

The attraction of the DNS method is that individual DNS-amino acid derivatives may be

This result is in contrast with the experience of Cole, Fletcher & Robson (1965) and of Deyl & Rasmus (1965), who used slow-running non-volatile solvent systems, which had to be removed thoroughly to prevent quenching. In addition, Morse & Horecker (1966) found that removal of non-volatile solvents by heating led to partial destruction of derivatives and loss of sensitivity.

In spite of the current interest in the DNS techniques, no quantitative studies have been published except for the statement that on paper strips there is a linear relationship between fluorescence and concentration of DNS-amino acid standards (Boulton & Bush, 1964). We have shown that a similar relationship exists with thin-layer chromatography, but individual standards after chromatography fluoresced at different intensities. These variations were studied in detail (Table 2), and it is clear that the differences are significant ( $P < 0.05$ ).

It will be recalled that glutamic acid yielded two DNS derivatives; since the stronger spot only was eluted, a low fluorescence reading was not unexpected. However, such an explanation does not suffice for the difference between glutamine and alanine. From qualitative considerations of the reaction of DNS chloride with one amino group, the fluorescence yield for equimolar DNS derivatives should be the same. The most probable explanation for these differences is that put forward by Neadle & Pollitt (1965) to explain the origin of 1-dimethylaminonaphthalene-5-sulphonamide formed as a by-product from the reaction of amino acids and the DNS reagent. The amount of 1-dimethylaminonaphthalene-5-sulphonamide produced varied for each amino acid and hence the larger the concentration of this by-product, the smaller the concentration (and fluorescence) of DNS-amino acid.

The concentrations of DNS-amino acids in samples were estimated visually; as shown in Table 3, these semiquantitative values provide a reasonable indication of the relative concentrations of each amino acid present in a given sample. Further, the values for the amino acids in plasma all show a gross

similarity to the reported values (Tallan, Moore & Stein, 1954).

Twenty-one amino acids were detected in superfusates of the cerebral cortex of anaesthetized cats. The relatively constant plasma superfusate ratio of 50-100:1 suggests that the plasma is the main source of the amino acids. Van Harreveld & Kooiman (1965) support this interpretation since they found that acute changes in blood pressure significantly modified the release of ninhydrin-positive material from the pia-arachnoid membrane. Further, there is good evidence that amino acids applied to the surface of the membrane penetrate sufficiently slowly into the underlying cortex as to indicate the presence of a definite barrier. By using  $^{14}\text{C}$ -labelled amino acids (10 mM), Nogueira, Argiz & Levin (1965) showed that the penetration of these acids from cups applied to the cerebral cortex of anaesthetized cats followed a first-order curve and that the half-life varied from 300 to 570 min. Thus at 10 and 20 min. there was little or no significant passage of amino acids from the cortical surface. Whether such a barrier exists in the reverse direction remains to be determined, but it is significant that Jasper *et al.* (1965) could only measure  $\gamma$ -aminobutyric acid release after first disrupting the pia-arachnoid membrane. Although  $\gamma$ -aminobutyric acid was detectable in superfusates without recourse to pricking the pia-arachnoid membrane, it was also evident in both cerebrospinal fluid and plasma, probably owing to the sensitivity of the DNS method; however, as expected the values reported above for  $\gamma$ -aminobutyric acid in cortical superfusates are lower than those observed by Jasper *et al.* (1965) (Table 5). The correlation between the two sets of data with respect to other amino acids is striking and suggests that the bulk of these acids, unlike  $\gamma$ -aminobutyric acid, are derived mainly from the plasma.

Although  $\alpha$ -aminobutyric acid,  $\beta$ -aminobutyric acid and histidine were present in plasma and cerebrospinal fluid, these acids were absent from the superfusates. Conversely, all the amino acids detected in superfusates have been reported present

Table 5. Comparison of rates of release of amino acids from the cerebral cortex

Cat preparation	Rate of release ( $\text{m}\mu\text{moles}/10 \text{ min.}/\text{cm.}^2$ of cortex)			
	$\gamma$ -Aminobutyric acid	Glutamine	Glutamic acid	Aspartic acid
Anaesthetized with sodium pentobarbitone*: left cortex superfused	0.5	5.0	2.5	1.8
Sleeping, after left midcollicular section†: chamber on left cortex, pia-arachnoid pricked	4.3	2.5	5.4	2.8

\* Figures derived from Table 3 and corrected for 60% loss.

† Jasper *et al.* (1965).

in plasma by Tallan, Moore & Stein (1954) except for hydroxyproline; their data, however, indicate the presence of an unknown acid with similar chromatographic properties to hydroxyproline.

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