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# CYSTEINE BIOSYNTHESIS IN BEET DISCS

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**Abstract**—The incorporation of [$^{35}$S] sulphate into cysteine by discs of red beetroot has been studied, and the amino acid isolated by adding N-ethyl maleimide to the ethanolic extracting solution and subsequent paper chromatography of the N-ethyl maleimide–cysteine adduct. The addition of L-serine (1–10mM) to beet discs that have been washed in aerated water to lower their endogenous pools of amino acids caused an increase (maximum observed 80–90 per cent) in the incorporation of radioactivity from [$^{35}$S] sulphate into cysteine. D-Serine and glycine do not have this effect; in fact D-serine inhibits the uptake of sulphate by the tissue. The discs convert L-[$^{14}$C] serine to [$^{14}$C] cysteine. No [$^{35}$S] cysteine sulphinic acid was detected after feeding with [$^{35}$S] sulphate.

## INTRODUCTION

ALTHOUGH information is available about the enzymes concerned in the reduction of sulphate and subsequent incorporation into cysteine by micro-organisms, very little is known about the intermediate reactions involved in the reduction of sulphate by green plants.[1] Excised tobacco leaves reduce [$^{35}$S] sulphate supplied through the petiole to [$^{35}$S] sulphite,[2] and excised mung bean leaves reduce [$^{35}$S] sulphate supplied by vacuum infiltration to [$^{35}$S] sulphite, [$^{35}$S] sulphide, [$^{35}$S] cysteine, and [$^{35}$S] methionine.[3-6] The origin of the carbon skeleton of cysteine was not determined in these studies. Nutritional and isotopic competition data obtained with *Escherichia coli*, *Neurospora crassa*, and *Torulopsis utilis* have shown that serine provides the carbon skeleton of cysteine in these organisms.[7] Recently an enzyme that condenses sulphide with L-serine to give cysteine has been reported in extracts from several micro-organisms and animals, and has been purified 20-fold from spinach leaves.[8] This evidence supports the view that plants reduce sulphate to the level of sulphide which is then condensed with serine to yield cysteine.

One of the difficulties previously experienced in studying the synthesis of thiol compounds has been the ease of autoxidation of the thiol group, which leads to secondary spots and tailing when chromatographic procedures are used for their isolation. The adducts of thiol compounds with N-ethyl maleimide are more stable than the thiols themselves, and can be separated by paper chromatography.[9,10] This work describes the use of N-ethyl maleimide in an examination of the synthesis of cysteine from sulphate in a plant tissue; some of the effects of the addition of possible intermediates on this synthesis are reported.

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[1] L. G. WILSON, *Ann. Rev. Plant Physiol.* **13**, 201 (1962).
[2] P. FROMAGEOT and H. PEREZ-MILAN, *Biochim. Biophys. Acta* **32**, 457 (1959).
[3] T. ASAHI and T. MINMIKAWA, *J. Biochem. (Tokyo)* **48**, 548 (1960).
[4] T. ASAHI and N. HARADA, *Bull. Agr. Chem. Soc. Japan* **21**, 243 (1957).
[5] T. ASAHI, *J. Biochem. (Tokyo)* **48**, 772 (1960).
[6] N. KAWASHIMA and T. ASAHI, *J. Biochem. (Tokyo)* **49**, 52 (1961).
[7] R. B. ROBERTS, P. H. ABELSON, D. B. COWIE, E. T. BOLTON, and R. J. BRITTEN, *Studies of Biosynthesis in* Escherichia coli. Washington D.C. Carnegie Institution of Washington Publication 607 (1957).
[8] J. BRUGGEMAN, K. SCHLOSSMANN, M. MERKENSCHLAGER and M. WALDSCHMIDT, *Biochem. Z.* **335**, 392 (1962).
[9] C. S. HANES, F. J. R. HIRD and F. A. ISHERWOOD, *Nature* **166**, 288 (1950).
[10] V. F. COTTY, S. M. HENRY and J. D. HILCHEY, *Contrib. Boyce Thompson Inst.* **19**, 379 (1958).

## RESULTS

### *Products of [$^{35}S$] sulphate metabolism*

Radioautographs of chromatograms of extracts made in the presence of N-ethyl maleimide from pea shoots and washed red beet discs fed with [$^{35}S$] sulphate revealed radioactive bands corresponding to N-ethyl maleimide-cysteine and N-ethyl maleimide-glutathione markers. The unchanged [$^{35}S$] sulphate and any cystine or oxidized glutathione present in the tissue extracts remained close to the origin of the chromatograms. If N-ethyl maleimide was omitted from the extracting solution, only a trace of radioactivity was found corresponding with the N-ethyl maleimide–cysteine marker in the case of the pea extracts, and none in the case of the beet extracts. Radioactivity was still present opposite the N-ethyl maleimide–glutathione marker in both cases, but the amount was less than that present when N-ethyl maleimide was added to the extracting medium. No radioactivity was observed in the areas of the chromatograms corresponding to the cysteine sulphinic acid, methionine, or N-ethyl maleimide–homocysteine markers.

Pea apices took up [$^{35}S$] sulphate at varying rates, and hence the time of exposure of the meristematic cells to the isotope varied from apex to apex. The beet disc system was therefore chosen as the most satisfactory for the present study. The labelled material from beet discs that corresponded with the N-ethyl maleimide–cysteine marker in the butan-1-ol–acetic acid–water solvent was identified as N-ethyl maleimide–[$^{35}S$] cysteine as described in the experimental section. Since only traces of other labelled compounds were found in this area of the chromatogram, it was considered satisfactory to use the radioactivity of the material as a measure of cysteine synthesis. The amount of soluble cysteine present in 25 beet discs was insufficient to estimate by the ninhydrin method, and so specific activity data could not be obtained.

### *Time course of sulphate uptake and reduction*

Figure 1 shows the time course of sulphate uptake and cysteine synthesis by beet discs as estimated by measuring the radioactivity of the soluble extract. The residue after

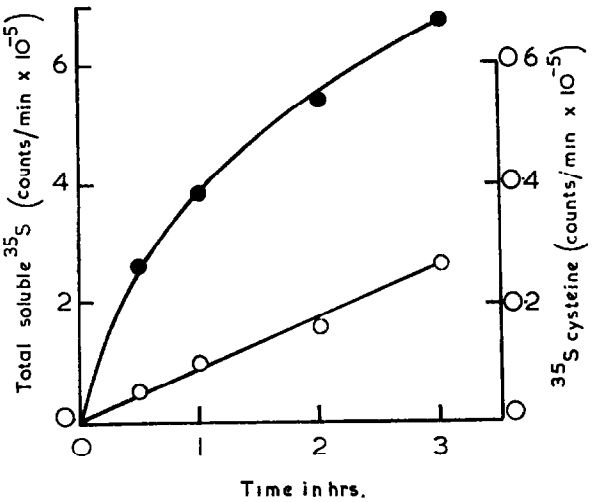


FIG. 1. TIME COURSE OF SULPHATE UPTAKE AND REDUCTION

Samples of 25 two day-washed red beet discs were shaken in 3 ml water containing $Na_2{}^{35}SO_4$ (mM. $85 \times 10^5$ counts/min) in Warburg flasks at 30°. The discs were washed and their content of total soluble $^{35}S$ (●) and [$^{35}S$] cysteine (O) determined as described in the text.

extraction contained less than 5 per cent of the radioactivity of the extract and, since it is difficult to obtain accurate estimates of the insoluble radioactivity, only that of the soluble fraction is reported. The time course of sulphate uptake shows the curved shape characteristic of salt uptake by plant cells, whereas the radioactivity in cysteine rises in a linear fashion. About 8 per cent of the sulphate supplied to the discs was taken up in 3 hr, and about 4 per cent of this fraction was present as soluble cysteine.

*Effect of washing and of added amino acids*

The effects of washing the discs, and of added L-serine on the radioactivity found in cysteine after feeding the discs with [$^{35}$S] sulphate are shown in Table 1. The amount of

TABLE 1. EFFECT OF WASHING, AND OF L-SERINE ON CYSTEINE SYNTHESIS

Red beet discs were washed in aerated distilled water at 25°. Samples of 25 discs were shaken in 3 ml of water containing $Na_2{}^{35}SO_4$ (3 $\mu$mole, $99 \times 10^5$ counts/min) with and without L-serine (15 $\mu$mole) for 3 hr at 30°, and the radioactivity of the soluble extract and of the cysteine determined as described in the text. The soluble amino nitrogen was estimated in ethanolic extracts of samples of 25 discs taken from the washing flask. The figures are averages derived from duplicate flasks.

| Days washed | Compound added | (counts/min $\times 10^{-5}$) A Total soluble $^{35}$S | B [$^{35}$S] cysteine | % B/A | Soluble $\alpha$-amino nitrogen ($\mu$g) |
|---|---|---|---|---|---|
| 0 | — | 1·80 | <0·004 | — | 139 |
| | L-serine | 1·92 | <0·004 | — | |
| 2 | — | 7·59 | 0·19 | 2·5 | 40 |
| | L-serine | 6·81 | 0·30 | 4·4 | |
| 4 | — | 12·48 | 0·14 | 1·12 | 34 |
| | L-serine | 11·46 | 0·18 | 1·57 | |
| 8 | — | 16·14 | 0·035 | 0·022 | 19 |
| | L-serine | 13·29 | 0·056 | 0·042 | |

soluble sulphate taken up by the discs in a 3 hr period (column A) was increased by longer periods of washing, as expected,[11] and this uptake was decreased slightly by L-serine. The maximum radioactivity in cysteine (column B) was found in the two day-washed discs. The addition of L-serine in each case increased the radioactivity present in the cysteine. The ratio B/A (counts in cysteine/total soluble counts) is used in Table 1 and in succeeding tables to minimise any effect that the added amino acid may have on the uptake of sulphate

TABLE 2. EFFECT OF L-SERINE ON CYSTEINE SYNTHESIS

Samples of 25 two day-washed beet discs were shaken in 3 ml water containing $Na_2{}^{35}SO_4$ (3 $\mu$mole, $87{\cdot}6 \times 10^5$ counts/min) with and without L-serine for 3 hr at 30°. The radioactivity of the soluble extract and of the cysteine was estimated as described in the text. The figures are averages derived from duplicate flasks.

| L-serine added (mM) | (counts/min $\times 10^{-5}$) A Total soluble $^{35}$S | B [$^{35}$S] cysteine | % B/A |
|---|---|---|---|
| 0 | 4·09 | 0·136 | 3·33 |
| 1 | 3·56 | 0·216 | 6·06 |
| 5 | 3·84 | 0·232 | 6·05 |
| 10 | 3·88 | 0·247 | 6·36 |

[11] F. C. STEWARD and J. F. SUTCLIFFE, *Plant Physiology* (Edited by F. C. STEWARD), Vol. 2, p. 253. New York: Academic Press Inc.

by the discs. This correction is only approximate since the counts in cysteine do not vary with the amount of sulphate taken up in a strictly linear fashion (Fig. 1).

Washing the discs reduces their content of soluble amino compounds (Table 1); this phenomenon is well known.[11]

The effect of additions of L-serine over a range of concentrations from 1–10mM is given in Table 2; increasing the concentration in this range does not appreciably affect the degree of stimulation of radioactivity in cysteine. The D-isomer of serine caused no increase in the radioactivity in cysteine, but produced a 40–50 per cent reduction in the sulphate uptake (Table 3); the nature of this effect is under investigation. Glycine did not affect the radio-

TABLE 3. EFFECT OF L-SERINE AND D-SERINE ON SULPHATE UPTAKE AND CYSTEINE SYNTHESIS

Samples of 25 beet discs washed for two or four days were shaken in 3 ml water containing $Na_2{}^{35}SO_4$ (3 $\mu$mole, $99{\cdot}2\times10^5$ counts/min) with and without either L-serine or D-serine (15 $\mu$mole) for 3 hr at 30°. The figures are averages derived from duplicate flasks.

| Days washed | Compound added | (counts/min $\times 10^{-5}$) A Total soluble $^{35}S$ | (counts/min $\times 10^{-5}$) B [$^{35}S$] cysteine | % B/A |
|---|---|---|---|---|
| 2 | — | 7·43 | 0·158 | 2·13 |
| | L-serine | 6·51 | 0·218 | 3·35 |
| | D-serine | 4·43 | 0·095 | 2·14 |
| 4 | — | 11·84 | 0·117 | 0·99 |
| | L-serine | 8·94 | 0·168 | 1·88 |
| | D-serine | 6·15 | 0·074 | 1·20 |

activity of cysteine, whereas L-alanine caused a reduction without a concomitant effect on sulphate uptake (Table 4). L-Methionine inhibited both the uptake of sulphate and its incorporation into cysteine (Table 4).

TABLE 4. EFFECT OF GLYCINE, L-ALANINE, AND L-METHIONINE ON CYSTEINE SYNTHESIS

Samples of 25 two day-washed beet discs were shaken in 3 ml water containing $Na_2{}^{35}SO_4$ (3 $\mu$mole, $96{\cdot}1\times10^5$ counts/min) with and without amino acid (15 $\mu$mole) for 3 hr at 30°. The figures are averages derived from duplicate flasks.

| Compound added | (counts/min $\times 10^{-5}$) A Total soluble $^{35}S$ | (counts/min $\times 10^{-5}$) B [$^{35}S$] cysteine | % B/A |
|---|---|---|---|
| — | 4·37 | 0·10 | 2·29 |
| L-serine | 4·23 | 0·145 | 3·43 |
| glycine | 4·09 | 0·099 | 2·42 |
| L-alanine | 4·18 | 0·065 | 1·55 |
| L-methionine | 2·77 | 0·039 | 1·41 |

*Uptake of amino acids by the discs*

To interpret these feeding experiments it is necessary to establish whether the added compound is taken up by the tissue. Table 5 shows the uptake of each amino acid in the presence of sodium sulphate. The two isomers of serine, glycine, and L-alanine are absorbed at high rates; L-methionine is absorbed more slowly, and L-cysteine sulphinic acid is hardly absorbed at all. Adjustment of the pH of the solution of the latter compound from 5·0 to 3·5 did not result in a greater uptake.

TABLE 5. UPTAKE OF AMINO ACIDS BY RED BEET DISCS

Samples of 25 three day-washed beet discs were shaken in 3 ml water containing $Na_2SO_4$ (3 $\mu$mole) and amino acid (15 $\mu$mole) for 3 hr at 30°. The L-cysteine sulphinic acid was adjusted to pH 5·0 with NaOH. Duplicate samples (20 $\mu$l) of the solution were removed at 0 and 3 hr and the uptake of amino acid calculated by measuring the disappearance of $\alpha$-amino nitrogen as described in the text. Discs shaken in $Na_2SO_4$ with no added amino acid did not release any detectable $\alpha$-amino compounds into the solution.

| Amino acid added | Amino acid taken up ($\mu$mole/3 hr) |
|---|---|
| L-serine | 6·8 |
| D-serine | 5·4 |
| glycine | 9·7 |
| L-alanine | 10·4 |
| L-methionine | 2·3 |
| L-cysteine sulphinic acid | 0·25 |

*Conversion of L-[$^{14}C$] serine to [$^{14}C$] cysteine*

Beet discs that had been washed for two days were found to convert added L-[$^{14}C$] serine to [$^{14}C$] cysteine (Table 6). Serine of high specific activity (17 mc/mmole) was used because the synthesis of cysteine was small and a high proportion (70–80 per cent) of the counts absorbed by the discs was not recovered in the soluble extract. N-Ethyl maleimide-[$^{14}C$] cysteine was identified by co-chromatography and electrophoresis as described in the experimental section. Chromatography in the butan-1-ol–acetic acid–water solvent of ethanolic extracts of discs fed with L-[$^{14}C$] serine revealed some radioactivity in the area

TABLE 6. CONVERSION OF L-[$^{14}C$] SERINE TO [$^{14}C$] CYSTEINE BY BEET DISCS

Samples of 30 two day-washed beet discs were shaken in 5 ml water containing $Na_2SO_4$ (5 $\mu$mole) and L-[$^{14}C$] serine (1·18 $\mu$mole, 20 $\mu$C, $44{\cdot}7 \times 10^5$ counts/min) for 6 hr at 30°. Portions (50 $\mu$l) of the solution were removed at 0 and 6 hr to estimate the uptake of isotope. The samples of discs were then washed with 250 ml of $Na_2SO_4$ (mM) on a Buchner funnel, and scraped into 20 ml of either hot ethanol (80%, v/v) or hot ethanol containing N-ethyl maleimide (50 mg). After two further extractions with 5 ml portions of ethanol (50%, v/v) the combined extracts were chromatogrammed in butan-1-ol–acetic acid–water (4 : 1 : 5, by vol) for 24 hr to obtain the N-ethyl maleimide-[$^{14}C$] cysteine.

| | (counts/min $\times 10^{-5}$) | | | |
|---|---|---|---|---|
| Extracting solution | [$^{14}C$] serine taken up | A Total soluble $^{14}C$ | B $^{14}C$ in position of N-ethyl maleimide-cysteine | % B/A |
| Ethanol | 42·59 | 9·83 | 0·133 | 1·35 |
| Ethanol containing N-ethyl maleimide | 43·95 | 11·66 | 1·182 | 10·14 |

opposite the N-ethyl maleimide–cysteine marker. This radioactivity represented only 10–15 per cent of that present in the same area when N-ethyl maleimide was added to the extracting solution (Table 6); this contaminating radioactive band split into several unidentified components when chromatographed in the ethyl acetate–acetic acid–water solvent.

## DISCUSSION

The results of these experiments can best be viewed in light of the fact that as the beet discs are washed the concentrations of the endogenous amino acids decrease (Table 1).

This decrease is advantageous since it allows the effect of added amino acids to be studied in the absence of high endogenous pools. It is a disadvantage from the point of view of estimating cysteine synthesis, since rather large amounts of radioactivity have to be used to detect the products of sulphate reduction, and the estimation of specific activity is precluded. The increased synthesis of [$^{35}$S] cysteine in the presence of added L-serine suggests that cysteine is formed from some reduced form of sulphur and serine. This view is supported by the fact that the discs convert L-[$^{14}$C] serine to [$^{14}$C] cysteine. The work of Bruggeman *et al.*[8] suggests that this reduced form of sulphur is sulphide.

The fact that glycine does not affect cysteine synthesis (Table 4), although it is taken up by the discs in amounts comparable to that of L-serine (Table 5), suggests that L-serine is not exerting its effect after conversion to glycine, and that glycine is not converted to L-serine to any appreciable extent. The possibility that L-serine increases the radioactivity in cysteine by decreasing the rates of reactions removing cysteine cannot however be ruled out.

The effect of D-serine on sulphate uptake (Table 3) was expected since it has been found to inhibit the uptake of several cations and anions by plant cells (unpublished experiments). No explanation, however, can be offered for the inhibitory effect of L-alanine on cysteine synthesis (Table 4). In the case of L-methionine however, the discs absorb about twenty times as much of this amino acid as sulphate (Tables 4 and 5), and it is possible that the inhibitory effect of methionine on both sulphate uptake and cysteine synthesis is due to its oxidation to sulphate. This so-formed sulphate could then partially saturate the uptake mechanism, thus decreasing the uptake of external sulphate, and lowering the specific activity of the labelled sulphate that is absorbed.

An alternative pathway of sulphate reduction was proposed by Singer and Kearney[12] who suggested that reversal of the desulphination of $\beta$-sulphinylpyruvate may be the step by which sulphur is attached to a carbon skeleton. $\beta$-Sulphinylpyruvate is the $\alpha$-oxo acid analogue of cysteine sulphinic acid which is readily transaminated by the glutamic–oxaloacetic transaminase of plant and animal tissues.[13] It has been claimed that [$^{35}$S] cysteine sulphinic acid was formed after infiltration of mung bean leaves with [$^{35}$S] sulphite, but the identity of the cysteine sulphinic acid was not rigorously confirmed.[3] The injection of [$^{35}$S] cysteine sulphinic acid into cockroaches, the intracellular symbionts of which reduce sulphate to cysteine, did not result in the formation of [$^{35}$S] cysteine, but labelled cysteine was formed after injection of [$^{14}$C] serine.[14] No labelled material having the $R_F$ of cysteine sulphinic acid was observed after feeding beet discs with [$^{35}$S] sulphate, and the discs did not take up a significant amount of added cysteine sulphinic acid. No support has therefore been found for the view that cysteine sulphinic acid is an intermediate in the reduction of sulphate by plant tissues.

## EXPERIMENTAL

### *Chemicals*

N-Ethyl maleimide was obtained from Light & Co., Ltd. Carrier-free sodium [$^{35}$S] sulphate and L-[U-$^{14}$C] serine (17mc/mmole) were obtained from the Radiochemical Centre, Amersham.

[12] T. P. SINGER and E. B. KEARNEY, *Biochim. Biophys. Acta* **11**, 276 (1953).
[13] R. J. ELLIS and D. D. DAVIES, *Biochem. J.* **78**, 615 (1961).
[14] S. M. HENRY and R. J. BLOCK, *Contrib. Boyce Thompson Inst.* **21**, 129 (1961).

*Preparation and feeding of plant tissues*

Alaska peas were sown in John Innes Compost and grown for fourteen days under fluorescent lights with a 16 hr photoperiod. The apices of the pea seedlings were severed just above a node and the cut ends placed at once into glass vials containing 20–50 $\mu$l of carrier-free [$^{35}$S] sulphate (6–10 $\mu$c) and kept under the fluorescent lights.

Red beetroot (Globe variety) were purchased from local markets and discs (5mm × 1mm) cut from the centre of the roots. Discs (400–600) were washed in twice glass-distilled water (400 ml) at 25° for two to eight days; the water was aerated and changed ten times on the first day and five times on subsequent days. Samples of 25 discs were blotted lightly and shaken in 3 ml of water containing sodium [$^{35}$S] sulphate (mM, 30–60 $\mu$c) at 30° for 3 hr in Warburg flasks. All treatments were run in duplicate.

*Measurement of cysteine synthesis*

Cysteine present in the plant tissue was extracted and stabilized in one operation by dropping the tissue into hot 80% ethanol (20 ml) containing excess N-ethyl maleimide (50 mg). The reaction between N-ethyl maleimide and cysteine is isomolar and proceeds to completion very rapidly at neutral or slightly acid pH values.[15,16] Alkaline conditions must be avoided since at pH 8 and above N-ethyl maleimide undergoes hydrolysis at an appreciable rate;[17] on standing at pH 9 the adduct of cysteine and N-ethyl maleimide cyclizes to the thiazane derivative.[18] The pH of ethanolic beetroot extracts was found to be in the range 6·3–6·5; the addition of N-ethyl maleimide (50 mg) lowered the pH by about 0·8 of a unit.

Beet discs were tipped out of the Warburg flasks on to a Buchner funnel, washed rapidly (about 1 min) with 250 ml of sodium sulphate (mM) to remove the adhering medium, and transferred to the extracting solution. Five pea apices (fresh weight about 600 mg) or 25 beet discs (fresh weight about 740 mg) were extracted in 20 ml of the ethanolic N-ethyl maleimide solution. The solution was reduced to 1–5 ml by boiling and decanted. The tissues were further extracted with two successive portions of hot 50% ethanol (5 ml) and the extracts combined.

*Identification of N-ethyl maleimide–cysteine*

Samples (2 ml) of the combined tissue extracts (5–10 ml) were transferred quantitatively to Whatman 3MM paper as 3 in. streaks. Extracts from duplicate treatments were placed on the same chromatogram. The paper was run in butan-1-ol-acetic acid–water (4 : 1 : 5, by vol top phase) for 24 hr by the descending method, dried, and placed against Kodirex X-ray film for 6–10 days. Markers were made by mixing solutions of glutathione or L-cysteine with a 20% molar excess of N-ethyl maleimide. The markers were revealed by spraying the chromatograms with 0·1% ninhydrin in butan-1-ol and heating at 100°. The radioactive band corresponding to the N-ethyl maleimide–cysteine marker was eluted with water and identified by co-chromatography with the authentic material in six other solvents. The average $R_F$ values of the N-ethyl maleimide–cysteine were:

[15] E. Roberts and G. Rouser, *Anal. Chem.* **30**, 1291 (1958).
[16] J. R. Whitaker, *Nature* **189**, 662 (1961).
[17] J. D. Gregory, *J. Am. Chem. Soc.* **77**, 3922 (1955).
[18] D. G. Smyth, A. Nagamatsu and J. S. Fruton, *J. Am. Chem. Soc.* **82**, 4600 (1960).

| *Solvent* (top phase in two phase systems) | $R_F$ |
|---|---|
| butan-1-ol–acetic acid–water (4 : 1 : 5, by vol) | 0·28 |
| propan-1-ol–ethyl acetate–water (7 : 1 : 2, by vol) | 0·32 |
| ethyl acetate–acetic acid–water (9 : 2 : 2, by vol) | 0·39 |
| 2-methyl-propan-2-ol–formic acid–water (70 : 15 : 15, by vol) | 0·53 |
| ethanol–ammonium acetate (M) containing EDTA (mM) (7 : 3, by vol) | 0·60 |
| methanol–pyridine–water (20 : 1 : 5, by vol) | 0·63 |
| water–saturated phenol | 0·95 |

The identity of the labelled material was further confirmed by paper electrophoresis. Electrophoretograms were run using either ammonium acetate buffer (0·1M, pH 5·6) or acetic acid (M, pH 2·5), using 22 v/cm, 2·5 mA for 16 or 6 hr respectively when the N-ethyl maleimide–cysteine had moved about 10 cm towards the cathode in each case.

*Radioactivity measurements*

Samples (up to 0·2 ml) were counted at infinite thinness on duralumin planchets ($\frac{3}{4}$ in. diam.) under an end-window counter of about 5 per cent efficiency. To ensure even spreading the planchets were pretreated with 0·1% Teepol in 50% ethanol (0·2 ml) and dried. Each solution was assayed in duplicate and at least 4000 counts above background recorded. All counts were corrected.

*Estimation of amino acids*

The uptake of amino acids and the content of $\alpha$-amino nitrogen in tissue extracts were determined by the ninhydrin procedure of Yemm and Cocking.[19]

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[19] E. W. YEMM and E. C. COCKING, *Analyst* **80**, 209 (1955).