Thin Layer Chromatography Abstracts 1968-1971

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PREFACE

This volume carries forward the project begun with THIN LAYER CHROMATOGRAPHY, AN ANNOTATED BIBLIOGRAPHY: 1964-1968 into the literature of 1971. Although the pattern of presentation of the earlier volume has been generally maintained some simplification of the Table of Contents has been done to bring it more into balance with the recent literature.

Of particular note in the period covered by this volume is the extensive use of the TLC technique for the study of lipids and of steroids. Clearly these compounds are analyzed in a rapid and easy fashion far better by TLC than by any competing technique. Multiple development represents a refinement which extends the method to more complex mixtures.

Other developments are worthy of mention. The extensive exploration of TLC to separate inorganic ions, particularly in the German literature, represents an application which with refinement could supplant the commonly used but more laborious ion exchange analyses. High levels of interest in TLC as an adjunct to environmental studies continues as new insecticide, pesticide, herbicide, and airborne pollutant methods are reported. The search for a rapid and dependable technique for the detection of harmful drugs and their metabolites in the urine continues. Finally some of the frontiers in research such as the assay of cyclic AMP are reflected in the TLC literature.

The assistance of Mei Lan Lin, Joanna Yii, and particularly of Joni Parker in the assembling of the abstracts is gratefully acknowledged. It is hoped that this volume will save time and energy and perhaps open a few new doors for analysts using TLC.

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CONTENTS

Chapter 1

AMINO ACIDS AND PEPTIDES

1. Gibbs, C.C.J., S.J. Saunders, and G.D. Sweeny. Quantitative Estimation of Tryptophan in Plasma and Urine Using TLC and Induced Florescence. Clin. Chim. Acta 17, 317 (1967).

   Tryptophan was isolated from urine or plasma on 0.02-inch layers of silica gel G using methyl acetate - isopropyl alcohol - 25% ammonia (9:7:4). Urine was spotted directly. The plate was sprayed with formaldehyde - 25% HCl - ethanol (1:1:2) and a photometric measurement of induced UV fluorescence was used for quantitative estimation. 95.8% of added tryptophan was recovered from plasma, and 93.7% from urine.

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2. Gloede, J., K. Poduska, H. Gross, and J. Rudinger. Amino Acids and Peptides LXXIX. Coll. Czech. Chem. Comm. 33, 1307 (1968).

   By reacting glycine, L- or Dl-leucine, L- or DL-phenylalanine, L-tyrosine, L-serine, and L-glutamate or the corresponding esters with 2,5-diethoxytetrahydrofuran, the corresponding α-pyrrolo-derivatives have been obtained. These were chromatogrammed on silica gel G using n-butanol - acetic acid - water (4:1:1), detecting the amino acids with ninhydrin and the derivatives with Ehrlich's reagent.

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3. Chimiak, A., K. Eisler, K. Jost, and J. Rudinger. Amino Acids and Peptides LXXX. Coll. Czech. Chem. Comm. 33, 2918 (1968).

The unambiguous synthesis of N-carbamyl-oxytocin and N-carbamyl-2-O-methyltyrosine-oxytocin was reported. N-carbamyl-S-benzylcysteine methyl ester, N-carbamyl-S-benzylcysteine amide, N-carbamyl-S-benzyl cysteine, S-benzylcysteine amide, and S-benzylthiomethylhydantoin were chromatogrammed on silica gel G using butanol - acetic acid - water (4:1:1) or butanol - acetic acid - pyridine - water (15:3:10:6). Detection was with Ehrlich's reagent.

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4. Babel, I., R.C.R. Stella, and E.S. Prado. Action of Horse Urinary Kallikrein on Synthetic Derivatives of Bradykinin. Biochem. Pharm. 17, 2232 (1968).

The action of horse urinary kallikrein on bradykinyl serine, methionyllsyl-bradykinin, and lysyllysyl-bradykinin was reported. DNS derivatives of these compounds, or their fragments, were separated on 0.25-mm layers of silica gel H using 2-propanol - methyl acetate - ammonia (9:7:4). Detection as pinkish fluorescent spots occurred under UV.

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5. Neher, R., B. Riniker, H. Zuber, W. Rittel, and F.W. Kahut. Thyrocalcitonin II. Helv. Chim. Acta 51, 917 (1968) (German).

The sequence of α-thyrocalcitonin and its sulfoxide (32 amino acids) was studied. PTH derivatives were chromatogrammed on silica gel G using chloroform: chloroform - methanol (9:1), chloroform - formic acid (20:1), chloroform - ethyl acetate - water (6:3:1) (lower phase), chloroform - isopropyl alcohol - formic acid (35:4:1), or chloroform - methanol - formic acid (70:30:2). Dansyl amino acids were chromatogrammed on polyamide layers using 90% formic acid - water (3:200), 25% ammonia - water (3:200), n-heptane - n-butanol - acetic acid (3:3:1), toluene - acetic acid (9:1), and chlorobenzene - acetic acid (9:1). Detection was by UV. Amino acids and peptides were chromatogrammed on silica gel G or cellulose using sec-butanol - 3% ammonia (7:3), butanol - acetic acid - water (150:15:42), sec-butanol - isopropyl alcohol - 9% monochloroacetic acid (29:4:17), nitromethane - dimethyl formamide - water (5:3:2), n-butanol - pyridine - acetic acid - water (17:12:4:15), n-butanol - pyridine - acetic acid - water (17:12:2:15), chloroform - methanol - 17% ammonia (41:41:18), and isopropyl alcohol - 25% ammonia - water (7:1:2).

6. Pataki, G., and K. Wang. Quantitative TLC VII. J. Chromatog. 37, 499 (1968).

DANS-, DNP-, and PTH-amino acids were separated on silica gel with starch [Chimia 20, 361 (1966)], silica gel-zinc silicate with starch [Pataki, G. *Techniques of TLC in Amino Acid and Peptide Chemistry* (1968)], or polyamide layers. The amino acid derivatives were then quantitated by direct fluorimetric scanning, using fluorescence or fluorescence quenching techniques. Special reference was given to the scanning of closely neighboring spots.

DANS-Amino Acids

silica gel G (starch)	benzene - pyridine - acetic acid (40:10:1)
silica gel G (starch)	chloroform - methanol - acetic acid (15:4:1)
polyamide	heptane - n-butanol - acetic acid (3:3:1)

DNP-Amino Acids

silica gel G (starch)	chloroform - benzyl alcohol - acetic acid (70:30:3)
silica gel G (starch)	n-propanol - 25% ammonia (7:3)
polyamide	benzene - acetic acid (4:1)

PTH-Amino Acids

silica gel-zinc silicate	chloroform - formic acid (20:1)
silica gel-zinc silicate	chloroform - methanol (9:1)
polyamide	formic acid - water (1:1)

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7. Simard-Savoie, S., L.M. Breton, and M. Beaulieu. Rapid Thin Layer Chromatographic Microassay of ε-Aminocaproic Acid in Urine. J. Chromatog. 38, 143 (1968).

ε-Aminocaproic acid was conveniently and rapidly assayed in urine by TLC on 0.25-mm layers of silica gel G and dried for one hour at 100°C, using n-butanol - acetic acid - water (4:1:1). Visualization was with 0.5% ninhydrin in methanol or water followed by heating at 80°C for 20 minutes.

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8. Shellard, E.J., and G.H. Jolliffe. The Identification of the Free Amino Acids Present in Some Grass Pollens by TLC. J. Chromatog. 38, 257 (1968).

The free amino acid content of aqueous extracts of eleven grass pollens was investigated by TLC on 0.25-mm layers of silica gel G, using the following solvents: 96% ethanol - water (7:3), phenol - water (3:1) + 2 mg NaCN/100g, n-butanol - acetic acid - water (4:1:1), and 96% ethanol - water - diethylamine (70:29:1). After air-drying the layers overnight, visualization was with modified ninhydrin [Anal. Chem. 31, 926 (1959)].

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9. Fawcett, C.P., M. Reed, H.M. Charlton, and G.W. Harris. The Purification of Luteinizing-Hormone-Releasing-Factor with Some Observations on its Properties. Biochem. J. 106, 229 (1968).

Preliminary experiments with bullock median-eminence tissue prepared for development a five-stage procedure with commercial sheep hypothalamic tissue to isolate luteinizing-hormone-releasing-factor. The active peptide was separated into its components on cellulose with the upper layer of n-butanol - acetic acid - water (4:5:1). Detection was with the ninhydrin-cadmium spray reagent.

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10. Grant, P.T., and K.B.M. Reid. Isolation and Partial Amino Acid Sequence of Insulin from the Islet Tissue of Cod. Biochem. J. 106, 531 (1968).

The isolation and sequencing of 39 of the 51 amino acids of cod insulin was presented. DNP- and DNS-amino acids were separated on 0.25-mm layers of silica gel G using chloroform - benzyl alcohol - acetic acid (70:30:3)*, chloroform - methanol - acetic acid (95:5:1)*, benzene - pyridine - acetic acid (40:10:1)**, chloroform - 2-methylbutan-2-ol - acetic acid (70:30:3) or (140:60:1), and chloroform - 2-methylbutan-2-ol - formic acid (70:30:1). Singly starred systems were used for DNP-amino acids, and doubly starred for both DNP- and DNS-derivatives.

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11. Makinen, K.K., and K.U. Paunio. Demonstration of Biosynthesis of Collagen in Rat Skin. Acta. Chem. Scand. 22, 1371 (1968).

Amino acids from hydrolysis of collagen were chromatogrammed on silica gel using phenol - water (3:1). Detection was by ninhydrin. Proline and hydroxyproline were separated.

+ + +

12. Samuelsson, G., L. Seger, and T. Olson. The Amino Acid Sequence of Oxidized Viscotoxin A3 from the European Mistletoe. Acta Chem. Scand. 22, 2624 (1968).

Oxidized viscotoxin A3 was digested with trypsin and chymotrypsin. The resulting peptides were separated by ion exchange chromatography and subjected to the Edman degradation. Peptide mapping was on 20 x 40-cm plates with 0.25-mm layers of silica gel G, using TLC in the short dimension with propanol - ammonia - water (30:3:17), then electrophoresis with pyridine - acetic acid - water (1:10:500, pH 3.5) at 2000 V for 2.5 hours. Preparative work utilized 1-mm layers of silica gel H. Visualization was with 0.05g ninhydrin - 50 ml ethanol - 2 ml collidine - 15 ml acetic acid followed by heating 5 minutes at 105°C.

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13. Granroth, B. Separation of Allium Sulfur Amino Acids and Peptides by Thin Layer Electrophoresis and TLC. Acta Chem. Scand. 22, 3333 (1968).

Sulfur metabolism in Allium species was studied. The following were separated on cellulose MN - silica gel H (15:2.5): Gly., Ala., Val., Ilen., Leu., Ser., Thr., Cys_2, Asn., Gl ., Glu., Asp., Phe., MeCys., Arg.-His., Lys., s-(carboxymethyl)-cys., S-(2-carboxyethyl)-cys., S-(2-carboxypropyl)-cys., S-(carboxyisopropyl)-cys., S-(2-carboxypropyl) glutathione, S-methyl cys., S-propyl cys., S-(propen-1-yl)-cys., S-methylcysteine sulfoxide, S-(propen-1-yl) cysteine sulfoxide, S-allylcysteine sulfoxide, and cycloalliin. Electrophoresis was run at 2100 V, 60 mA for 15-20 minutes using formic acid - acetic acid, pH 2.0. Chromatography was by two successive solvents: butanone - pyridine - water - acetic acid (710:5:15:2), then propanol - water - propyl acetate - acetic acid - pyridine (120:60:20:4:1).

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14. McManus, I.R., and M. Jackson. Isolation and Assay of Radioactive Urinary Kynurenic and Xanthurenic Acids. Anal. Biochem. 23, 163 (1968).

3-^{14}C-L-Tryptophan was injected intravenously, and kynurenic acid and xanthurinic acid were isolated from urine by ion-exchange chromatography and TLC. Various tryptophan derivatives were separated on 0.25-mm layers of cellulose, including kynurenic acid, kynurenine, xanthurenic acid, tryptophan, indole-3-acetic acid, 5-hydroxyindole acetic acid, 5-hydroxytryptophan, and tryptamine using 8% NaCl - acetic acid (100:1) as solvent. Visualization was by UV and quantitation by scintillation.

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15. Smith, G.F., and M. Murray. Direct Spectrophotometric Quantitation of Phenylthiohydantoin Derivatives of Amino Acids from TLC on Silica Gel. Anal. Biochem. 23, 183 (1968).

The chief deterrant to spectrophotometric quantitation of compounds separated on silica gel thin layers was the inability to obtain pure spectra from eluates. Plates were therefore pretreated with methanol. The spots were removed by placing the mouth of a tube over the spot and twisting. The spots were eluted with methanol and good spectra were obtained. TLC was on 0.25-mm layers of silica gel with organic binder and manganese-activated zinc silicate indicator. Solvents used were chloroform - methanol (9:1), chloroform - formic acid (20:1), and chloroform - methanol - formic acid (50:35:1).

+ + +

16. Ber, A., and L. Wasserman. Pigment Formation from Tyrosine Derivatives by UV Irradiation in TLC. Experientia 24, 224 (1968).

Thyroxine is known to be sensitive to light, forming on exposure a yellow pigment due to oxidation. The occurrence of this reaction on thin layer plates was investigated using 3-monoiodo-tyrosine, 3,5-diiodo-tyrosine, 3,5-diiodothyronine, 3,5,3'-tri iodothyronine, thyroxine and tyrosine. These were run on 0.25-mm layers of silica gel G using ethyl acetate - methanol - 2N ammonia (5:2:3). Detection was with 0.2% ninhydrin in acetone followed by heating for 5 minutes at 100°C.

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17. Levis, G.M., D.A. Koutras, A. Vagenakis, G. Messaris, C. Miras, and B. Malamos. Thyroidal Iodinated Compounds in Nodular Goiter. Clin. Chim. Acta 20, 127 (1968).

Iodinated compounds in nodular and paranodular tissues of 19 cases of nodular goiter were separated by TLC on silica gel G - DEAE cellulose (3:2) in two dimensions using n-butanol - acetic acid - water (78:5:17) in the first direction and n-butanol saturated with 2N ammonia in the second. Spots were detected by autoradiography. Lipids were extracted with chloroform - methanol (2:1). Phospholipids were analyzed on silica gel G using chloroform - methanol - acetic acid - water (80:15:3:2).

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18. Katter, D., and R. Humbel. Screening for Sulfite Oxidase Deficiency. Clin. Chim. Acta 24, 211 (1969).

Screening methods for sulfite oxidase deficiency involve assay for sulfite using commercial test paper. S-sulfo-L-cysteine was assayed on cellulose layers. Urine was applied directly and the plate was developed with phenol - water (3:1), which leaves the S-sulfo-L-cysteine at the origin. It was then developed again using acetone - acetic acid - water (7:1:2). Visualization was with ninhydrin. Normal urines had as high as 1 mg% S-sulfo-L-cysteine; 20 mg% was typical in pathological urine.

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19. Herrmann, J., H.L. Kruskemper, and H. Muller. Zur Methodik der Bestimmung von Freiem, Dialysablem Thyroxin im Serum. Clin. Chim. Acta 24, 457 (1969) (German).

Whether or not radioactive contaminants in ^{125}I thyroxine affect the free thyroxin estimations in human serum was studied. Iodothyronines were chromatogrammed on two different systems: on 0.25-mm layers of silica gel using acetone - pentene hydrate - 1M ammonia (4:1:1), and 0.5M acetic acid - acetone (4:1) on cellulose-coated aluminum foil.

+ + +

20. Teuchy, H., and C.F. Van Sumere. Quantitative TLC Determination of Hippuric Acid in Rat Urine. Clin. Chim. Acta 25, 79 (1969).

One-ml urine samples were extracted with ethyl acetate, and the concentrated extract was chromatogrammed on 0.25-mm layers of silica gel G - cellulose (1:1) using toluene -

ethyl formate - water (2:10:1). Plates were sprayed with a 5% solution of p-dimethylaminobenzaldehyde in acetic acid anhydride - acetone. Zones were scraped and extracted, and the concentration determined by colorimetry.

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21. Wadman, S.K., H.F. deJonge, and P.K. de Bree. Rapid High-Resolution Two-Dimensional Amino Acid Chromatography on Micro Scale Chromatograms. Clin. Chim. Acta 25, 87 (1969).

Cellulose-coated aluminum foil was reported to have unique properties for amino acid separation in two dimensions. For most amino acids, n-butanol - pyridine - water (1:1:1) was used in the first direction and 88% phenol - 25% ammonia - water (10:0.8:1) + 1 mg/200 ml 0-oxychinoline was used in the second. For aromatic and branched amino acids or for methionine, 96% ethanol - water (86:14) was used in the first direction and t-butanol - butanone - 25% ammonia - water (5:3:1:1) was used in the second. Both systems involved short migration paths. Detection was with 0.2% ninhydrin in ethanol. Where necessary (as with urine) samples were desalted before chromatography by passage through Dowex 50-W X-8 (50-100 mesh). The amino acids were eluted with 2N ammonia.

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22. Hyanek, J., H.J. Bremer, and M. Slavik. "Homocystinuria" and Urinary Excretion of β-Amino Acids in Patients Treated with 6-Azauridine. Clin. Chim. Acta 25, 288 (1969).

Marked urinary excretion of β-alanine and other unidentified amino acids has been described in patients with generalized psoriasis treated with high doses of 6-azauridine triacetate. After desalting of the urine [J. Am. Chem. Soc. 74, 5954 (1952)], β-alanine, homocystine, β-aminoisobutyric acid, and α-aminobutyric acid were chromatogrammed in two dimensions on cellulose using either first ethanol - water (83:17) then t-butanol - butanone - 25% ammonia - diethylamine - water (125:75:25:1:50) or first acetone - acetic acid - water (7:1:2) then phenol - 15% formic acid (250 g: 83 ml). Detection methods included ninhydrin, isatin, hexachloroplatinate, and Sakaguchi reagents.

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23. Niederwieser, A., and H.C. Curtius. Aminosauren-Analyse in der Klinischen Chemie. Z. Klin. Chem. Biochem. 7, 404 (1969) (German).

The paper is a review of the methods used for qualitative, semiquantitative, and quantitative determinations of amino acids in biological materials. Included is information about the interpretation of pathological amounts of specific amino acids, methods for paper, thin layer, and electrophoretic analysis, quantitation, and 272 references.

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24. Roelcke, D., and H. Weiker. Physikochemische, Immunologische, und Biochemische Charakteristierung des Proteinanteils der Low-Density Lipoproteins. Z. Klin. Chem. Biochem. 7, 467 (1969) (German).

Amino acids from protein hydrolysis (6N HCl, 12 hours, 120°C) were chromatogrammed in two dimensions on cellulose using n-butanol - acetic acid - water (12:3:5) and phenol - ammonia - water (640:3.4:44).

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25. Kraffczyk, F., R. Helger, and H. Lang. Ein Einfacher Dunnschichtchromatographischer Suchtest zur Erkennung von Hyperaminacidamien. Z. Klin. Chem. Biochem. 7, 521 (1969) (German).

A TLC screening test for all important hyperaminoacidaemia was proposed. Plasma was chromatogrammed on cellulose with butanol - acetone - acetic acid - water (7:7:2:4) next to normal standards. Histidinaemia was detected with t-butanol - butanone - 25% ammonia - diethyl amine - water (50:30:10:0.4:20).

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26. Benson, A.M., and K.T. Yasunobu. Non-heme Irone Proteins X. J. Biol. Chem. 244, 955 (1969).

The amino acid sequence of ferredoxin from Leucaena glauca, a 96 residue protein, was reported. In sequence studies done by the Edman subtractive method, amides were determined by direct identification of their phenylthiohydantoins on silica gel G using chloroform - methanol (9:1). Dansyl or DNP derivatives of N-terminal amino acids were determined using two-dimensional TLC as described in J. Chromatog. 20, 514 (1965).

27. Keresztes-Nagy, S., F. Perini, and E. Margoliash. Primary Structure of Alfalfa Ferredoxin. J. Biol. Chem. 244, 981 (1969).

Edman degradation of peptides was performed. The thiazolinones were extracted with diethyl ether, heated 10 minutes at 80°C for 1 hour in 1N HCl to convert them to hydantoins, and identified by TLC on Eastman Chromagram K 301R sheets (silica gel) using the solvents of Anal. Biochem. 18, 264 (1967). The Pauly reagent was used for detection. Heating the sheets 2-3 minutes at 125°C caused the serine, threonine, glycine, and tyrosine to turn bright yellow, phenylalanine to turn pale yellow, and aspartate, glutamate, and carboxymethyl cystein to turn blue to violet.

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28. Reichert, L.E., Jr., M.A. Rasco, D.N. Ward, G.D. Niswender, and A.R. Midgley, Jr. Isolation and Properties of Subunits of Bovine Pituitary Luteinizing Hormone. J. Biol. Chem. 244, 5110 (1969).

Dansyl derivatives were prepared of subunits. These were hydrolyzed, and the amino acids were chromatogrammed on polyamide sheets in two dimensions using the systems of Biochim. Biophys. Acta 133, 369 (1967). Visualization was by UV light.

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29. Wang, S.S., and R.B. Merrifield. Preparation of Some New Biphenylisopropyloxycarbonyl Amino Acids and Their Application to the Solid Phase Synthesis of a Tryptophan Containing Heptapeptide of Bovine Parathyroid Hormone. Int. J. Prot. Res. I, 235 (1969).

Several new 2-p-biphenyl-2-propyloxycarbonyl amino acids were synthesized and characterized. Products were shown to be homogenous on silica gel (Eastman 6061) using n-butanol - acetic acid - pyridine - water (15:3:10:12), chloroform - methanol (4:1), and n-propanol - water (7:3). Visualization was with 0.06% ninhydrin in n-butanol containing 4% acetic acid.

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30. States, B., and S. Segal. The Separation of Cystine and the N-Ethylmaleimide Adducts of Cysteine and Glutathione. Anal. Biochem. 27, 323 (1969).

S(N-Ethylsuccinimido) glutathione, S(N-ethylsuccinimido) cysteine, and cystine were separated on silica gel (Eastman 6061) and MN-300 cellulose using the following proportions of n-butanol - pyridine - acetic acid - water:

on cellulose	(3:2:0.6:2.4)
on cellulose	(6:2:0.6:2)
on cellulose	(3:2:0.6:1.5)
on cellulose	(3:2:0.6:1)
on cellulose	(9:2:1:2)
on silica gel	(3:2:0.6:2.4).

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31. Wieland, T., and A. Buku. Chromatographic MicroMethod for Determining Amino Acid Configurations. Anal. Biochem. 27, 378 (1969).

Diasterioisomeric dipeptides separate on cellulose thin layers. To determine the configuration of an amino acid, it was coupled with an n-protected L-amino acid producing an LL or DL dipeptide. t-Butyloxycarbonyl L-alanine was coupled, the dipeptides spotted, and the plate heated to 110°C for 1 hour to remove the protecting group. The plate was then developed with chloroform - methanol - ammonia (2:2:1).

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32. Sen, N.P., E. Somers, and R.C. O'Brien. TLC and Gas Chromatographic Determination of α, ε-Diaminopimelic Acid in E. Coli. Anal. Biochem. 28, 345 (1969).

α, ε-Diaminopimelic acid (DAP) was obtained from hydrolysates of bacterial cell walls. Assay by TLC proved to be rapid and sensitive. Separation was accomplished on 0.25-mm layers of Adsorbosil-1 and air dried using a 95% ethanol - 28% NH_3 - water (15:2:3) solvent and 0.3% ninhydrin and 2% pyridine in 95% ethanol as the spray reagent. Plates were heated 5 minutes at 90-100°C to develop the color. Two-dimensional TLC is necessary to separate DAP from other amino acids.

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33. Hoye, A. Radiochemical Breakdown of an Iodine-131-Labeled L-Thyroxin Preparation. Acta Chem. Scand. 23, 1040 (1969).

The radiochemical stability of ^{131}I-labeled thyroxine was investigated. The purity of thyroxine was checked on

cellulose with t-butanol - 2N ammonia - chloroform (188:35:30) or on silica gel with t-amyl alcohol - acetone - ammonia (28:8:7) or t-butanol - acetone - 10N ammonia (50:25:18). The cellulose system gave the best separation of T_4 and 3,5,3$_1$-triiodothyronine. Quantitation was with a scaler.

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34. Blomback, B., and E. Zetterqvist. Incorporation of ^{131}I into Dog Fibrinopeptides. Acta Chem. Scand. 23, 1137 (1969).

Iodine appeared in fibrinopeptide B upon iodination of dog fibrinogen. Iodo-amino acids were identified on 0.75-mm layers of cellulose using butanol - 2M acetic acid (77:23) or pyridine - 2M acetic acid (4:1). Visualization was with ninhydrin.

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35. Onger, G. An Improved Preparation of Cellulose Layers for the TLC of Amino Acids. Acta Chem. Scand. 23, 2185 (1969).

Cellulose layers were prepared as follows: 50 ml water and 40 ml ethanol were used to suspend 15 g cellulose powder. The suspension was stirred for two minutes. The layers were spread 0.3-mm thick, dried, and predeveloped with the first solvent system.

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36. Zintel, J.A., A.J. Williams, and R.S. Stuart. Some Enzymic Syntheses of ^{15}N-L-Aspartic Acid and ^{15}N-L-Glutamic Acid. Can. J. Chem. 47, 411 (1969).

^{15}N-L-Aspartate was prepared by adding $^{15}NH_3$ to fumarate in the presence of aspartase. Labeled glutamate was then prepared by transferring the ^{15}N from aspartate using aspartate aminotransferase. The amino acids were separated on silica gel (Eastman K301R) with n-butanol - acetic acid - water (4:1:1) and were visualized with 0.2% ninhydrin in butanol.

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37. Holbrook, J.J., and R. Jeckel. A Peptide Containing a Reactive Lysyl Group from Ox Liver Glutamate Dehydrogenase. Biochem. J. 111, 689 (1969).