tissue 10 μ thick and for the X-rays used would fall in the range 0–0·04, and the contrast would be very low.

It must be emphasized that many precautions must be taken before data so obtained may be translated into concentrations of intracellular protein. Further experiments are in progress to overcome some of the difficulties involved.

A. Engström
M. A. Jakus

Department for Cell Research,
Karolinska Institutet,
Stockholm.
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Micro Estimation of Amino-Nitrogen and its Application to Paper Partition Chromatography

The present interest in the quantitative estimation of amino-acids in protein and peptide hydrolysates has prompted an early description of a method which it is felt may make some contribution to this problem.

The method described by Pope and Stevens[1] using copper phosphate for determining amino- and peptide-nitrogen has been successfully adapted to the determination of micro amounts (1–25 μgm.) of α-amino-nitrogen. The essential points of the method are that after the amino-acid has been allowed to react with copper phosphate, the soluble copper complex in the filtrate is decomposed by means of sodium diethyl-dithio-carbamate, the resultant characteristic golden yellow colour is extracted into amyl alcohol and the amount of copper determined absorptiometrically. The theoretical value of 0·44 for the ratio of α-amino-nitrogen to copper in the complex A_2Cu is not obtained under the experimental conditions in use, and it is essential to construct a standard curve for each individual amino-acid. Such curves are reproducible, and over their linear portion (10–25 μgm. α-amino-nitrogen) the amino-acids methionine, hydroxyproline, aspartic acid, leucine, *iso*leucine, histidine, phenylalanine, valine, glutamic acid, tryptophane and tyrosine give ratios lying between 0·50 and 0·55; alanine, glycine, threonine, serine, cystine and cysteine ratios between 0·55 and 0·80; and lysine, arginine and glucosamine ratios between 1·25 and 1·60.

The application of the method to the paper chromatograms of Consden, Gordon and Martin[2] has enabled quantitative recoveries of the component amino-acids of simple mixtures to be obtained. The technique employed is to localize the amino-acid on the chromatogram by use of a trial strip, as suggested by Dent[3], then cut out the square of paper containing the amino-acid. The square is folded, dropped into a test tube and the amino-acid treated with a suitable amount of copper phosphate sludge. The excess copper phosphate is filtered off, the copper in the filtrate determined by means of sodium diethyl-dithio-carbamate and its equivalent in α-amino-nitrogen obtained by reference to a standard curve for the amino-acid under investigation. Using this technique, a single-dimensional chromatogram of a valine/glycine mixture using *n*-butanol/water as the solvents gave recoveries of 94 per cent for glycine and 97 per cent for valine.

It has been found possible for ninhydrin to react with glycine and valine under conditions where almost no destruction of amino-nitrogen occurs, although enough colour is obtained to determine the position of the amino-acid on the chromatogram. Such development of colour did not affect appreciably the subsequent quantitative determination of α-amino-nitrogen. With single-dimensional chromatograms of a glycine/valine mixture, recoveries of 91 per cent for glycine and 93 per cent for valine have been obtained after reacting with ninhydrin. If the principle is found capable of extension to other amino-acids, it may be possible to avoid the use of trial chromatograms altogether, and this would be a great advantage in two-dimensional chromatography.

At the present time the errors of the estimation are such as to be well within the limits required for establishing the ratios of the amino-acid components of peptides.

A fuller report of this work will be published elsewhere.

A. J. Woiwod
Wellcome Physiological Research Laboratories,
Langley Court,
Beckenham.
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Solvation of Hydrogen Ion in Oxygen-containing Solvents

The nature of non-aqueous solutions of the so-called 'strong' acids has received a good deal of attention; but although much information is now available regarding a number of individual solvent systems, comparatively little progress has been made in establishing quantitative scales of comparison of properties such as proton-availability in different solvents. A quantity suitable for this purpose is the acidity function first introduced by Hammett[1] to deal with concentrated aqueous solutions. The acidity function is a measure of the tendency of the medium to donate a proton to a neutral base and may be defined by

$$H = \log[BH^+]/[B] - \log K_B^{H_2O}, \quad . \quad (1)$$

where B is the proton-acceptor and BH^+ its conjugate acid, and where $K_B^{H_2O}$ is the equilibrium constant of the reaction

$$B + SH^+ \rightleftharpoons BH^+ + S \quad . \quad . \quad . \quad (2)$$

in water, SH^+ representing the solvated proton. It can be readily shown that $H = \log (\alpha c_A K_B^S / K_B^{H_2O})$, where c_A and α are the concentration and degree of dissociation of the acid, and K_B^S the equilibrium constant of reaction (2). The ratio $K_B^{H_2O}/K_B^S$ will be a measure of the proton-affinity of the solvent relative to water and can be calculated from H if α is known.

Using *m*- and *p*-nitranilines as proton-acceptors, H has been determined spectrometrically for solutions of hydrogen chloride in water, dioxan, ethanol and acetone, and their binary mixtures. The values of H for the four single solvents vary in the order dioxan < water < ethanol < acetone. These differences cannot be accounted for by differences in α, which can be estimated from conductivity measurements. The deduced values of $K_B^{H_2O}/K_B^S$ vary in the