2-acylaminofluorenes in the intestine and the carcinogenicities of these compounds after oral feeding to the rat. Thus, the relative rates of formation of AF follow the same order as the relative carcinogenicities of 2-acylaminofluorenes (AAF > SAF > BAF > TSAF)[16,*]. These observations support the view that AF is a key compound in carcinogenesis by AAF and related derivatives and that the small intestine is the principal site for its release. The ready liberation of AF in the intestine would also explain the carcinogenicity of compounds, such as SAF, which are not attacked by liver deacylase.

SUMMARY

The deacylation of the carcinogen 2-acetamidofluorene and several related 2-acylaminofluorenes to 2-aminoflorene by rat liver slices, intestinal strips, and aqueous extracts of intestine, was studied. Intestinal strips deacylated all substrates at faster rates than did liver slices. Aqueous extracts of intestine likewise cleaved 2-acetamidofluorene at appreciable rates. Evidence is presented that the observed hydrolyses were due to a deacylase of the intestine and not to bacterial action. The relative rates of release of 2-aminofluorene by intestinal strips from various 2-acylaminofluorenes closely parallels the relative carcinogenicities of these compounds.

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QUANTITATIVE DETERMINATION OF 0.5–5 μg OF AMINO ACID NITROGEN ON PAPER CHROMATOGRAMS AND IN SOLUTION

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Numerous methods have been evolved for the quantitative determination of amino acids on paper chromatograms and in solution[1]. Most of them depend on measurements of the intensity of the blue colour produced on treatment with ninhydrin. In the application of this technique to paper chromatograms, the intensity of colour of the blue

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area is either measured directly or the colour is eluted and measured photometrically.

These methods suffer from a number of disadvantages, chief of which is the variable interference of ammonia, present either in the solvents used or in the atmosphere. This becomes particularly serious when it is attempted to apply such methods to material obtained from micro-organisms grown on relatively high concentrations of ammonium salts[2], samples of which are chromatographed in acid–phenol solvents[3]. Under these conditions, the removal of ammonia by the technique of FOWDEN[4] has not been found, in our hands, to be quantitative.

The present method utilises the preferential absorption of ammonia by the polysulphonic acid resin Dowex-50[5], at pH 5.0, to eliminate its interference in the quantitative ninhydrin procedure. Quantities of amino acids containing as little as 0.5 μg α-amino nitrogen can be determined with accuracies of $\pm$ 5% whilst larger quantities (1.5–5 μg) can be determined with accuracies of $\pm$ 2%. The method is particularly sensitive when applied to ^{14}C-labelled amino acids, where the position of the material on paper chromatograms can be accurately located by autoradiography. With unlabelled amino acids, at least 1 μg α-amino nitrogen per spot has to be present in order to locate the material[4]. The method can also be directly applied to the determination of the amino acid nitrogen of solutions, such as acid protein hydrolysates. In all such determinations, proline, hydroxyproline and the basic amino acids have to be separately determined.

METHODS

Elution of amino acid from chromatograms. The amino acid is located either by the fluorescence technique of FOWDEN[4] or, if labelled with radioactive isotope, by autoradiography. The area of the spot is cut out and shredded into a 10 ml stoppered graduated test-tube containing about 150 mg of dry Dowex-50, which has been washed according to the procedure of MOORE AND STEIN[5] and equilibrated with 0.2 *M* Na citrate buffer, pH 5.0 before drying. An area of the paper devoid of amino acids, of approximately the same size as the test spot, is treated similarly. The material in the tubes is well mixed with 2 ml of 0.05 *M* citrate buffer, pH 5.0, the tubes stoppered, and left to stand for 1–2 hours.

Determination of amino acid nitrogen. Samples (0.5–1 ml) of the material in the tubes are removed and placed into separate tubes. The amino acid nitrogen content of these samples, made up to 1.5 ml with 0.2 *M* citrate buffer, pH 5.0, is determined by the method of YEMM AND COCKING[6].

Treatment of amino acid solutions. Solutions of amino acids, such as are produced by hydrolysis of proteins at 110° for 20 h in sealed tubes, are evaporated to dryness under a stream of H_2SO_4-washed N_2. The dry material is dissolved in 2.0 ml of 0.05 *M* citrate buffer, pH 5.0, and samples (0.05–1.0 ml) are treated with Dowex-50 as described for chromatographic eluates.

RESULTS

The method here described was tested on solutions of alanine, aspartate and glutamate of known concentration. When samples were spotted on test strips of Whatman No. 4 filter paper, which were either unwashed or which had been previously washed with water, citrate buffer or with the solvents routinely used for two-dimensional paper chromatography, high and variable blanks were produced, and no proportionality was observed between the amounts of amino acid applied and the intensity of ninhydrin colour produced. The use of Dowex-50 reduced these blanks to negligible and constant values. The intensities of the ninhydrin colours produced were proportional to the amounts of α-amino nitrogen applied, and were independent of the nature of the amino acid used. When ammonium chloride, in quantities equal to or three times the

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nitrogen content of the applied amino acid, was added to these spots, no interference with the ninhydrin colour was observed in the presence of Dowex-50 although high and erratic readings were obtained in its absence (Table I). The addition of the resin itself produced only a negligible increase above the buffer blank reading.

TABLE I

EFFECT OF TREATMENT OF PAPER, OF AMMONIUM CHLORIDE AND OF BUFFERED DOWEX-50 ON THE QUANTITATIVE DETERMINATION OF AMINO ACIDS

(The materials tested were applied to strips of paper, which were shredded and analysed for amino acid N by the quantitative ninhydrin procedure, as described under METHODS)

Spot No.	*Material tested*	*O.D. at 570 mμ*	*O.D. at 570 mμ corrected for Dowex blank*	*Amino acid-N measured (μg)*	*Amino acid-N applied (μg)*
–	Aspartate solution	0.395	—	2.45	2.44
–	Alanine solution	0.395	—	2.45	2.44
–	Glutamate solution	0.385	—	2.40	2.44
1	Unwashed paper	0.107	—	0.68	0
2	Water-washed paper	0.100	—	0.63	0
3	Solvent-washed paper	0.134	—	0.84	0
4	Buffer-washed paper	0.173	—	1.08	0
5	Buffer + Dowex-50	0.070	0.00	0.00	0
6	As 1 + Dowex-50	0.074	0.04	0.05	0
7	As 2 + Dowex-50	0.072	0.02	0.02	0
8	As 3 + Dowex-50	0.071	0.01	0.01	0
9	As 3 + 1.4 μg ammonia-N	0.271	—	1.70	0
10	As 3 + 3.5 μg ammonia-N	0.314	—	1.99	0
11	As 9 + Dowex-50	0.074	0.004	0.04	0
12	As 10 + Dowex-50	0.081	0.011	0.10	0
13	As 8 + aspartate	0.265	0.195	1.20	1.22
14	As 8 + alanine	0.270	0.200	1.25	1.22
15	As 8 + glutamate	0.270	0.200	1.25	1.22
16	As 13 + 3.5 μg ammonia-N	0.272	0.202	1.25	1.22
17	As 14 + 3.5 μg ammonia-N	0.260	0.190	1.19	1.22
18	As 15 + 3.5 μg ammonia-N	0.275	0.205	1.25	1.22

These results showed that the present method could be applied to the determination of amino acids in the presence of ammonium salts. When the quantities of α-amino nitrogen applied to paper strips were plotted against the optical density of the ninhydrin colour (570 mμ) of the eluates, a straight line was obtained which passed through the origin and which was independent of the nature of the amino acid tested (Fig. 1). The crosses show the erratic results obtained in the absence of Dowex-50; the open circles show that the presence of ammonia did not interfere with the method.

The utility of this technique in the analysis of solutions, such as acid protein hydrolysates, was tested on solutions in 6*N* HCl of alanine, aspartate and glutamate which had been heated for 20 h at 110° in sealed tubes, and which were analysed singly or together. The results obtained (Fig. 2) showed that only in the presence of Dowex-50 were consistent and reproducible results obtained, and that added ammonia did not interfere with the determination.

DISCUSSION

The quantitative determination of amino acid nitrogen on paper chromatograms or in protein hydrolysates is hampered by the interference of ammonia, particularly when

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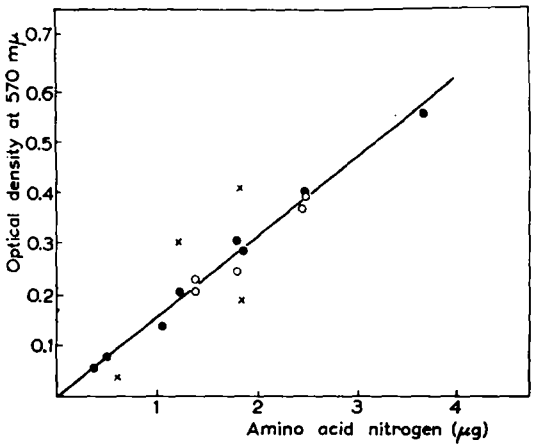

Fig. 1. Effect of buffered Dowex-50 and ammonium salts on the intensity of ninhydrin colour. Solutions of alanine, glutamate or aspartate were applied as spots to strips of Whatman No. 4 paper, which had been previously run in phenol–formic acid–water (500 g : 13 ml : 167 ml) and propionic acid–butanol–water (62 ml : 178 ml : 91 ml). The spots were shredded, eluted and analysed as described under METHODS. × = No Dowex-50 added to eluate; ● = 150 mg of Dowex-50 added to eluate; ○ = 14 μg ammonium chloride-N added to spots before eluting, 150 mg of Dowex-50 added to eluate

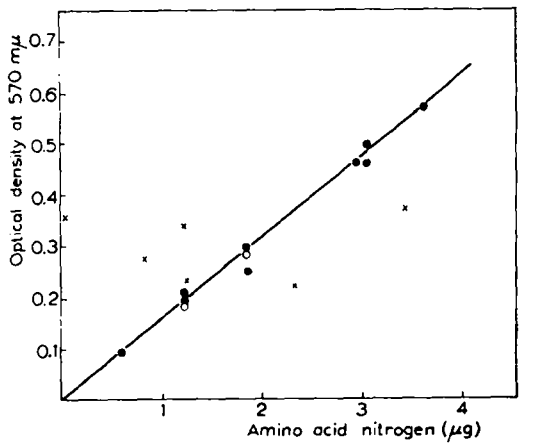

Fig. 2. Effect of buffered Dowex-50 and ammonium salts on the intensity of ninhydrin colour. Solutions of alanine, glutamate or aspartate, singly and in combination, were dissolved in 6*N* HCl, treated as described under METHODS, and analysed. × = No Dowex-50 added. ● = 150 mg of Dowex-50 added. ○ = 14 μg ammonium chloride-N added to solution before evaporation, 150 mg of Dowex-50 added.

the test solutions are obtained from experiments in which micro-organisms are grown on relatively high concentrations of ammonium salts[2]. Under such conditions and when acid–phenol solvents are used for chromatography[3] the removal of ammonia by the technique of FOWDEN[4] is often incomplete and variable. Removal of ammonia by distillation procedures or by the microdiffusion technique[7] lead to inconveniently great dilution of the samples and to the introduction of salts, making subsequent analysis cumbersome and difficult. The present method represents a rapid and convenient technique for the quantitative determination of amino acid nitrogen, which is not affected by the presence of ammonium salts in concentrations at least three times greater than the amino acid nitrogen to be determined.

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SUMMARY

1. A method is described for the rapid quantitative microdetermination of amino acids on paper chromatograms and in solutions. Its main advantage is that the interference of ammonia is eliminated by the use of buffered Dowex-50.

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2. Quantities of amino acids containing between 0.05 and 5 μg of α-amino nitrogen can be determined. The accuracy of a single determination is of the order of $\pm$ 5% at levels of 0.05–1 μg N, but is $\pm$ 2% when quantities of 1.5–5 μg N are determined.

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Short Communications

The *in vitro* inhibition of acetyl phosphatase by thyroxine

A study of the properties of purified acetyl phosphatase[1], an enzyme first described by LIPMANN[2], demonstrated that it is a hydrolase with specificity for acyl phosphates. Radioisotope and chemical studies indicated that it does not possess acetyl- or phosphotransferase activity. The possibility that an enzyme whose function leads to the loss of high energy phosphate compounds may take part in the cellular mechanisms regulating growth and metabolism has led to a study of this enzyme in varying metabolic states. It was also thought to be of interest to study the effect of hormones on the activity of purified preparations. This communication deals with the inhibition of acetyl phosphatase by L-thyroxine.

Enzymic activity was measured by incubating 20 μmoles acetyl phosphate, 100 μmoles sodium acetate buffer, pH 5.4, and enzyme in a final volume of 1.0 ml for 20 min at 36° C. One unit is defined as that amount of enzyme catalyzing the hydrolysis of 10 μmoles of acetyl phosphate, under these conditions, as measured by the hydroxamic method of LIPMANN AND TUTTLE[3]. Little effect of $5 \cdot 10^{-5} M$ L-thyroxine on the enzymic activity could be obtained if both substrate and L-thyroxine were added at the same time. However, upon preincubation of 0.32 units of the enzyme with $5 \cdot 10^{-5} M$ L-thyroxine for 30 min at 36° C, complete inhibition of the hydrolysis of added acetyl phosphate resulted, as shown in Table I. The enzyme used was purified 400 fold from a water extract of horse skeletal muscle.

TABLE I

EFFECT OF PREINCUBATION OF L-THYROXINE ON ACETYL PHOSPHATASE ACTIVITY

	% *activity*
Enzyme	100
Enzyme + L-thyroxine	92
Enzyme + L-thyroxine preincubated 30 min, 36° C, pH 6.0	0

Concentrations: L-thyroxine = $5 \cdot 10^{-5} M$; Enzyme = 0.32 units.
Test conditions: 20 μmoles acetyl phosphate, 100 μmoles sodium acetate, pH 5.4, vol. 1.0 ml, 36° C, 20 min.