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Displacement Chromatography on Synthetic Ion-exchange Resins

8. A SYSTEMATIC METHOD FOR THE SEPARATION OF AMINO-ACIDS

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In this paper it is intended to combine an account of recent work with a survey of the results described in some previous communications in this series (Partridge & Westall, 1949; Partridge, 1949*a*, *b*; Partridge & Brimley, 1951*a*, *b*) in such a way as to present a systematic procedure for the isolation of amino-acids from the hydrolysis products of proteins or from extracts of biological materials. In addition, the results obtained from model experiments with the hydrolysis products of two representative proteins are given; the proteins chosen were egg albumin and a protein fraction extracted from baker's yeast.

The experiments with egg albumin were carried out on what may be regarded as a large laboratory scale, and, in the model experiment described, a batch of 280 g. of the protein was used for hydrolysis. As large quantities of protein were required for these experiments it was convenient to use a commercial grade of egg albumin, but since this product contained considerable quantities of carbohydrate, significant losses of some of the amino-acids were to be expected, due to humin formation during the course of the hydrolysis. It should be emphasized that displacement chromatograms, unlike elution chromatograms, are very flexible as regards the scale on which the experiments are carried out; the amount of material which a column of given dimensions can handle is necessarily much larger with displacement development than it is with elution development, but the most important factor limiting an increase in the scale of an elution chromatogram is the disturbance due to uneven fronts, which is always encountered when broad columns are used. With displacement chromatograms this disturbance may be very largely eliminated by the use of a multiple column, in which the filtration tubes are connected as a series of units of diminishing diameter, and using this device there is no reason to anticipate difficulty in increasing the scale to meet requirements for large quantities of pure bases or amino-acids. In the second model experiment, that with yeast protein, the scale was reduced to approximately one-thirtieth. In this case the weight of the individual amino-acids isolated (from 10 g. of protein) was from 0·1 to 1·0 g., and the scale may be regarded as near the minimum for convenient crystallization and recovery of the less abundant amino-acids.

An important application of any simple method for the isolation of amino-acids from mixtures would lie in the possibility of preparing isotopically labelled amino-acids by a biosynthetic process. It has been suggested (cf. Burris, 1947) that a suitable source of amino-acids labelled with ^{15}N would be the protein from yeast grown with [^{15}N]ammonium salts as the source of nitrogen. The experiment with yeast protein described here was primarily intended as an exploratory step in this direction.

In presenting the systematic procedure it should be emphasized that no single scheme can be the most effective for all mixtures, and various modifications must be made to suit individual requirements. With this in mind, the two examples given show considerable variations in detail, some of which were designed to suit the difference in scale.

APPARATUS AND ION-EXCHANGE MATERIALS

Since columns packed with synthetic resinous ion exchangers may be regenerated and used again many times, it has become the practice to preserve packed filtration tubes as permanent pieces of equipment. In collecting together a set of ready packed columns, there are obvious advantages in a certain degree of standardization as regards height and diameter of filtration tube. It is also useful to supply each packed column with an identification mark and to record data connected with it for use in subsequent experiments. The use of coupled columns of diminishing diameter (Partridge, Westall & Bendall, 1947; Claesson, 1947) has now proved its value for most applications of displacement chromatography (Hagdahl, 1948) and the system has been adopted as a standard one in this laboratory. In addition to its great value in improving the sharpness of the boundaries, the use of standard column sections coupled together also leads to great flexibility. Table 1 shows data for the permanent column sections in use in this laboratory. The standard filtration tubes are numbered I–VII in order of decreasing size, and details are given for three sets of tubes packed respectively with Zeo-Karb 215, sulphonated polystyrene and Dowex 2. It has been shown empirically that the most advantageous column assembly for work with the majority of the amino-acid separations consists of three of the standard filtration tubes coupled in sequence. This scheme is flexible, since a set of three consecutive tubes can always be chosen to accommodate a given weight of mixture without incurring the waste of reagents and time that would be involved in using a column many times too large. Further, if for a particular separation (as, for instance, that of serine and threonine) a column with a very high resolving power is required, accommodation may be made for this by coupling four columns in series. However, in this case a greater quantity of reagents is required, and since the rate of flow must be adjusted to suit the diameter of the smallest column, the time required for the experiment will be much greater than that required for an assembly consisting of three columns.

In order to illustrate the use of the data in Table 1 it may be useful to consider what column assembly would be required for the primary separation of the hydrolysis products of 240 g. of protein using Zeo-Karb 215 as the resin. A rough calculation shows that the mixture would contain about 2000 m-moles of amino-acids and NH_3. The data in Part 1 of this series (Partridge & Westall, 1949, Fig. 3) shows that 1 g. of Zeo-Karb 215 adsorbs about 2·5 m-moles of amino-acid from isoelectric solution. Thus, provided no free mineral acid were present, 240 g. of protein would require 800 g. of resin for complete adsorption. However, the mixture resulting from the hydrolysis of a protein with HCl always contains a small amount of mineral acid even after careful vacuum evaporation, and as this will diminish the effective capacity of the resin, some accommodation must be made for it. Where amino-acids are adsorbed from isoelectric solution it is usual to leave at least one-third of the column unsaturated in order to secure complete resolution, but experience has shown that, in the primary separation of a protein hydrolysate, it is necessary to increase this proportion to at least one-half. Thus a column assembly containing 1600 g. (dry wt.) of Zeo-Karb 215 would be necessary for the 240 g. batch, and reference to Table 1 (column 4)

Table 1. *Data for a range of laboratory columns*

Filtration tube		Zeo-Karb 215 (capacity for 0·15 N-NH_3, 4·25 mg. equiv./dry g.)					Sulphonated polystyrene (capacity, 5·25 mg. equiv./dry g.)					Dowex 2 (capacity, 3·7 mg. equiv./dry g.)				
(1) No.	(2) Diameter (cm.)	(3) Depth of bed (cm.)	(4) Dry wt. of resin (g.)	(5) Particle size (mesh/in.)	(6) Volume 0·15 N-NH_3 required (ml.)	(7) Flow rate 0·15 N-NH_3 (ml./min.)	(8) Depth of bed (cm.)	(9) Dry wt. of resin (g.)	(10) Particle size (mesh/in.)	(11) Volume 0·075 N-NaOH (ml.)	(12) Flow rate 0·075 N-NaOH (ml./min.)	(13) Depth of bed (cm.)	(14) Dry wt. of resin (g.)	(15) Particle size (mesh/in.)	(16) Volume 0·1 N-HCl required (ml.)	(17) Flow rate 0·1 N-HCl (ml./min.)
I	7·6	61	1100	60–80	30000	—	46	830	60–100	58000	—	—	—	—	—	—
II	5·1	41	335	60–80	9500	40	31	255	60–100	17500	50	—	—	—	—	—
III	3·8	30	138	80–100	3900	20	23	106	60–100	7300	25	23	100	100/200	3700	7·5
IV	2·5	20	41	80–100	1200	10	15	30	60–100	2100	12	15	28	100/200	1000	3·0
V	1·7	13·6	12	80–100	340	4	10	9	60–100	620	6	10	8·3	250/500	310	1·5
VI	1·2	9·6	4·2	80–100	120	1·5	7·2	3·2	80–100	220	2	7·2	3·0	250/500	111	0·75
VII	0·8	6·4	1·3	100–120	37	0·5	4·8	1·0	100–120	70	0·6	4·8	0·9	250/500	33	0·33

shows that the three filtration tubes nos. I–III, coupled in series, would be just sufficient. Column 5 shows the particle size of resin recommended for each filtration tube, and the volume of 0·15 N-NH_3 required as displacement developer may be obtained from the data in column 6; in this case the figure is 43·4 l. The rate of flow of displacement developer chosen for the experiments must be that listed for the smallest column section, i.e. 20 ml./min. (column 7) and thus the time required for application of 43·4 l. of displacement developer will be 36 hr.

was supported by the loosely fitting cork *D*. Closure of the top of the filtration tube was carried out in a similar way.

In order to support the resin bed, and to secure uniformity of flow and thorough mixing, the column was provided with a filtration plate *G*, which fitted loosely inside the filtration tube and rested upon the rubber gasket *E*. The filtration plate was turned from a disk of Perspex and is shown in plan and section in the figure. It was provided with radial and annular grooves in the underside, and was perforated with a large number of evenly spaced holes which

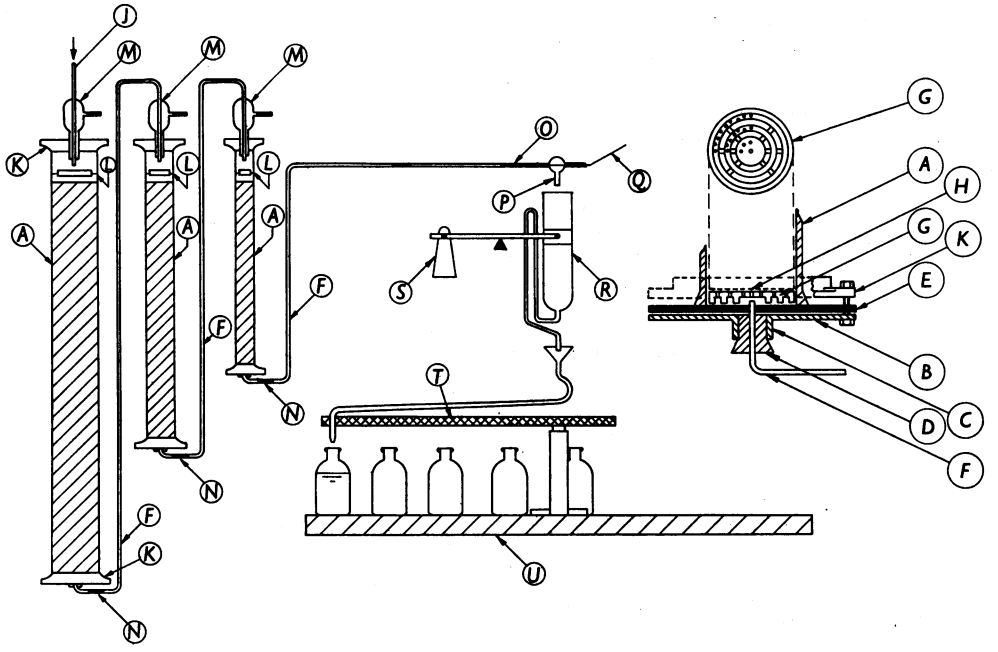


Fig. 1. Construction of the column assembly for the primary fractionation. The key is given in the text.

Construction of the columns. For the smaller column sections (i.e. tubes V–VII, Table 1) a conventional type of construction is suitable. The filtration tubes were made by sealing a glass tap to the end of a glass tube of suitable diameter, and the resin was supported on a plug of glass wool carefully packed into the base. However, with filtration tubes of larger diameter, it was found necessary to make some special provision for ensuring uniformity of flow at the bottom of the column.

The type of construction adopted for larger columns is shown in Fig. 1. The filtration tubes (*AAA*) were made from sections of industrial glass pipeline which were cut to length and provided with standard tapers at both ends by the manufacturers. When used in industrial pipelines the sections are connected together by means of standard metal flanges, and the same flanges (*KK*) were employed for bolting on the endplates in the construction of filtration tubes.

The inset to Fig. 1 shows the endplate *B* which was constructed in metal and provided with a collar *C* to hold the cork *D*. The end of the filtration tube was closed by a thick rubber gasket *E*. This was provided with a small central hole through which was forced the glass outlet tube *F*, thus forming a tight seal. The outlet tube was of narrow bore and passed through the thin sections of the plate left by the grooves. On top of this rested a disk of heavy Fibreglass filter cloth (Fibreglass Ltd., Firhill, Glasgow, N.W.) which prevented the holes in the plate becoming clogged with fine particles of resin. The disposition of the grooves in the plate may be varied to some extent, but should be designed to ensure thorough mixing; however, the provision of a continuous outer ring of full thickness is important since it forms a seal with the rubber gasket and so prevents the passage of fine grains of resin round the edge of the disk.

The arrangement of the apparatus for a large-scale separation experiment is illustrated in Fig. 1. The filtration tubes (*AA*) were arranged side by side and connected by the outflow tubes (*FF*). The solution passed into the apparatus at *J* from a reservoir placed on a shelf arranged at a height about 2 m. above floor level. Each packed column was provided with a float (*LL*) made from a disk of Polythene, which prevented disturbance of the top of the resin by the inflowing solution. In use, the level of solution above the top of the resin was kept as low as was consistent with the proper action of the float. Adjustment of the liquid level was carried out by adjusting the amount of air held at the top of each filtration tube by means of clips placed on the outlet

tubes to the air traps (*MM*). Further clips were supplied at the rubber connections (*NN*) in order to facilitate this operation.

The effluent from the columns passed through a flow regulator (*O*) which consisted of a capillary tube blown to a bulb at one end and provided with an outlet tube (*P*). Regulation of the flow was carried out by varying the depth to which the stainless steel wire (*Q*) was inserted into the capillary. From the flow regulator, the solution passed into the siphon of an automatic fraction collector. This was constructed as described by Brimley & Snow (1949) and consisted of a siphon (*R*) supported on a balance arm carrying a weight (*S*). When the siphon was half full, the balance tipped and operated electrical switches controlling the movement of the arm (*T*). The fraction collector accommodated fifty-six bottles each containing 250 ml. of solution, or a smaller number of 500 ml. bottles. These were arranged in spiral formation on the table (*U*) which was supported about 25 cm. above floor level.

Specification and pre-treatment of the resins

Zeo-Karb 215. The resin, as obtained from the makers (Permutit Co. Ltd., Gunnersbury Avenue, London, W. 4), was dried by spreading it on a sheet of paper in a hot room at 37°. It was then passed through a hammer mill, using a coarse screen, and the product graded by sieving in the dry condition. The sieved material was cleaned by soaking overnight in 5 N-HCl, followed by washing in a beaker with a jet of tap water to remove fine particles.

In packing the column, the filtration tube was first filled with water, and the resin transferred to it in small quantities as a suspension in water. As the particles of resin fell to the bottom of the column water was allowed to flow from the outlet tube below, in order to avoid the disturbance caused by the upward displacement of water by the falling resin. In this way the column was built up as a series of flat laminae, and no attempt was made to consolidate it by tamping or tapping. The column, once made, was not allowed to drain dry, and was always kept with a small depth of water above the resin. Before use, the column was treated two or three times alternately with 2 N-HCl and N-NH_3, and after finally regenerating with 2 N-HCl, was washed with distilled water until the reaction of the effluent reached pH 4.

Sulphonated cross-linked polystyrene. Since the degree of cross linkage of most commercial samples of sulphonated polystyrene is too high for maximum efficiency in the separation of organic bases and amino-acids, the sample of resin used was prepared in the laboratory according to the procedure given by Partridge, Brimley & Pepper (1950). The resin was a cream-coloured substance in the form of microbeads. Its capacity was 5·3 mg. equiv. NaOH/g. of dry H^+ resin. The nominal content of divinylbenzene was 4·5% and the water regain at saturation was 1·8 g. water/g. of dry H^+ resin. The procedure for cleaning the resin and packing the columns was the same as that described for Zeo-Karb 215.

Since completion of the work described here, a sample of lightly cross-linked polystyrene resin, which conformed closely to the above specification, has been received from Permutit Co. Ltd. (London), and this has been used to replace the laboratory-prepared material.

Dowex 2. The resin is a cross-linked polymer containing strongly basic quaternary groups, and was supplied as a cream-coloured material in the form of microbeads (R. W. Greeff and Co. Ltd., 12 Finsbury Circus, London, E.C. 2). In the chloride form the saturation regain was 0·83 g. water/g. dry resin and the capacity for HCl was 3·7 mg. equiv./g. dry resin. Before use the resin was regenerated with carbonate-free 2 N-NaOH until the effluent was free from chloride. (CO_2-free alkali was prepared by adding 10 g. $Ba(OH)_2.8H_2O$/l. of 2 N-NaOH, the solution being filtered immediately before use.) After regeneration the resin was washed with carbonate-free distilled water until the alkalinity of the effluent fell below pH 8. Columns were packed in the same way as described for Zeo-Karb 215, but before use the columns were treated alternately with 0·2 N-HCl and CO_2-free 2 N-NaOH in order to remove impurities and compact the resin bed. Carbonate-free solutions were used at all stages, since it was found that if any carbonate became absorbed on the resin, CO_2 was released by the acid used as displacement developer, and this seriously disturbed the chromatogram.

Although most of the experiments calling for a strongly basic resin have been carried out with Dowex 2, a few of the separations have been successfully repeated with a new product, Deacidite FF (Permutit Co. Ltd.). The capacity of this material was 2·0 mg. equiv. HCl/g. dry Cl^- resin and the water regain at saturation was 1·02 g. water/g. dry Cl^- resin.

EXPERIMENTAL PROCEDURES

In order to effect the complete separation of a complicated mixture of amino-acids and bases by a displacement method it is necessary first to carry out a primary fractionation in which the components are separated as a series of groups; the final separation of the components of the individual groups can then be carried out independently, using for preference a resin with different properties from that used for the group separation. For the primary fractionation the cation-exchange resin Zeo-Karb 215 has many advantages. It is inexpensive and the rate of exchange for large organic cations is very high; large columns can therefore be constructed, and they can be used with high rates of flow. The order of displacement of the amino-acids from Zeo-Karb 215 is also particularly fortunate in that it is markedly different from that given by the strongly basic resins (Partridge & Brimley, 1951*b*) and for this reason most of the amino-acids may be separated by use of the two resins only. However, Zeo-Karb 215 has the disadvantage that it is unsuitable for use at high pH. The resin is of the sulphonated phenol-formaldehyde type and contains phenolic hydroxyl groups. These, at high pH, slowly exchange cations, with the result that the resin shows some degree of irreversible adsorption towards strongly basic substances such as arginine and lysine. Consequently these substances are not quantitatively displaced by stronger bases (Partridge *et al.* 1950).

For this reason, where it is necessary to isolate the strong bases in a mixture, it is desirable to use a cation exchanger which does not contain phenolic hydroxyl groups, and in the present experiments a sulphonated polystyrene resin has been used. However, not all the primary column assembly need consist of polystyrene columns, since during the application of the mixture of amino-acids the strong bases will be concentrated in the top section only; if the top section contains a quantity of sulphonated polystyrene just sufficient to retain all the strongly basic substances, it may afterwards be disconnected from the remainder of the

assembly and developed independently. This procedure has been adopted for the large-scale fractionation described below. For work on a smaller scale it is usually more convenient to carry out the primary fractionation in two stages: in the first stage the whole of the mixture of amino-acids is fractionated on a column of sulphonated polystyrene for the purpose of isolating the basic amino-acids; in the second stage the fraction consisting of the neutral and acidic amino-acids is refractionated on a column of Zeo-Karb 215.

Fractionation of the hydrolysis products of commercial egg albumin

Hydrolysis of the protein. Commercial egg albumin (H_2O, 13·8%; ash, 3·4%; 280 g.) was hydrolysed for 40 hr. under reflux with 3 l. of 5·5 N-HCl. The bulk of the HCl was removed by repeated evaporation with water under reduced pressure and the residue, after addition of water (2 l.), was filtered to remove humin.

Treatment with charcoal. Since the aromatic amino-acids phenylalanine and tyrosine behave rather irregularly on columns of Zeo-Karb 215, it has been found convenient to remove them from the mixture at the outset. This was carried out by treating the slightly acid solution with prepared charcoal. The application of charcoal also served to decolorize the solution.

The charcoal (Activated Charcoal, British Drug Houses Ltd.; 520 g.) was first prepared by shaking for 1 hr. with 5% (v/v) aqueous acetic acid (8 l.) on a mechanical shaker. The mixture was then filtered and the charcoal washed with about 5 l. of distilled water. The protein hydrolysate was made up to 8 l. with distilled water, and the prepared charcoal, which was in the form of a wet filter cake, was transferred to it with stirring. The mixture was then shaken gently for 1 hr. and the charcoal removed by filtration, leaving a colourless filtrate. Finally the charcoal was thoroughly washed with water (8 l.) and the washings added to the filtrate.

Recovery of phenylalanine and tyrosine from the charcoal. The procedure adopted has already been described (Partridge, 1949 *a*). The yields of phenylalanine and tyrosine obtained are given in Table 2, together with the analyses of the crystallized products.

Primary fractionation. The column assembly used consisted of three filtration tubes, sizes I–III (Table 1), packed with Zeo-Karb 215 and coupled in series (Fig. 1). Above this was coupled a sulphonated polystyrene column containing 81 g. (dry wt.) of resin (60–100 mesh/in.; filtration tube, size III). The amount of polystyrene resin used for the first column section was calculated to retain the whole of the arginine, lysine and histidine from the protein, assuming these to be 5·7, 6·1 and 2·4% respectively.

The decolorized solution was diluted with distilled water to 40 l.; the pH was then 1·86 (glass electrode). The solution was passed through the column assembly at a rate of

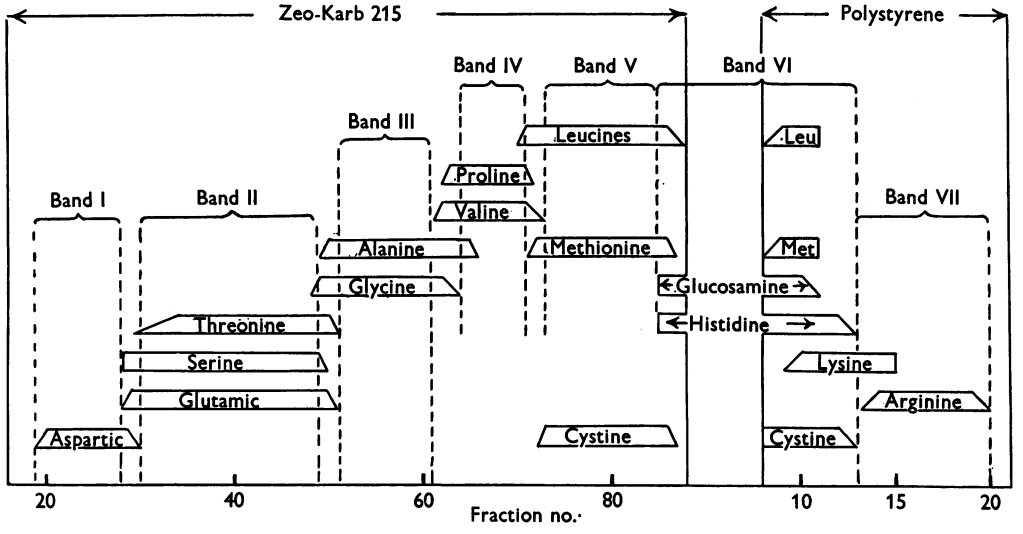


Fig. 2. Primary fractionation of the hydrolysis products of 280 g. of commercial egg albumin. The top section of the column was packed with sulphonated polystyrene and the lower sections with Zeo-Karb 215. The right-hand portion of the figure is the chromatogram resulting from independent displacement of the top section. For experimental details, see text.

40 ml./min., and the effluent solution ran to waste. The column was then washed with a little water and the top section, containing the sulphonated polystyrene resin, was disconnected. The remainder of the column was developed with 0·15 N-NH_3 (44 l.), the solution being passed at a rate of 20 ml./min. The effluent was collected in 250 ml. fractions and 1 drop from each fraction taken for qualitative paper chromatography (Consden, Gordon & Martin, 1944). The result of the qualitative analysis is shown in the left-hand part of Fig. 2.

The sulphonated polystyrene section of the column, which now contained the basic amino-acids, was set up as the top section of a new multiple column, the two lower sections of which were also packed with sulphonated polystyrene resin using filtration tubes IV and V. The amino-acids were then displaced through the two new column sections by applica-

tion of 0·075 N-NaOH as displacement developer; the developer (10 l.) was passed at the rate of 6 ml./min. and the effluent collected in 250 ml. fractions. Analysis of the fractions by paper chromatography gave the result shown in the right-hand part of Fig. 2.

The chromatographic data shown in Fig. 2 were used as the basis for grouping the fractions. The amino-acids fell into a series of mixed bands (numbered I–VII in the figure) which overlapped one another to the extent of two or three fractions. The grouping of the fractions adopted in this experiment is indicated by the dotted lines in the figure; the fractions in each band which lay between the dotted lines were collected together and stored for further fractionation, while those falling in the overlaps between contiguous bands were rejected.

Treatment of the mixed bands. Band VI. This band consisted of the final fractions (126–128) from the Zeo-Karb 215 column and the first fractions (19–23) from the polystyrene column. The combined fractions contained small quantities of leucine, methionine and cystine in addition to the main components, glucosamine, histidine and lysine, but the subsequent fractionation was designed to secure the isolation of the three last-named amino-acids only.

It had been shown previously (Partridge, 1949*a*) that by use of a dilute solution of NaCl as displacement developer the basic amino-acids, including histidine, may be separated sharply from the neutral amino-acids. Under the appropriate conditions, glucosamine and neutral amino-acids are displaced by the front due to Na^+, while histidine, lysine and arginine are retained on the column. The mixed fractions of band VI were accordingly passed through a column of sulphonated polystyrene resin in the hydrogen form (filtration tube III; wt. of resin, 81 g.) and NaCl solution (0·1 N, 5·5 l.) was used as displacement developer. The position of the band comprising glucosamine, leucine and methionine was determined by paper chromatography, and the fractions containing these substances were collected together and set aside for further treatment. The remainder of the fractions were rejected. The glucosamine-rich solution, which contained appreciable quantities of NaCl, was then passed through a column of Zeo-Karb 215 (filtration tubes V–VII) and the organic bases displaced in the usual way by means of 0·15 N-NH_3. The chromatogram showed two bands, the first containing leucine and methionine and the second pure glucosamine. The glucosamine was recovered as the crystalline hydrochloride, yield 1·2 g. The analysis is given in Table 2.

Table 2. *Yields and analyses of amino-acids isolated from commercial egg albumin and from yeast protein*

	Commercial egg albumin (280 g.)				Yeast protein (10 g.)	
	Yield		Nitrogen		Yield	
	(g.)	(% of dry protein)	% found	% calc.	(g.)	(% of dry protein)
Aspartic acid	19·1	8·3	10·6	10·5	0·55	6·4
Glutamic acid	22·0	9·5	9·6	9·5	0·67	7·8
Serine	5·7	2·5	13·4	13·3	0·15	1·7
Threonine	4·9	2·1	11·6	11·8	—	—
Threonine + serine	—	—	—	—	0·43	5·0
Alanine	7·9	3·4	15·7	15·7	0·37	4·3
Glycine	3·3	1·4	18·4	18·7	0·20	2·3
Valine	10·6	4·6	11·9	12·0	0·41	4·8
Proline	2·3	1·0	12·2	12·2	0·26	3·0
Methionine	3·5	1·5	9·5	9·4	—	—
Leucine + isoleucine	18·8	8·1	10·5	10·7	0·23	2·7
Histidine	2·7	1·2	25·6	27·1	0·08	0·9
Lysine	6·8	2·9	12·9*	12·8	0·85	10·0
Arginine	7·8	3·4	31·7	32·2	0·56	6·5
Phenylalanine	5·0	2·2	6·9†	7·0	0·22	2·6
Tyrosine	4·4	1·9	7·8	7·7	0·15	1·7
Glucosamine	1·2	0·5	6·4†	6·5	—	—
Total	126·0	54·2			5·13	59·7

* Analysed as dihydrochloride. † Analysed as hydrochloride.

The basic amino-acids that remained on the sulphonated polystyrene column were recovered by displacement with 0·1 N-NaOH. The fractions collected were free from leucine, methionine and glucosamine but contained a small amount of cystine. Part of the latter crystallized out on storing the solution and the crystals were removed by filtration. A further crop of cystine was then obtained by concentrating the solution under vacuum and allowing it to stand for 2–3 days at 0°. After filtration the solution was decolorized with a little charcoal and mixed with the fractions of band VII for recovery of histidine, lysine and arginine.

Band VII. The solution of basic amino-acids was passed through a column of sulphonated polystyrene resin (filtration tubes III–V) and the column developed with 0·1 N-NaOH. The chromatogram showed three sharply separated bands which contained histidine, lysine and arginine in that order. The fractions forming the narrow overlaps between the bands were rejected, and the pure amino-acids were recovered by crystallization (arginine as the free base, lysine as the dihydrochloride). The yields and analyses are given in Table 2.

Band I. This contained aspartic acid only. The solution was concentrated by vacuum evaporation and set aside to crystallize. The analysis and yield of amino-acid obtained are given in Table 2.

Band II. This contained glutamic acid, serine and threonine. The glutamic acid was separated and recovered by the

method already given (Partridge, 1949*a*). The solution that remained contained serine and threonine only, and these were recovered as a crystalline mixture by addition of warm ethanol to the concentrated solution (yield 21·3 g.). A portion of this mixture (4·9 g.) was taken for the final isolation of L-serine and L-threonine.

Since the isolation of these amino-acids by a displacement method has not been reported previously, the procedure is now given in detail. A survey of the chromatograms obtained with several different resins showed that the serine and threonine bands were in no case completely resolved in experiments at room temperature. Increase in temperature produced rather better resolution in some cases, but after a number of exploratory experiments the conclusion was reached that a separation based on the use of a strongly acidic resin with an aqueous organic solvent offered the best promise. A partial separation on these lines, using Zeo-Karb 215 with 50% acetone-water as the solvent, has already been reported (Partridge, 1949*a*). It was then found that substitution of sulphonated polystyrene resin for Zeo-Karb 215 effected an improvement; however, with short columns the degree of separation of the two bands was still unsatisfactory, and it appeared that even under the best conditions obtainable, the difference in the distribution coefficients of the two amino-acids was insufficient to result in the complete separation of bands of normal length using the standard column assembly.

In order to overcome this difficulty, a column with a higher theoretical-plate equivalent was adopted. This was constructed from routine equipment by coupling a fourth and larger filtration tube above the standard set of three. The new column assembly was operated with the load and rate of flow appropriate to the ordinary assembly consisting of the three lower sections only; thus, while the efficiency of separation was greatly increased, the time required for the separation experiment was increased by a factor of three.

Under these conditions useful separations were obtained, and both serine and threonine were recoverable in a pure condition. However, there was still much overlapping, and in order to ensure a reasonable yield of the two amino-acids it was necessary to carry out the separation in three stages. Using 0·1 N-NH_3 in 50% (v/v) aqueous acetone as displacement developer, a serine-rich and a threonine-rich fraction were first separated on the multiple column, the centre fraction being rejected: the two enriched fractions were then chromatographed independently using the same column and conditions. In this way, 1·3 g. of L-serine and 0·83 g. of L-threonine were obtained from 4·9 g. of the mixture, the yields of the two substances together representing 38% of the weight of mixture taken.

Isolation of L-*serine.* The acetone-water solution from the column experiment was slightly coloured. Acetone was removed by vacuum evaporation, and the solution was decolorized by addition of a little charcoal. After filtration, the solution was concentrated to small bulk and an equal volume of hot ethanol was added. L-Serine crystallized in small prisms, and the product was shown to be free from other amino-acids by paper chromatography. (Found: N, 13·4. Calc. for $C_3H_7O_3N$: 13·3%.) $[\alpha]_D^{21°} - 6·45° \pm 0·15°$ in water (*c*, 7·5; *l*, 2 dm.). The yield represented 1·9% of the dry wt. of protein taken.

Isolation of L-*threonine.* After removal of acetone, the solution containing threonine was heated to 70° and treated with a little charcoal. Paper chromatograms confirmed the absence of serine and other amino-acids. The solution was evaporated to the point at which a few crystals appeared and two volumes of hot ethanol were then added. The solution was set aside to crystallize and the product collected by filtration. (Found: N, 11·6. Calc. for $C_4H_9O_3N$: 11·8%.) $[\alpha]_D^{19°} - 28·1°$ in water (*c*, 5·1; *l*, 2 dm.). The yield represented 1·6% of the dry wt. of protein taken.

Band III. This consisted of a mixture of glycine and alanine and was uncontaminated with other amino-acids. The mixture was separated by use of a column of Dowex 2 following the procedure given by Partridge & Brimley (1951*b*). The yields and analyses are given in Table 2.

Band IV. The separation and recovery of proline and valine from this band was carried out by fractionating the mixture on a column of Zeo-Karb 215 at 55°, following the method already given (Partridge & Brimley, 1951*a*). The yields and analyses are given in Table 2.

Band V. Two different methods for the isolation of L-methionine and a mixture of the isomeric leucines from this band have already been described. The alternatives are (*a*) the use of a column of Zeo-Karb 215 at a temperature of 55° (Partridge & Brimley, 1951*a*) or (*b*) the use of a column of the strongly basic resin Dowex 2 (Partridge & Brimley, 1951*b*). In this experiment the former method was used since the cation-exchange resin was more readily available in large quantities. No attempt was made to separate the two isomeric leucines, which were obtained as a crystalline mixture free from other amino-acids. The yields and analyses are given Table 2.

Fractionation of the hydrolysis products of yeast protein

Preparation of the yeast protein. Since baker's yeast contains large amounts of carbohydrate it was considered advisable to attempt to extract the protein from the cells, rather than to subject the whole unfractionated material to hydrolysis. However, yeast cells are difficult to disintegrate by the usual procedures, and the results of a few exploratory experiments indicated that partial acid hydrolysis, followed by trichloroacetic acid precipitation of the extract so obtained, offered the simplest procedure for the preparation of the yeast protein in a state of sufficient purity. The procedure proved to be satisfactory for the purpose in mind, but the yield of protein obtained was low.

Wet-pressed baker's yeast was stored for 2–3 weeks at 0° and a portion (500 g.) was heated on a boiling-water bath with 2 l. of 0·2 N-HCl for 90 min. The cell debris was then separated by centrifugation at 3000 rev./min. It was found that when stored yeast was used the supernatant solution was clear, but with fresh yeast an opalescent suspension remained which was only partially cleared by filtration through a layer of Celite (Johns-Manville Co. Ltd., Artillery House, Artillery Row, London, S.W. 1).

The clear solution was mixed with 1 l. of 10% (w/v) trichloroacetic acid solution and the precipitated protein collected by centrifugation. This was washed several times with acetone and finally with dry ether. The residual ether was removed under reduced pressure at 60° and the product dried *in vacuo*. In this way the crude protein (10 g.) was obtained as a fine white powder. (Found: N, 14·1; moisture, 12·6; ash, 0·9%.) The product gave a positive Molisch test.

Hydrolysis of the protein. The crude yeast protein (10 g.) was hydrolysed with 5·5 N-HCl following the procedure

given for egg albumin. A two-dimensional chromatogram showed the presence of aspartic acid, glutamic acid, serine, threonine, proline, valine, glycine, alanine, leucine, histidine, lysine, arginine, tyrosine and phenylalanine, but cystine and methionine were present as traces only. The hydrolysis product was treated with prepared charcoal (17·2 g.) following the procedure described above. A paper chromatogram carried out with the colourless filtrate after charcoal treatment showed the absence of tyrosine and phenylalanine.

Bands VI–VIII. At this stage, bands VI–VIII were worked up for histidine, lysine and arginine respectively. The lysine and arginine bands required no further fractionation and the crystalline amino-acids were recovered by the methods already given. The histidine band contained, in addition, leucine, lysine and a small amount of cystine. The solution was passed through a column of Dowex 2 (filtration tubes V and VI) and the column was displaced with 0·075 N-HCl collecting approx. 4 ml. fractions. The first 10 fractions (fractions 40–49) contained lysine only, and these were

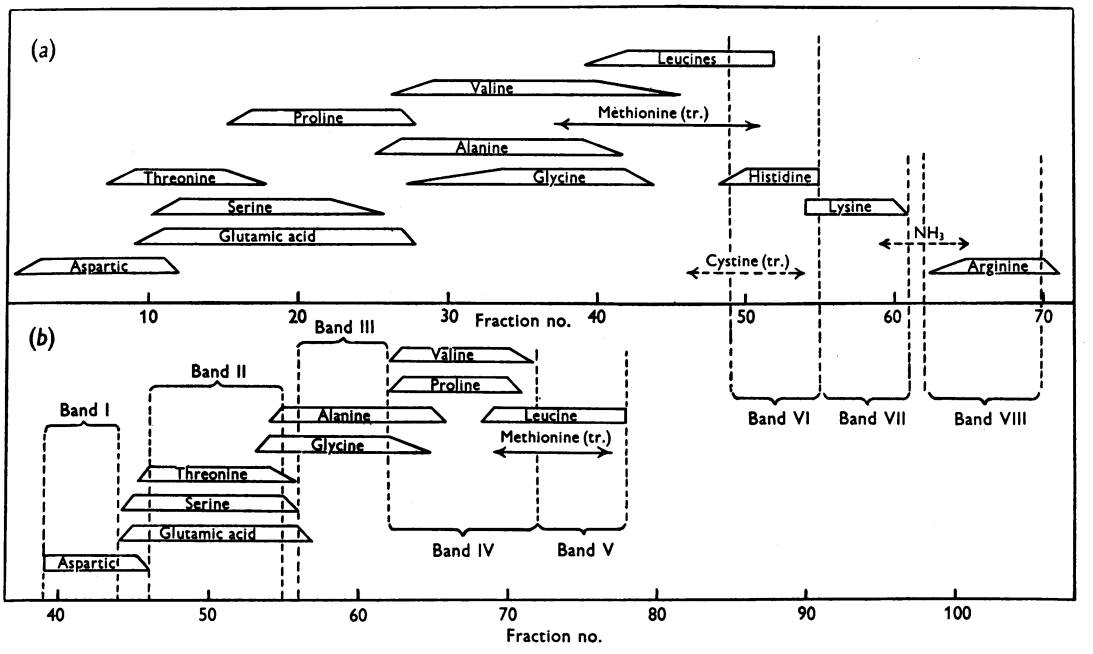


Fig. 3. Primary fractionation of the hydrolysis products of 10 g. of yeast protein. (*a*) Chromatogram prepared by displacing the mixture from a column of sulphonated polysytrene with 0·075 N-NaOH. (*b*) Fractions 1–49 from chromatogram (*a*) adsorbed on a column of Zeo-Karb 215 and displaced with 0·15 N-NH_3. For experimental details see text. tr. = trace.

Recovery of phenylalanine and tyrosine. Crystalline phenylalanine and tyrosine were recovered from the charcoal by elution with phenol-acetic acid mixture following the method given above; the yields are given in Table 2. A paper chromatogram carried out on the phenol-acetic acid eluate showed no amino-acids other than phenylalanine and tyrosine.

Primary fractionation: first stage. In this experiment the primary fractionation was carried out in two stages. In the first stage the charcoal-treated filtrate (1·5 l.) was passed through a column of sulphonated polystyrene resin (filtration tubes IV–VI) and the column developed with 0·075 N-NaOH. The fractions (15 ml.) were collected by use of a clockwork-driven device (Snow, 1951) which changed the receivers at fixed intervals of time (20 min.). The fractions were analysed by paper chromatography with the result shown in Fig. 3 (*a*). The chromatogram shows complete separation of arginine and lysine, and it is noteworthy that the ammonia band falls between the two amino-acids, thus improving the resolution by acting as a 'carrier' (cf. Tiselius, 1943; Tiselius & Hagdahl, 1950).

added to the fractions of band VII for lysine recovery. Fractions 50–61 contained lysine, leucine and a small amount of histidine, and were rejected. The histidine appeared in pure solution in fractions 62–65 and was followed by a further four fractions containing cystine and HCl. Crystalline histidine was recovered from fractions 62–65; the yields of the three basic amino-acids are given in Table 2.

Isolation of serine. Fig. 3 (*a*) shows considerable separation of serine from threonine, and advantage was taken of this to secure the isolation of part of the serine present in the mixture. Fractions 19–25 (Fig. 3 (*a*)) were passed through a column of Dowex 2 (filtration tubes V and VI) and displaced with 0·075 N-HCl. A clear separation of proline, serine and glutamic acid was obtained, the serine appearing in the centre fraction. The serine was crystallized from the pure solution and the mother liquors were combined with the fractions containing proline and glutamic acid for readdition to the main stock. The yield of serine was 0·15 g.

Primary fractionation: second stage. Fractions 2–49 (Fig. 3 (*a*)) were bulked together and passed through a

column of Zeo-Karb 215 (filtration tubes IV–VI). The displacement developer was 0·15 N-NH_3 and the chromatogram obtained (15 ml. fractions) was as shown in Fig. 3 (*b*). The chromatogram showed five sharp bands with very little dislocation of the individual amino-acids within the bands. The fractions were collected together as indicated by the dotted lines in the figure, and the overlapping portions of the bands were rejected. The treatment of the individual bands is summarized below, and the yields of the crystalline amino-acids isolated from each band are given in Table 2.

Band I. The aspartic acid contained in this band was free from other amino-acids and was isolated by crystallization.

Band II. The mixture of serine, threonine and glutamic acid was passed through a column of Dowex 2 (filtration tubes V and VI) and displaced with 0·075 N-HCl. The amino-acids separated sharply into three bands. The first contained the small amount of glycine and alanine present in the mixture and was rejected. The second band contained serine and threonine in pure solution, and the two amino-acids were recovered together as a crystalline mixture. The final band consisted of pure glutamic acid which was recovered by crystallization.

Band III. The mixture of glycine and alanine was separated on a column of Dowex 2 (filtration tubes V and VI) using 0·1 N-HCl as displacement developer. Separation was sharp and the alanine band appeared first. The glycine band was followed by a trace of glutamic acid, which was rejected. The two amino-acids were recovered from their pure solutions by crystallization.

Band IV. This consisted of fractions 63–73 (Fig. 3 (*b*)). The separation of the valine-proline band was insufficient to allow the recovery of the two amino-acids in good yield. Accordingly, the mixed fractions from the band were treated again with Zeo-Karb 215, using a column assembly of just sufficient size to accommodate the mixture (filtration tubes V–VII). Ammonia solution (0·15 N) was used as displacement developer, and the chromatogram obtained (8 ml. fractions) showed a sharp separation into three bands. The first band contained glycine and alanine (fractions 3–5) and the pure solution containing these amino-acids was returned to the stock from Band III. Fractions 6–9 represented the overlapping portion of the first and second bands, and were rejected. Fractions 10–20 contained the mixture of proline and valine free from other amino-acids, while fractions 21–23 contained leucine in addition to valine and were rejected.

The mixture of valine and proline was then passed through a column of Dowex 2 (filtration tubes V and VI) and displaced with 0·1 N-HCl. A sharp separation was obtained and valine and proline were recovered by crystallization.

Band V. This contained leucine, isoleucine and a small amount of methionine. The mixture was passed through a column of Dowex 2 (filtration tubes V and VI) and displaced with 0·1 N-HCl. The chromatogram showed a long band consisting of the isomeric leucines followed by a short band containing methionine and a trace of phenylalanine. The amount of methionine was too small for convenient recovery, and the solution was rejected. A crystalline mixture of the two isomeric leucines was recovered from the bulked fractions comprising the first band.

DISCUSSION

The two examples given in the experimental part of this paper were intended to illustrate the application of a systematic procedure suitable for the isolation of individual amino-acids and bases from the complicated mixtures occurring in protein hydrolysates, tissue extracts or secretory fluids. The procedure adopted in the two examples differed in detail, and it is not possible or desirable to lay down a completely finalized routine that could be applied inflexibly to all problems: such a routine would necessarily prevent full advantage being taken of the favourable circumstances of individual problems. Nevertheless, the second example represents a less empirical approach, and has so far proved to be a convenient procedure for all but very large-scale work. In this procedure three resins were used, and the separation was carried out in three main stages. (1) The amino-acid hydrochlorides were adsorbed on a column of sulphonated polystyrene resin and displaced with a solution of sodium hydroxide. This step was included for the specific purpose of isolating arginine, lysine and histidine; if these three amino-acids or other strong bases are absent, or their isolation is not desired, the stage may be omitted. (2) The fractions of the eluate from stage (1) containing the acidic and neutral amino-acids were combined, and the solution passed through a column of Zeo-Karb 215. The column was then displaced with dilute ammonia solution. This completed the 'primary separation' and resulted in a series of mixed bands which were very suitable in composition for the final separation by means of a strongly basic resin. (3) Each of the mixed bands from stage (2) was passed through a column of Dowex 2 and displaced with dilute hydrochloric acid.

The application of this procedure resulted in the isolation of all the amino-acids of the protein hydrolysate except two pairs which were not readily resolved. These were leucine-isoleucine and threonine-serine. The separation of the isomeric leucines was not attempted, but in view of the difficulty of obtaining pure L-serine and L-threonine by conventional methods, the separation of these two substances was studied in some detail. After a number of trials, the most convenient procedure found consisted in displacing the mixture from a column of sulphonated polystyrene resin, using sodium hydroxide dissolved in 50 % aqueous acetone as the displacement developer.

No example of the separation of amino-acids from biological extracts has been given in this paper, but an excellent procedure for the primary separation has been described by Westall (1950). Biological extracts usually contain materials, in part of high molecular weight, such as proteins, polysaccharides, tannins and anthocyanins, which must first be removed. The procedure adopted for deproteinization should not result in the addition of large amounts of inorganic salts to the extracts, and

for small-scale work dialysis, or the addition of ethanol to a concentration of about 80% (v/v), is suitable. Deproteinization may be followed by treatment with charcoal to remove phenolic substances, colouring matter and the aromatic amino-acids. On a large scale, the use of ethanol for deproteinization is inconvenient; the addition of a predetermined amount of basic lead acetate, following the procedure given by Westall (1950) is preferable.

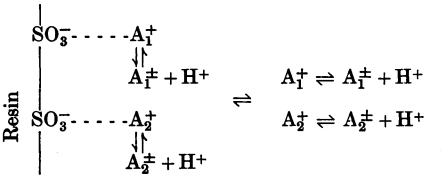
Once the components of high molecular weight have been removed from the mixture, the treatment applied may be substantially the same as that adopted for protein hydrolysates. The solution will usually contain inorganic salts, organic acids, sugars and other neutral substances in addition to amino-acids and bases; but on passing the mixture through a column of a strongly acidic resin in its hydrogen form, the inorganic cations remain at the top of the column while the organic and inorganic anions are not adsorbed and pass through as free acids together with the neutral substances. After washing with water, the column contains only cations and ampholytes, and since the inorganic cations do not interfere with displacement when sodium hydroxide or ammonia solution is used as developer, the isolation of the organic bases and amino-acids may proceed in the usual way.

It should be noted that the use of a cation-exchange column in this way affords a valuable procedure for desalting, and for the preliminary separation of organic extractives into groups based on their ionic charge (cf. Platt & Glock, 1943; Partridge, 1948). The first fraction of the effluent from the cation-exchange column contains the organic and inorganic acids and the sugars, and these groups may again be separated by passage through an anion-exchange column. It is often convenient to carry out a qualitative analysis on each of the groups separately by means of paper chromatography, and to follow this by the application of further column procedures for the isolation or quantitative determination of individual components.

Another important application of the procedure would lie in the preparation of amino-acids and bases labelled with ^{15}N, ^{13}C or ^{14}C. Proteins so labelled may readily be prepared biosynthetically by growing micro-organisms on synthetic media containing the required labels in inorganic form. The experiment with yeast protein was carried out as an exploratory step in this direction, and the example given demonstrates the practicability of isolating the amino-acids in good yield from amounts of crude protein of the order of 10 g. Yeast is not an ideal organism for work of this kind, since it contains a large amount of carbohydrate which is difficult to remove from the protein; it may be more convenient in future work to choose an organism containing a lower amount of carbohydrate, and to subject the whole organism to hydrolysis without prior isolation of a protein fraction. This procedure has been adopted with success in recent work carried out in this laboratory in connexion with the preparation of amino-acids labelled with ^{14}C.

The relationship between the ionic charge of a solute and its position in the order of displacement has been discussed by Davies (1949), and further discussion has since appeared in papers in this series. Extension of the experimental work to cover a larger range of solutes and several different resins has provided confirmatory evidence for the mechanism proposed by Davies and permitted its extension to the behaviour of anion- and cation-exchange resins with mixtures containing multivalent ampholytes.

Conditions during the separation of a mixture of monocarboxylic monoamino-acids using a strongly acidic cation-exchange resin may be illustrated by a scheme such as the following:



The resin adsorbs the amino-acid only in the form of the cation A^+ and this form is in reversible equilibrium with hydrogen ions and uncharged molecules in the surface layer. The system at the surface of the resin is again in equilibrium with the solution remote from it, but since the resin surface is charged, the concentration of hydrogen ions at the interface will be different from that in the ambient solution.

In the scheme given above, the displacement of the weaker base A_2 will depend primarily upon the partial suppression of the cationic form of A_2 by the presence of the stronger base A_1. However, differences in the affinity of the resin for the cations A_1^+ and A_2^+ will also be important and must be taken into account. This 'adsorption affinity' may be regarded as the sum of two factors: the electrostatic attraction of the two charged centres and the contribution of van der Waals forces. The electrostatic term contributes very largely to the total adsorption energy and may be modified by the valency of the ion, by hydration and by factors affecting the ion diameter. The van der Waals term, on the other hand, will increase with increasing molecular weight and will be very sensitive to structural factors such as the presence or absence of an aromatic ring.

A study of the order of displacement of a range of solutes confirms that the main factor in determining

Table 3. *Dissociation equilibria governing adsorption on cation- and anion-exchangers, given for a number of ion types*

	Cation exchange		Anion exchange	
Monocarboxylic monoamino	$A^+ \rightleftharpoons A^{+,-} + H^+$	pK_1	$A^{+,-} \rightleftharpoons A^- + H^+$	pK_2
Dicarboxylic monoamino	$A^+ \rightleftharpoons A^{+,-} + H^+$	pK_1	$A^{+,-} \rightleftharpoons A^{+,2-} + H^+$	pK_2
Monocarboxylic diamino	$A^{2+,-} \rightleftharpoons A^{+,-} + H^+$	pK_2	$A^{+,-} \rightleftharpoons A^- + H^+$	pK_3
Dicarboxylic diamino	$A^{2+,-} \rightleftharpoons A^{2+,2-} + H^+$	pK_2	$A^{2+,2-} \rightleftharpoons A^{+,2-} + H^+$	pK_3
Univalent base or acid	$BH^+ \rightleftharpoons B + H^+$	pK	$A \rightleftharpoons A^- + H^+$	pK
Bivalent base or acid	$BH^+ \rightleftharpoons B + H^+$	pK_2	$A \rightleftharpoons A^- + H^+$	pK_1

the order is the basic or acidic strengths of the solutes, and it has become the practice to regard any departures from this order based on pK values as 'anomalous'. An estimate of the expected position of a solute in the order of displacement is of considerable value in experimental work, and this may be obtained provided the dissociation constants of the solute are known. Table 3 lists the relevant equilibria and ionization constants governing the adsorption of a number of ion types by a strongly acidic and a strongly basic resin. The dissociation exponents given are the negative logarithms of the acid dissociation constant for each reaction.

In Table 4 the experimentally determined order of displacement of a range of ionic solutes from a strongly acidic cation-exchange resin and a strongly basic anion-exchange resin is given. The relevant pK values, taken from Cohn & Edsall (1943), are included for comparison. It will be observed that the order of displacement is substantially the order of pK values, but in several cases (those marked with an asterisk) a solute is more strongly retained than would be expected from its dissociation constant. The aromatic amino-acids show marked anomalous adsorption, and proline and methionine are also retarded on both types of resin. In Table 4 amino-acids which form mixed bands in the routine procedure are bracketed together, and it will be observed that, with the cation-exchange resin, the resolution of six discrete bands may be observed over the pK range 1·88–2·36. Since successful resolution may be obtained over the full range to about pK 10·6 it is clear that the displacement method is capable of resolving very complicated mixtures of bases, and that the separation of bases in the range pK 3–9 could proceed without interference from neutral or acidic amino-acids present in the mixture. A similar situation holds for the strongly basic resin Dowex 2, where separations in the range pK 8–3 have received little investigation.

Recently, Stein & Moore (1950) and Moore & Stein (1951) have described a very elegant procedure for the chromatographic fractionation of mixtures of amino-acids by elution analysis, using columns of a commercial sulphonated polystyrene (Dowex 50). This procedure is more suitable for

Table 4. *The sequence of displacement of a range of solutes from two resins at room temperature*

(The solutes are arranged in sequence of displacement from the resins. Relevant pK values are from Cohn & Edsall (1943). Values not falling in the sequence are marked with asterisks (see text).)

	Sulphonated cross-linked polystyrene (4·5% divinylbenzene)	
Aspartic acid	pK_1	1·88
Hydroxyproline	pK_1	1·92
Threonine	—	—
Serine	pK_1	2·21
Glutamic acid	pK_1	2·19
Proline	pK_1	1·99*
Glycine	pK_1	2·34
Alanine	pK_1	2·34
Valine	pK_1	2·32
Methionine	pK_1	2·28*
Leucine	pK_1	2·36
Cystine	pK_2	2·26*
Creatine	pK_1	approx. 3·0
Phenylalanine	pK_1	1·83*
β-Alanine	pK_1	3·60
Trimethylamine oxide	pK	approx. 4·5
Creatinine	pK	approx. 4·8
Histidine	pK_2	6·0
Methylhistidine	pK_2	6·48
Carnosine	pK_2	6·83
Anserine	pK_2	7·04
Hydroxylysine	—	—
Lysine	pK_2	8·95
Ammonia	pK	9·27
Arginine	pK_2	9·04
Methylamine	pK	10·64
	Dowex 2	
Lysine	pK_3	10·53
Proline	pK_2	10·60*
β-Alanine	pK_2	10·19
Alanine	pK_2	9·69
Valine	pK_2	9·62
Leucine	pK_2	9·60
Glycine	pK_2	9·60
Carnosine	pK_3	9·51
Threonine	—	—
Serine	pK_2	9·15
Histidine	pK_3	9·17*
Methionine	pK_2	9·21*
Methylhistidine	pK_3	8·85
Cystine	pK_3	7·85
Tyrosine	pK_2	9·11*
Acetic acid	pK	4·75
Glutamic acid	pK_2	4·25
Aspartic acid	pK_2	3·65
Hydrochloric acid	—	—

quantitative analysis than the displacement method, and it may also be used for isolation work on a small scale (cf. Ehrensvärd, Reio, Saluste & Stjernholm, 1951).

The formation of elution peaks depends upon the presence in the developing solution of cations capable of competing with the cationic form of the amino-acids. The competing cations are hydrogen ions in the case of development with acids, or sodium ions in the case of development with sodium buffers; a peak may thus be caused to travel more rapidly by increasing the ionic strength of the developing solution in either case. As would be expected from the theory of Davies (1949), acceleration of a peak may also be induced by increasing the pH of the developer, thus suppressing the cationic form of the amino-acid. When the solvent used for elution development is a buffer solution, the pH of the ambient fluid is predetermined, and in the procedure worked out by Moore & Stein the developing solvents consist of a range of buffers from pH 3·4 in the early stages to pH 11·0 in the final stage. In the displacement chromatogram, on the other hand, the pH of the ambient fluid is controlled only by the presence of the amino-acids themselves; here likewise it is found that during the fractionation of a range of amino-acids the pH of the first fractions (containing aspartic acid) is about 3·6; the pH then rises by a series of steps to about pH 10 in the region of lysine. It is not surprising therefore that the sequence of the elution peaks in the method of Moore & Stein is very similar to that obtained in displacement chromatography, and that both sequences suffer the same alterations and reversals when experimental conditions such as temperature are changed. This situation is a convenient one since it may prove that experience gained with one method may be applied to the other.

It is encouraging to observe that a very large range of ampholytes and bases may be separated by the use of synthetic resins of very simple structure. In the preparation of these resins no deliberate attempt has been made to introduce chemical configurations which are likely to confer specific adsorptive effects, and the fact that the application of both the elution and the displacement procedures has resulted in the separation of closely related homologues suggests that the methods are capable of far wider application. The majority of substances of physiological importance are water-soluble and ionic in character, and there appears to be every reason for the expectation that many other groups of substances of biological origin could be fractionated chromatographically with only minor modifications of the same procedures.

SUMMARY

1. A systematic procedure is described for the fractionation and isolation of amino-acids and bases using displacement chromatograms carried out with synthetic ion-exchange resins.

2. The procedure is illustrated by two model experiments. In the first example 16 amino-acids were isolated from 280 g. of commercial egg albumin with an overall recovery of 54 % of the dry weight of protein taken. In the second example 10 g. of a protein derived from yeast were hydrolysed and fractionated. Thirteen crystalline amino-acids were recovered, the total weight representing 60 % of the dry weight of the protein.

3. A new procedure for the isolation of threonine and serine is described.

4. The relationship between the dissociation constants of a solute and its position in the sequence of displacement is discussed.

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