a primary chromatogram and then resolved by a secondary chromatogram upon one or other of these adsorbents. Filter-paper chromatography, it may be recalled, does not differentiate between the members of this group (Nicholas & Rimington, 1949).

It is unfortunate that none of the systems we have studied has been capable of separating sharply the I and III series porphyrin isomers. It may be noticed, in particular, that we failed to separate uroporphyrin esters I (from bone) and III (Waldenström ester), whereas Fischer & Hofmann (1937) claimed to have done this by chromatographing uroporphyrin ester from Petry urine upon talc. They ascribed to uroporphyrin ester I a melting point of 311° (corr.) and to the series III isomer a melting point of 261° (uncorr.), but Grinstein *et al.* (1945) have cast serious doubt upon this claim. The material with m.p. 311° is thought by them to have been a uroporphyrin metal complex (probably copper) and the existence in nature of a uroporphyrin belonging to the aetioporphyrin III series is considered unproven. The Waldenström ester is thought by Grinstein *et al.* (1945) to be a mixture or molecular compound of uroporphyrin I with a heptacarboxylic porphyrin (probably of series III).

In a later paper the application of chromatographic methods to the isolation of new porphyrins from various sources will be described.

SUMMARY

1. The chromatographic behaviour has been studied of porphyrin esters under standardized conditions.

2. Magnesium oxide and magnesium carbonate possess certain advantages for porphyrin chromatography.

3. The naturally occurring dicarboxylic porphyrins may be separated chromatographically upon calcium carbonate or on magnesium oxide columns.

The author wishes to thank Prof. C. Rimington for his continued help and advice, the Medical Research Council for a personal grant, and Miss A. Benson for technical assistance. The Hartridge reversion spectroscope was purchased out of a grant from the Central Research Fund of London University.

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Displacement Chromatography on Synthetic Ion-exchange Resins

6. EFFECT OF TEMPERATURE ON THE ORDER OF DISPLACEMENT

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During the course of development of the work described in this series of papers it has become increasingly clear that non-ionic adsorption effects play an important part in determining the order of displacement of bases and ampholytes from columns of synthetic cation exchangers. In Part 4 of this series (Partridge, 1949*b*) the various factors then known to affect the order of displacement were discussed in some detail. Previously Davies (1949) had shown, from theoretical considerations, that an ideal cation-exchange process would be expected to result in the separation of a series of monoamino-acids in an order depending upon their pK_1 values; that is to say, upon the negative logarithms of the acid dissociation constants for the reaction: $A^+ \rightleftharpoons A^{\pm} + H^+$. For the purposes of illustration Davies assumed a

constant and low pH at the surface of the resin throughout the major part of the separation, but his arguments apply equally to the case where a mixture of monoamino-acids, free from mineral acid, is applied to a column packed with a strongly acidic cation-exchange resin in its hydrogen form.

As pointed out by Davies, the order of displacement from the sulphonated phenol-formaldehyde resin Zeo-Karb 215 at room temperature (Partridge, 1949*a*) does in fact reflect the order of pK_1: but there are certain exceptions. Thus, valine (pK_1, 2·32) and proline (pK_1, 1·99) form a mixed band and are displaced by a mixed band containing leucine (pK_1, 2·36) and methionine (pK_1, 2·28). Another exception concerns cystine (pK_1, 1·0; pK_2, 2·0), the behaviour of which is complicated by its capacity to form a bivalent cation. It seems probable that the high affinity of methionine for the resin is due to a strong contribution from van der Waals forces since methionine is adsorbed more strongly than leucine on charcoal (Tiselius, 1947).

Changes in the temperature at which a displacement experiment is carried out would be expected to modify the system in a complex way. Thus the dissociation constants both of the insoluble resin acid itself and of the ionizable solutes in the liquid phase would be altered. In addition, the van der Waals contributions and the degree of hydration of the resin and the solutes in the liquid phase would also be expected to change.

Measurements of pK_1 for various amino-acids over a temperature range up to 50° are available (Cohn & Edsall, 1943), and from these it appears that changes in temperature over this range do not greatly affect the relative values. It may thus be assumed that relative changes in the pK_1 values of the amino-acids due to increasing temperature are unlikely to be large enough to result in changes in the order of displacement.

The effect of hydration on the ion-exchange behaviour of a series of inorganic cations on natural zeolites has been discussed by Taylor & Urey (1938), who point out that any change in conditions affecting the solvation of the ions, such as change in the nature of the solvent or temperature, changes the behaviour of the ions. Similar considerations are applicable to the ion-exchange behaviour of organic ampholytes, and it may well be that the anomalous behaviour of certain of the amino-acids is due to the effects of hydration. Thus, proline is unique among the amino-acids in its high solubility in both water and ethanol, and its anomalous ion-exchange behaviour may be due to a high degree of solvation in aqueous solution.

These considerations have led us to attempt to resolve certain of the mixed bands reported in Part 3 of this series (Partridge, 1949*a*) by carrying out a further fractionation using the same resin but at a higher temperature. An increase in temperature would be expected to provide a situation in which the order of displacement of the amino-acids follows that suggested by Davies more closely, and should thus result in the separation of proline from valine and methionine from leucine.

EXPERIMENTAL AND RESULTS

Materials

The sulphonated-polystyrene resin used in these experiments was prepared in the laboratory following the procedure given by Partridge, Brimley & Pepper (1950). A lightly cross-linked product was used, the nominal divinylbenzene content of the mixture of monomers, before polymerization, being 4% (w/w). The spherical particles of resin were graded by sieving in the air-dry condition before use, and in the experiments described here the columns were packed with the 40–70 mesh/in. fraction.

Zeo-Karb 215, as received from the makers (Permutit Company, London), was ground in a hammer mill and graded by sieving in the air-dry condition. The sieved powder was allowed to stand overnight in 5N-HCl and was then thoroughly washed in an upward flow of water in order to remove fine particles. The columns were packed with the washed resin and treated alternately with N-NH_3 and 2N-HCl several times in order to complete the removal of impurities from the resin and to compact the bed.

Apparatus

The construction and packing of the columns was generally as given in Part 1 of this series (Partridge & Westall, 1949). However, since it has been shown (Hagdahl, 1948; Partridge, 1950) that considerable improvement in the sharpness of separation is obtained by use of a system of coupled columns of diminishing diameter, this device has been employed for both the experiments at room temperature and those carried out at higher temperatures.

For small-scale work, where columns 1·5 cm. or less in diameter were employed, it was found that steady temperatures up to 90° could be maintained by enclosing the whole column assembly in an air jacket. In the apparatus used, air was continuously circulated, by means of a rotary blower, through a closed system consisting of the column jacket and a small electric furnace. Temperature was controlled to $\pm 2°$ by regulating the output of the furnace by means of an external resistance.

The use of an air jacket in this way has the merit of simplicity, but is unsuitable for large-scale work involving large-diameter columns and high rates of liquid flow. For this purpose it is necessary to pre-heat the inflowing liquid as well as to maintain the temperature of the surfaces of the columns and connecting tubes. A water-jacketed column appeared to offer the simplest arrangement, and Fig. 1 shows a jacketed column constructed for operation at temperatures up to 60°. The column consisted of three sections (*A*, *B*, *C*) connected in series one above the other. The sections were of diameter 5, 3·3, 2·3 cm., and height 50, 22·5, 12·5 cm., respectively, and each was packed with resin to about 4 cm. from the open top. Each section was provided with a float (*D*, *E*, *F*), made from disks of 'polythene', in order to prevent disturbance of the top surface of the resin by the jet of liquid flowing into the

section. The jacket (*G*) was made from a length of industrial glass pipeline with flanged ends. Standard flanged joints were used at the top and bottom of the jacket in order to bolt on the metal faceplates (*H*, *I*). The jacket was made water-tight by employing thick rubber gaskets under both face-plates.

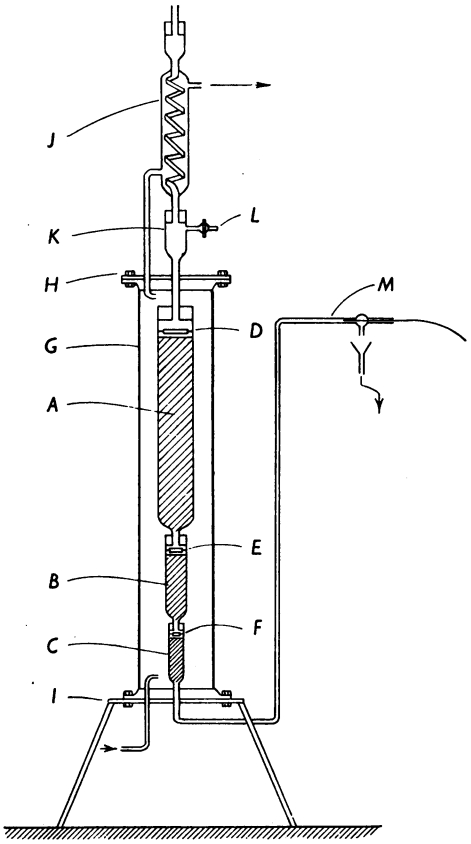

Fig. 1. Jacketed column assembly for use at high temperatures, showing set of three coupled columns and flow regulator. A description is given in the text.

Liquid entering the column passed first through a spiral condenser (*J*) which was heated by water from the jacket. The pre-heated solution passed to the column through the air trap (*K*), air being released from time to time by means of the tap (*L*). The rate of flow through the column was regulated by the valve (*M*) which consisted of a piece of stainless steel wire inserted into a closely fitting glass capillary carrying a bulb and side arm near the open end. It was found that the rate of flow could be adjusted accurately by varying the depth to which the wire was thrust into the capillary.

Displacement of a mixture of leucine and methionine from sulphonated-polystyrene resin at room temperature, 60 *and* 80°

In order to avoid disturbances due to the marked swelling and shrinkage of the sulphonated cross-linked polystyrene (cf. Partridge *et al.* 1950) the column was constructed in two sections, the lower section being very short in relation to its diameter. Wall effects are reduced as the height of the resin bed decreases, and the provision of a short independent section at the lower end of the column tends to correct any boundary disturbance arising in the long upper section.

Displacement at room temperature. The main column was 27 cm. high (packed in a glass tube of 11 mm. bore), while the lower section was 2·5 cm. high (bore 13·5 mm.). The weight of resin (40–70 mesh/in.) packed into the two filtration tubes was 8·5 g. (dry wt., sodium form). A solution of L-leucine (10 mmol.) and DL-methionine (10 mmol.) made up to 130 ml. with water was passed through the column at a rate of 60 ml./hr. The column was rinsed with a little distilled water (10–20 ml.). This was followed by 0·20 N-sodium hydroxide solution (200 ml.) passed at a rate of 40 ml./hr. Both the lower amino-acid boundary and the upper boundary due to sodium hydroxide could be followed by observing the slight colour change in the light-coloured resin. The effluent solution was collected in 5 ml. fractions, and a drop from each fraction applied to the base line of a filter-paper partition chromatogram using butanol-acetic acid as the solvent. Fig. 2*a* is a diagrammatic representation of the resulting chromatogram, and shows that no separation was observed under these conditions.

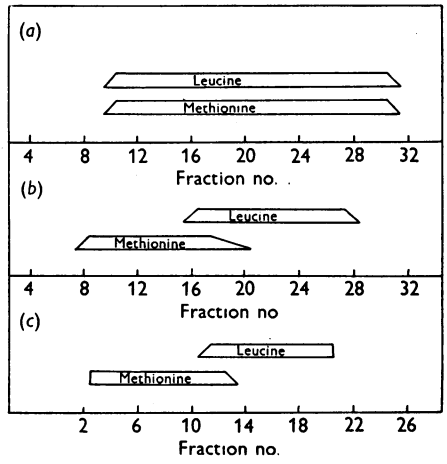

Fig. 2. Fractionation of a mixture of leucine and methionine by displacement from a column of sulphonated polystyrene (40–70 mesh/in.) with 0·2 N-NaOH. (*a*) Displacement at room temperature. (*b*) Displacement at 60°. (*c*) Displacement at 80°.

Separation of leucine and methionine at 60°. The column used in the experiment described above was regenerated with 2 N-hydrochloric acid and washed with distilled water. It was placed in an air jacket maintained at 60°. Fractions 9–31 from the previous experiment (Fig. 2*a*) containing the mixture of

leucine and methionine were mixed together and the solution (110 ml.) was heated to 100° for a few minutes in order to expel dissolved air. The solution was cooled to 60° and applied to the column at the same rate as before. The column, after washing with a little air-free distilled water, was displaced with 0·2N-sodium hydroxide which had previously been heated to 100° and cooled to 60°. The sodium hydroxide solution was applied at a rate of 40 ml./hr. and the effluent collected in 5 ml. fractions. The results of a paper chromatogram of the fractions (Fig. 2*b*) showed that a clear separation was obtained.

Separation of leucine and methionine at 80°. The experiment described above was repeated under identical conditions except for an increase in the temperature of the jacket to 80°. The chromatogram is shown in Fig. 2*c* and indicates a rather sharper separation than that obtained at 60°.

The series of experiments described above show that although leucine and methionine may be separated quite readily at 60° they have no tendency to separate at room temperature. It is known that the boundaries of the bands are reduced in width as temperature increases (Partridge, 1949*c*) and indeed the boundary width observed at 80° was less than at 60°. However, the effect of increasing temperature cannot be explained on this basis alone, since, as the next section shows, leucine and methionine form a mixed band which separates from valine at room temperature, while at higher temperatures valine and methionine form a mixed band which separates from leucine.

Separation of a mixture of valine, leucine and methionine at room temperature and at 60°

The column used was the same as that employed in the previous series of experiments. A solution (120 ml.) containing DL-valine (8 mmol.), L-leucine (8 mmol.) and DL-methionine (8 mmol.) was applied to the column and the mixture displaced with 0·2N-sodium hydroxide, the rate of flow being adjusted to 40 ml./hr. The effluent was collected in 5 ml. fractions. A paper chromatogram using butanol-acetic acid as the solvent was prepared as before, but since, in this solvent, the R_F value of methionine (0·47) lies close to that of valine (0·50), each spot, after application to the base line of the chromatogram, was treated with a drop of hydrogen peroxide solution in order to convert the methionine to its sulphone (Dent, 1948).

The result of the experiment at room temperature is shown in Fig. 3*a*. No separation was observed between leucine and methionine, but there was a partial separation of valine which tended to be displaced by the other two components of the mixture. The experiment was repeated under identical conditions but with a jacket temperature of 60°. The analysis of the effluent solution (Fig. 3*b*) showed that at 60° valine and methionine formed a mixed band and were displaced by leucine.

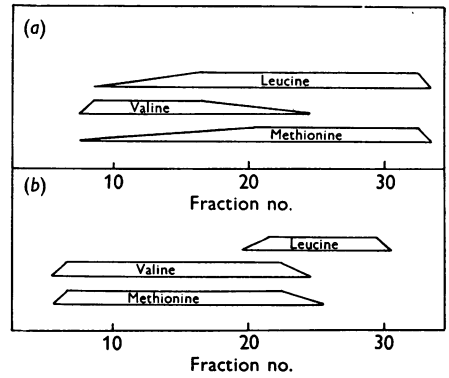

Fig. 3. Fractionation of a mixture of leucine, valine and methionine by displacement from a column of sulphonated polystyrene (40–70 mesh/in.) with 0·2N-NaOH. (*a*) Displacement at room temperature, (*b*) displacement at 60°.

Isolation of methionine and a mixture of the isomeric leucines from a protein hydrolysate

A number of the experiments described above were repeated using a commercial sulphonated phenol-formaldehyde resin, Zeo-Karb 215, in place of the laboratory-prepared sulphonated polystyrene. The results were essentially the same, and it proved more convenient to use the commercial resin for larger scale work.

The primary fractionation of the hydrolysis product of egg albumin has already been described (Partridge, 1949*a*, 1950). The starting material used in this experiment was the leucine-methionine fraction taken from a primary fractionation carried out with the hydrolysis product from 232 g. (dry wt.) of commercial egg albumin. The solution (1500 ml.) contained leucine, isoleucine, methionine and a little cystine and was approximately 0·10M with respect to the amino-acids. The column used was that illustrated in Fig. 1; a full description has already been given (p. 314).

The solution containing the mixture of amino-acids was filtered to remove a deposit of cystine; it was then brought to the boil and cooled to 60°. The jacket temperature of the column was adjusted to 56° and the solution passed through at a rate of 500 ml./hr. This was followed by about 50 ml. of distilled water to rinse the reservoir and connecting tubes. Ammonia solution (0·16N, 10 l.) was then passed to the column at 500 ml./hr., the effluent solution being collected in fractions of 45 ml. by means of an automatic apparatus. Fig. 4 shows the analysis of the fractions.

Recovery of L-*methionine.* Fractions 4 and 5 (Fig. 4) were mixed, concentrated to about 50 ml. and allowed to stand at

0° for 2 or 3 days. A small crop of cystine was removed, and the filtrate treated with washed charcoal until free from cystine. The filtrate was then added to fractions 1–3 (Fig. 4) containing methionine only, and the whole was concentrated to 90 ml. Hot ethanol (90 ml.) was added and the L-methionine crystallized in fine needles (2·44 g.). Two further crops were obtained by further evaporation and the addition of ethanol. Total yield, 3·46 g. (Found N, 9·5. Calc. for $C_5H_{11}O_2NS$: N, 9·4%); $[\alpha]_D^{20°}$ + 19·11° (*c*, 3·3 in 3 N-HCl; 2 dm.). Paper chromatograms carried out on the recovered methionine showed it to be free from other amino-acids. The recovery represented a yield of 1·5% calculated on the dry weight of protein taken. This figure is much lower (as judged by an analysis of pure ovalbumin; Chibnall, 1945) than can be accounted for by losses during the chromatographic procedure, and it is thought that serious loss may have

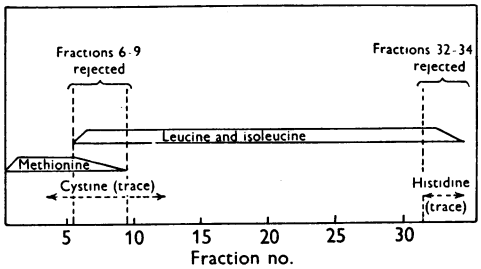

Fig. 4. Isolation of L-methionine and the isomeric leucines from the hydrolysis product of egg albumin. Displacement of band V (from the primary fractionation) by means of 0·16 N-NH_3 at 55°. Column packed with Zeo-Karb 215.

occurred during the hydrolysis of the impure protein. Commercial egg albumin contains a considerable amount of carbohydrate which may give rise to loss of methionine by side reactions of the type investigated by Lugg (1938; cf. Mohammad, Fraenkel-Conrat & Olcott, 1949).

Recovery of the mixture of leucine and isoleucine. Fractions 10–12 (Fig. 4) contained a little cystine in addition to leucine and isoleucine. They were mixed and concentrated by evaporation, when a small crop of cystine was deposited on standing. This was removed and the filtrate treated with successive small quantities of washed charcoal until a paper chromatogram showed the filtrate to be free from cystine. The filtrate was then added to the mixed solution from fractions 13–31 (Fig. 4), and the whole evaporated under reduced pressure to the crystallization point. An equal volume of hot ethanol was then added and the mixture of leucines allowed to crystallize. A second crop was obtained by further evaporation and the addition of ethanol. Total yield 18·8 g. (Found N, 10·7. Calc. for $C_6H_{13}O_2N$: N, 10·7%.) The yield represented a recovery of 8·2% calculated on the dry weight of protein taken; the mixture of leucines was shown, by paper chromatography, to be free from other amino-acids.

Isolation of L-*valine and* L-*proline from the hydrolysis products of a protein*

The primary separation of the products of hydrolysis of commercial egg albumin (dry wt. 64 g.) was carried out as previously described (Partridge, 1949*a*). The fractions containing valine and proline (band IV) were collected together so as to include smaller amounts of glycine and alanine from the overlapping boundary of band III together with a little leucine and isoleucine from band V. The solution was refractionated at room temperature, using a smaller column of Zeo-Karb 215 and following the details of procedure already given (Partridge, 1949*a*). The analysis of the effluent solution is shown in Fig. 5*a*. Fractions 9–23 (Fig. 5*a*) were mixed and

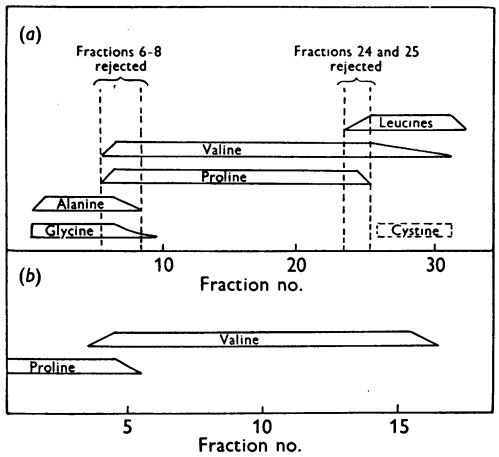

Fig. 5. Isolation of L-valine and L-proline from the hydrolysis product of egg albumin. (*a*) Displacement of band IV (from the primary fractionation) by means of 0·16 N-NH_3 from a column of Zeo-Karb 215 at room temperature. (*b*) Displacement of the mixture of valine and proline from experiment (*a*) (fractions 9–23) from a column of the same resin at 53°. Some of the proline was accidentally lost in this experiment.

the solution (after de-aeration by boiling) was refractionated on the same column at 60°, using 0·16 N-ammonia solution as the displacement developer. The resulting chromatogram (Fig. 5*b*) showed that, at 60°, valine was well separated from proline; some of the L-proline was accidentally lost during this experiment.

Recovery of L-*proline.* Fractions 1–3 (Fig. 5*b*) were collected and the solution evaporated to dryness under reduced pressure. The residue was dissolved in hot ethanol and the yellow solution decolorized with a little charcoal. Ether was added to the warm filtrate and the solution set aside to crystallize. Yield of L-proline, 0·66 g. (Found, N, 12·2. Calc. for $C_5H_9O_2N$: N, 12·2%) $[\alpha]_D^{20°}$ − 84·0° (*c*, 1·22 in water; 2 dm.). The recovery represented 1·03% dry weight of protein taken.

Recovery of L-*valine.* Fractions 6–15 (Fig. 5*b*) were collected and the solution evaporated under reduced pressure until a few crystals appeared. An equal volume of hot ethanol was then added and the L-valine crystallized immediately in large plates. A second crop of crystals was obtained by further evaporation with the addition of ethanol. Total yield

1·8 g. (Found N, 11·9. Calc. for $C_5H_{11}O_2N$, 12·0%); $[\alpha]_D^{20°}$ +27·6° in 6·07 N-HCl (*c*, 3·33; 2 dm.). The recovery represented 2·8% dry weight of protein taken.

Separation of proline and valine: effect of reversing the order of the steps

The preceding section describes the isolation of proline and valine from a protein hydrolysate in the following steps: (1) The primary separation (Zeo-Karb 215 at room temperature) gave the proline-valine band heavily overlapped with glycine, alanine and leucine. (2) Fractionation using a narrower column of Zeo-Karb 215 at room temperature allowed a band consisting of proline and valine only to be isolated. (3) The proline was separated from the valine by fractionation at 60°.

It appeared that there would be some advantage in reversing the order of steps (2) and (3), but it could not be assumed that such a reversal of order would necessarily give satisfactory results since it has been observed that the separation of two components is frequently modified by the presence or absence of a third component of similar affinity for the resin. The point was therefore investigated experimentally.

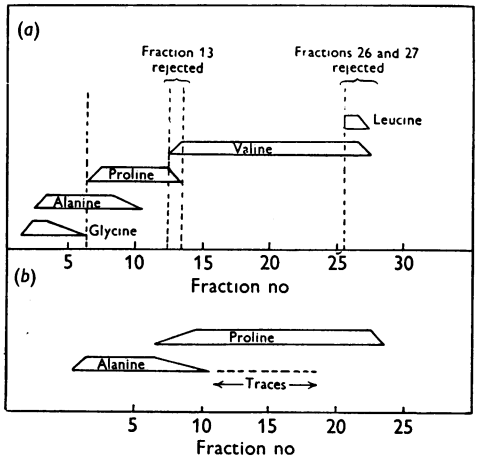

Fig. 6. Isolation of L-valine and L-proline from the hydrolysis product of egg albumin. (*a*) Displacement of band IV (from the primary fractionation) by means of 0·15 N-NH_3 from a column of Zeo-Karb 215 at 55°. (*b*) Displacement of a mixture of alanine and proline from experiment (*a*) (fractions 7–12) from a column of the same resin at room temperature.

The proline-valine band from a primary fractionation carried out with the hydrolysate from 232 g. (dry wt.) of commercial egg albumin was selected for the experiment. The solution was fractionated at 55° using 0·15 N-ammonia as the displacement developer. The analysis of the effluent solution is given in Fig. 6*a*. Fractions 14–25 (Fig. 6*a*), containing valine only, were collected and the valine recovered by crystallization. The yield of L-valine was 10·55 g.; N, 11·9%; $[\alpha]_D^{20°}$ +27·1° in 6·07 N-HCl (*c*, 3·43; 2 dm.). This represented a recovery of 4·54% calculated on the dry weight of protein taken, and the yield was thus much higher than that obtained in the previous experiment.

Fractions 7–12 (Fig. 6*a*), containing proline and alanine only, were collected and refractionated at room temperature by displacement from a column of the same resin. The analysis of the effluent (Fig. 6*b*) shows that although the bulk of the alanine separated from the proline as expected, the proline band was contaminated by traces of alanine which persisted almost throughout its length. Separations by displacement development are usually very sharp, and effects of this kind are rarely observed except in the case of cystine. However, the effect appeared to be real since when the proline and alanine solutions were mixed together and refractionated using a freshly packed column the result was identical.

It thus appears that proline and alanine may be separated quite readily at room temperature when in the presence of valine and glycine, but in the absence of these two amino-acids the separation is difficult. The effect is reminiscent of the formation of azeotropes in fractional distillation and a review of the separations, so far achieved by displacement from columns of synthetic ion exchangers, leads to the view that the formation of 'azeotropic mixtures' may to a large extent explain the sharpness of separation between mixed bands so frequently observed.

DISCUSSION

So far there have been few publications dealing with the effects of temperature on ion-exchange reactions. Nachod & Wood (1944) showed that, for a cation exchanger of the sulphonated coal type, there was little increase in the rate of exchange between calcium and hydrogen ions when the temperature was increased from 27 to 60°, although the reaction was slower at 0°. On the other hand, Ketelle & Boyd (1947), using sulphonated-polystyrene resins (Dowex 50), showed considerable improvement in the sharpness of separation between two rare earth elements on increasing the temperature of an experiment from 25 to 100°. As far as the resins that have been used here are concerned, some preliminary experiments carried out in this laboratory have shown that the boundary width given by sodium hydroxide solution on columns packed with a laboratory-prepared sulphonated-polystyrene resin (nominal divinylbenzene content, 4%) were reduced by approximately 40% as the temperature was raised from 17 to 36°. A similar reduction of the boundary width of ammonia fronts has been observed when columns packed with Zeo-Karb 215 were used at higher temperatures (Partridge, 1949*c*).

The undoubted effect of higher temperature in increasing the reaction rate of some ion-exchange resins may be employed usefully where the resins themselves are sufficiently stable to withstand decomposition, and increasing the temperature should result in a useful reduction in the time required to effect a given separation. In other cases, such as for instance the isolation of L-aspartic acid, L-glutamic acid or L-leucine, when the component has a low solubility in cold water, an increase in temperature may be desirable in order to allow the use of a displacement developer of higher concentration than would otherwise be possible. With aspartic acid, for instance, it is rarely possible to displace the amino-acid at a concentration greater than 0·1 M since it tends to crystallize out in the column. Higher concentrations of displacement developer could be used at higher temperatures, and this would avoid the removal of much water when it is required to recover the component by crystallization.

However, the results reported here show that there is a further effect of increasing the temperature, other than simply increasing the reaction rate. In some cases there is a qualitative alteration in displacement. Thus, Fig. 3*a* shows that leucine and methionine will together displace valine at room temperature, while at 60° (Fig. 3*b*), the valine forms a mixed band with methionine which is displaced by leucine. This shift in the position of methionine, together with a similar shift observed with proline, offers a ready means of isolating the four amino-acids, proline, valine, methionine and leucine, when present in mixed solution.

In general the position in the order of displacement taken up at higher temperature by an amino-acid approaches more closely the position expected from its dissociation constant for the process:

$$A^+ \rightleftharpoons H^+ + A^{\pm}.$$

Changes in position are not observed among the majority of the amino-acids for which the order of displacement is already qualitatively identical with the order of pK_1. Thus, when a mixture of mono-amino-acids from a protein hydrolysate (excluding the aromatic amino-acids) was displaced from a column of sulphonated-polystyrene resin at 60° the order observed was identical with that found at room temperature except for methionine and proline, which were both displaced more readily and appeared higher in the order. Various attempts to separate pairs of amino-acids of similar pK_1 value (such as glycine, pK 2·34 and alanine, pK 2·34) were as unsuccessful at 60 or 80° as at room temperature, in spite of the improvement in the efficiency of the column resulting from the increased temperature. It thus appears that increasing the temperature reduces the relative effect of adsorptive forces tending to increase the affinity of the amino-acid or its ion for the resin. The effect of such forces will change with changing environmental conditions and will be sensitive to temperature, both directly and indirectly, through changes in the state of hydration of the ions and the resin.

SUMMARY

1. Chromatographic displacement of mixtures of amino-acids from columns containing synthetic resinous cation exchangers gives rise to a number of mixed bands containing small groups of amino-acids which cannot be resolved by further treatment with cation-exchange resins at room temperature.

2. The effect of displacement at higher temperature has been investigated, and it is shown that this results in a reversal of the order of displacement affecting methionine and proline. Use is made of this in order to isolate L-proline, L-valine, L-methionine and a mixture of the isomeric leucines from a protein hydrolysate.

3. Two synthetic resinous cation exchangers have been investigated, a phenol-formaldehyde resin containing nuclear sulphonic acid groups (Zeo-Karb 215) and a sulphonated polystyrene resin (prepared in the laboratory). Similar results were obtained with each.

4. In discussing the mechanism it is shown that at higher temperature the order of displacement conforms more closely with that deduced from consideration of the pK_1 values of the amino-acids. Deviations from the ideal affecting methionine and proline at room temperature may be due, in large part, to the combined effects of hydration and van der Waals forces. As temperature increases, these deviations would be expected to diminish.

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The Synthesis and Secretion of Amylase by Pigeon Pancreas *in vitro*

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In this investigation a new approach has been made to the study of protein synthesis in animal tissues *in vitro*. An incorporation of labelled amino-acids into proteins has been shown by previous workers to occur in tissue homogenates and slices, and this has been regarded as evidence of protein synthesis *in vitro*. However, no net increase in proteins has so far been reported.* In planning the present experiments, it was thought that the chance of detecting a net increase of a protein would be good if digestive glands were used as the experimental material. These glands are equipped to synthesize enzymes, i.e. proteins, at rapid rates, and many of these enzymes can be quantitatively determined.

The first series of experiments on the formation of pepsin by gastric mucosa proved unsuccessful. The formation of amylase by pigeon pancreas slices was next studied, and this proved a suitable system. Under appropriate conditions the amylase activity increased by over 100% on aerobic incubation.

Part of this work has been communicated to the Biochemical Society (Hokin, 1950).

EXPERIMENTAL

Media. The following media were used: (1) the bicarbonate saline of Krebs & Henseleit (1932), gassed with either 5% CO_2 + 95% O_2 or 5% CO_2 + 95% N_2; (2) medium III (without the organic constituents) of Krebs (1950), gassed with O_2; (3) phosphate saline of Krebs & Eggleston (1940), gassed with O_2; (4) sheep serum (inactivated by heating at 60° for 2 hr.), gassed with 5% CO_2 + 95% O_2; the heat treatment destroyed the serum amylase activity. NaOH (0·2 ml. of a 10% (w/v) solution) was placed in the centre well when medium 2 or 3 was used. All media contained 0·2% (w/v) glucose. In some experiments either 'supplemented casein hydrolysate' or a mixture of amino-acids was added. The supplemented casein hydrolysate consisted of acid-hydrolysed casein prepared by the method of McIlwain & Hughes (1944) with 1·5 parts of L-tryptophan added per 100 parts of original casein. The amino-acid mixture was added to the media to give a concentration of 20 mg./100 ml. of L-arginine, L-aspartic acid, L-citrulline, L-cystine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-tryptophan, L-tyrosine and L-valine, and 40 mg./100 ml. of DL-serine and DL-threonine.

When the effects of secretory stimulants were studied, carbamylcholine, pilocarpine or acetylcholine with eserine were either placed in the main compartment of the manometer flask initially or tipped from the side arm after 30 min. incubation. The latter procedure was followed when the effect of secretory stimulants on tissue respiration was studied.

Preparation and incubation of pigeon pancreas. Pigeon pancreas has the advantage over the pancreas of many other species of being relatively homogeneous; it contains very little adipose tissue, and its consistency lends itself well to the slicing technique.

The following procedure was found to give good results and was adopted for the preparation of the tissue. Pigeons were provided with an abundant supply of 'pigeon corn'. About 45 min. to 1 hr. prior to killing 0·09–0·15 mg. of carbamylcholine was administered intramuscularly. Salivation usually began within 5 min., and prostration occasionally occurred. The birds were killed by decapitation, the abdomen was immediately plucked and a substernal incision was made. The duodenal loop, which contains the pancreas, was exteriorized and both lobes of the pancreas were dissected from the duodenum with a pair of sharp scissors. The pancreas and duodenum of pigeons receiving carbamylcholine were usually hyperaemic. Care was taken to avoid puncturing the duodenum in order to prevent contamination of the tissue by the duodenal contents.

The lobes were carefully freed of connective tissue, and slices approximately 0·5 mm. thick were made with the slicer of Stadie & Riggs (1944). The base of the slicer was chilled before use. Unless otherwise specified, the tissue was placed immediately after slicing in a covered crystallizing dish lying in chipped ice. Wetted filter paper was kept in the dish to maintain a high humidity. When sufficient slices had been prepared, they were weighed on a torsion balance and placed in conical manometer flasks (18–26 ml. volume) containing

* Since this paper was submitted it has come to the author's attention that Peters & Anfinsen (1950) have reported a net synthesis of albumin by chicken-liver slices.