concentration of aneurin, riboflavin, nicotinic acid and biotin in the tissues. Increase in the concentration of riboflavin in the liver increases the activity of the lactic and succinic enzymes (with the exception of lactic dehydrogenase of aneurin-deficient livers), while a decrease in the riboflavin content decreases their activity. Similar results have been obtained for lactic and succinic dehydrogenases of heart and kidney in riboflavin series and for succinic dehydrogenase in aneurin series; while the enzyme make up of these tissues has been shown to be unaffected in biotin series.

4. The oxidations of acetaldehyde and pyruvic acid appear to involve all the vitamins, aneurin, riboflavin and biotin, since withdrawal of any one of these appears to decrease the activities of their respective enzymes.

5. The results are interpreted as evidence of an interrelationship between certain vitamins of the B group.

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## REFERENCES

Axelrod, A. E. & Elvehjem, C. A. (1941). *J. biol. Chem.* **140**, 725.
Axelrod, A. E., Potter, V. R. & Elvehjem, C. A. (1942). *J. biol. Chem.* **142**, 85.
Axelrod, A. E., Sober, H. A. & Elvehjem, C. A. (1939). *Nature, Lond.*, **144**, 670.
Axelrod, A. E., Swingle, K. F. & Elvehjem, C. A. (1942). *J. biol. Chem.* **145**, 297.
Ball, E. G. (1938). *Science*, **88**, 131.
Ball, E. G. (1939). *J. biol. Chem.* **128**, 51.
Bhagvat, K. (1943). *Ind. J. med. Res.* **31**, 145.
Braendstrup, P. (1940). *Ugeskr. Læg.* **102**, 95.
Corran, H. S., Dewan, J. G., Gordon, A. H. & Green, D. E. (1939). *Biochem. J.* **33**, 1694.
Green, D. E. & Brosteaux, J. (1936). *Biochem. J.* **30**, 1489.
Klopp, C. T., Abels, J. C. & Rhoads, C. P. (1943). *Amer. J. med. Sci.* **205**, 852.
Lehmann, J. & Nielsen, H. E. (1939). *Nord. med. Ark.* **1**, 289.
Lipmann, F. (1939). *Nature, Lond.*, **143**, 436.
Lu, G. D. (1939). *Biochem. J.* **33**, 249.
McCollum, E. V. & Davis, M. (1913). *J. biol. Chem.* **15**, 167.
Pilgrim, F. G., Axelrod, A. E. & Elvehjem, C. A. (1942). *J. biol. Chem.* **145**, 237.
Pilgrim, F. J. & Elvehjem, C. A. (1944). *J. biol. Chem.* **156**, 257.
Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
Russell, R. A. & Nasset, E. S. (1941). *J. Nutrit.* **22**, 287.
Salvesen, O. (1940). *Nord. med. Ark.* **5**, 279.
Singher, H. O., Kensler, C. J., Levy, H., Poore, E., Rhoads, C. P. & Unna, K. (1944). *J. biol. Chem.* **154**, 69.
Subrahmanyan, V., Gordon, A. H. & Green, D. E. (1939). *Nature, Lond.*, **144**, 1016.
Supplee, G. C., Jensen, O. G., Bender, R. C. & Kahlenberg, O. J. (1942). *J. biol. Chem.* **144**, 79.
Sure, B. (1944). *J. Nutrit.* **27**, 447.
Sure, B. & Ford, Z. W. jun. (1942). *J. biol. Chem.* **146**, 241.
Sure, B. & Ford, Z. W. jun. (1943). *J. Nutrit.* **26**, 659.
Swaminathan, M. (1942*a*). *Ind. J. med. Res.* **30**, 45.
Swaminathan, M. (1942*b*). *Ind. J. med. Res.* **30**, 397.
Sydenstricker, V. P. (1941). *Ann. intern. Med.* **15**, 45.
Thunberg, T. (1920). *Skand. Arch. Physiol.* **40**, 11.
Unna, K. & Clark, J. D. (1942). *Amer. J. med. Sci.* **24**, 364.

# The Fractionation of Weak Electrolyte Mixtures by Ion-Exchange Resins

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Ion-exchange resins are becoming of increasing importance in the separation of mixtures of weak electrolytes, and the object of the present paper is to enumerate some general considerations which are of importance in the planning and interpretation of separation experiments.

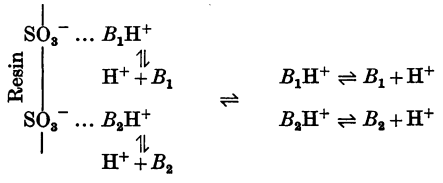
*Choice of resin.* Numerous experiments in these laboratories and elsewhere (see, for instance, Partridge* & Brimley, 1949) have established the fact that processes which involve the ionization of weakly acidic or basic groups in the resin structure are, comparatively, very slow; they also involve large swelling changes. The reverse processes are equally slow, probably because it is the surface of the granules that will be first attacked by a fresh reagent, and a reduction of charge and a desorption of water in the surface layers will hinder the diffusion of material to and from the interior of the gel. Procedures involving an alteration in the charge of the resin are therefore not well suited to chromatographic separations. For the types of resin in common use this means that cationic exchangers containing phenolic as well as sulphonic acid groupings should not be used at pH values greater than 8, and that the weakly acidic or weakly basic resins should if possible be employed

* The author is much indebted to Dr Partridge for an early view and discussion of his recent papers.

only for exchange processes in which the pH can be maintained at a suitable constant value.

Whatever the type of resin, separations will be the sharper the more nearly the processes at the resin surface approach to a state of equilibrium. Separations will, therefore, be improved by reducing the rate of flow and the grain size, and by working at elevated temperatures (within the range of stability of the resin) in jacketed columns.

*Separations through salt formation.* The separation of two weak bases $B_1$ and $B_2$ may be taken as a typical example. If these are applied, as such, to a column of sulphonic acid-type resin they will be quantitatively adsorbed: $R.SO_3^-...H^+ + B = R.SO_3^-...BH^+$, and subsequent development with a stronger base $B_3$ will give an effluent in which the bases should appear in the order of increasing basic dissociation constant. Conditions during the separation are illustrated in the following scheme:




The two cations initially adsorbed on the resin will be in reversible equilibrium with their dissociation products in the surface layer, and this system will be in equilibrium with the solution remote from the surface. On admitting the developing solution the new base will react with the hydrogen ions, and the cations previously held by the resin will be displaced; $B_1$ and $B_2$ will move down the column as uncharged molecules only to be adsorbed at fresh sulphonic acid groups and the whole process repeated. A separation will clearly require that the bases, in their movement down the column, are retarded by the resin to different extents. This retardation will depend on two factors, the strength of the attraction of the resin for the cation $BH^+$ and the dissociation constant of the base.

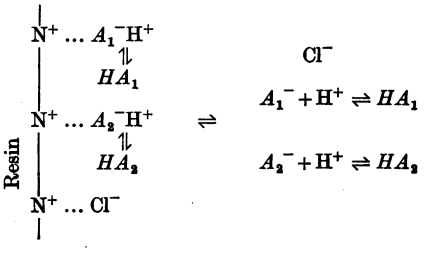
The first of these factors will be of primary importance if the cations differ in charge; bivalent ions, for instance, are much more strongly adsorbed than univalent ions of similar structure. If, on the other hand, the ions are of the same valency, and, assuming that the adsorption affinity is mainly electrostatic, this factor may be expected to be unimportant, and the separation will depend almost entirely upon a difference in dissociation constants. Quite a small difference is effective; complete separations have been achieved of constituents differing in pK by less than 0·2 unit (see the last section).

If the bases to be separated are of the same valency and of identical dissociation constant, a successful fractionation may still occur if differences are appreciable in the affinity of the resin for the various cations. The adsorption affinity may be regarded as made up of two parts, an electrostatic term and a 'van der Waals' term. The first of these makes an important contribution to the total adsorption energy, but one which is unlikely to show sufficiently large variations to be made the basis of separations. Variations in the 'van der Waals' term, on the other hand, may well be important, and information on this point can be gained from adsorption measurements using the uncharged electrolyte. The data so far available (Bhatnagar, Kapur & Bhatnagar, 1939; Thomas, unpublished) show that molecular adsorption on resins is often very large, that it is particularly marked with aromatic compounds, and that in a homologous series it increases as expected in molecular weight; the same rules should apply to the corresponding ions, which differ in chemical structure by only one proton. Separations believed to be based on this effect are quoted in the last section.

In the preceding paragraphs differences in pK and differences in adsorption affinity have been discussed separately. They will of course be superimposed, and in some cases the two factors may be of comparable importance. It should be added, also, that a very high adsorption affinity, such as is common with aromatic compounds, will in general have an adverse effect on separations. This is because the uncharged electrolyte travelling down the column will be adsorbed to a significant extent, and, as molecular adsorption and desorption are very slow processes compared with ion exchage, this will result in the highly adsorbed constituent being spread out on the column in the rear of its advancing band. It should be possible to minimize this effect by operating at elevated temperatures.

*Separations depending upon ion exchange.* With the advent of strong base resins the technique of displacement development discussed in the second section will become applicable to the separation of weak acids. There are separations, however, for which weak acid or weak base resins will be employed, and, as was pointed out in the first section, it will be best to restrict the use of these to exchange processes at a constant pH.

Let $HA_1$, $HA_2$ be two weak acids to be separated on a weak base resin. The resin will first be conditioned with, say, hydrochloric acid of the predetermined pH. On adding the acids conditions will be somewhat as shown below:



In a case where the adsorption affinities for $A_1^-$ and $A_2^-$ do not differ greatly, the separation of the acids will depend on a difference in the dissociation constants $K_{A_1}$ and $K_{A_2}$. If activity coefficients, which will be virtually equal for the two anions, are neglected, the proportion of the first acid which is present in the form of anion can be written as $\frac{K_A}{K_{A_1}+[H^+]}$, and similarly for the second. It can then be readily shown that these ionized fractions show their maximum difference when $pH^* = \frac{1}{2}(pK_{A_1}+pK_{A_2})$, and this condition gives the optimum pH for the separation. It must be noticed, however, that the argument applies to conditions at the surface of the resin, and here the ionization equilibrium is greatly modified by the surface charge. The effective pH to be used in the above equation will be given by $pH^* = pH + \frac{V}{0{\cdot}059}$, where the first term on the right-hand side is the pH of the solution remote from the resin surface, and $V$ is the potential difference in volts through which a hydrogen ion must be moved in bringing it from the remote solution up to the surface of the adsorbed anion. In general, the value of $V$ will be unknown, and even for the same resin it will vary with the pH and the ionic strength of the solutions employed. However, Hartley & Roe (1940), who have derived the above equation and applied it to a similar problem in measuring indicator shifts at the surface of a colloidal micelle, found values of $V$ varying over the range 50–100 mV.; and work in progress here on the electrokinetic potential at resin surfaces leads to similar values. It can be anticipated, therefore, that in working with a positively charged resin the best separation of two anions will be achieved when the pH of the solutions applied to the resin is 1–2 units lower than the value of $\frac{1}{2}(pK_{A_1}+pK_{A_2})$. In separating weak bases at a negatively charged resin the corresponding condition is that the pH of the solutions used should be 1–2 units higher than the value of $pK_w - \frac{1}{2}(pK_{B_1}+pK_{B_2})$, where $K_{B_1}$ and $K_{B_2}$ are the basic dissociation constants of the substances to be separated.

Interesting confirmation of these conclusions is provided by the work of Consden, Gordon & Martin (1948), who succeeded in separating glutamic from aspartic acid on Amberlite IR 4. These authors point out that the best separation would be expected at pH 4, but find that actually the solutions must be applied to the resin at pH 2·5, a value at which the acids carry a small net positive charge.

*Separation of amino-acids.* Partridge (1949) has had striking success in the separation of amino-acids on a resin column. The results appear to be in excellent agreement with the considerations advanced in the second section of this paper, and to throw some light on the relative importance of the factors discussed there. Partridge applied the amino-acid mixture as hydrochlorides to a column of sulphonic acid resin. The hydrogen-ion concentration at the resin surface was therefore high, and the monoamino-acids were present largely in the form of univalent cations, whilst the diamino-acids, including cystine, were present partly as bivalent cations, and consequently were much more firmly held at the resin surface. On displacement development with ammonia, fractions were collected in the order shown below.

(The figures given after the names of the acids are the negative logarithms of the acid dissociation constants for the reaction: $A^+ \rightleftharpoons A^\pm + H^+$, taken from Cohn & Edsall (1943); that is to say, they are the $pK_1$ values for the monoamino-acids and the $pK_2$ values for the diamino-acids; these dissociation constants are primarily responsible for controlling the movement of acid molecules down the column. The pK of threonine, which is not listed, may be presumed to lie close to that of serine.)

Fraction

I Aspartic acid, 1·88
II Glutamic acid, 2·19; serine, 2·21; threonine
III Glycine, 2·34; alanine, 2·34
IV Valine, 2·32; proline, 1·99
V Leucine, 2·36; isoleucine, 2·36; methionine, 2·28; cystine: $pK_1 = 1{\cdot}0$, $pK_2 = 2{\cdot}0$
VI Histidine, $pK_2 = 6{\cdot}00$
VII Lysine, $pK_2 = 8{\cdot}95$
VIII Arginine, $pK_2 = 9{\cdot}04$ (this is not displaced by dilute ammonia)

The monoamino-acids come through first, followed by the diamino-acids, and with two exceptions the order throughout is that to be anticipated from the pK values. Proline falls out of place, being more firmly held on the resin than would be expected from its pK value, but in view of its cyclic structure it is reasonable to attribute this anomaly to a specially large contribution from the 'van der Waals' adsorption factor. The other exception is cystine, where the low $pK_2$ value (which by itself would lead to the appearance of cystine in the earliest fractions) is offset by the existence of a considerable portion of the acid at low pH values in the form of bivalent cations.

Some numerical values may serve to illustrate the argument. Partridge (1949) found that the early fractions emerged from the column at pH of about 3, and the effective pH at the resin surface during a large part of the separation may be taken as being round about 1–2. In a mixture of amino-acids at pH 1·5, only 70 % of the aspartic acid will be in the form of cations; the corresponding figures for serine and leucine are 83 and 88 % respectively. Cystine will be 20 % un-ionized and only 80 % in the form of cations, but a quarter of these latter will be carrying two positive charges, and will require to lose two protons before being displaced from the resin

surface; the net rate of movement down the column may therefore be expected to be comparable with that of leucine. Finally, histidine and the other diamino-acids will be completely ionized and firmly held by the resin at this pH.

Fractions III, IV and V consist of acids whose pK values are so close that a separation depending on this factor would not be expected, and according to the argument given in the second section we should look for an explanation in terms of adsorption affinity. The molecular weights of the acids concerned are:

| Fraction | |
|---|---|
| III | Glycine, 75; alanine, 89 |
| IV | Valine, 117 |
| V | Leucine, isoleucine, 131; methionine, 149 |

The results do, therefore, seem to provide real evidence that, in the absence of significant pK differences, separations based on quite small differences in chain length and adsorption affinity can be carried out on ion-exchange columns.

Partridge (1949) found that tyrosine and phenylalanine showed anomalous behaviour, and had to be removed in a preliminary operation. This is in keeping with the strong adsorption of all aromatic compounds, a factor which seems at present to set the most important limitation to the use of ion-exchange resins.

SUMMARY

The theory of the separation of weak electrolytes by ion-exchange resins is discussed and illustrated by reference to recent work with the amino-acids.

REFERENCES

Bhatnagar, S. S., Kapur, A. N. & Bhatnagar, N. S. (1939). *J. Indian chem. Soc.* **16**, 249, 261.

Cohn, E. J. & Edsall, J. T. (1943). *Proteins, Amino Acids and Peptides.* New York: Reinhold.

Consden, R., Gordon, A. H. & Martin, A. J. P. (1948). *Biochem. J.* **42**, 443.

Hartley, G. S. & Roe, J. W. (1940). *Trans. Faraday Soc.* **36**, 101.

Partridge, S. M. (1949). *Biochem. J.* **44**, 521.

Partridge, S. M. & Brimley, R. C. (1949). *Biochem. J.* **44**, 513.

# Direct Transformation of Fumarate to Oxaloacetate, without Intermediate Formation of Malate, by *Clostridium saccharobutyricum*, Strain GR 4

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Annau, Banga, Göszy, Huszák, Laki, Straub & Szent-Györgyi (1935), on the basis of differences between fumarate and malate oxidation, thought that fumarate might be oxidized without intermediate formation of malate. Later, Laki (1936) abandoned this view. However, Kalckar (1939) showed that kidney extracts can dehydrogenate an addition product of fumarate and phosphate with formation of phosphoenoloxaloacetate. Lipmann (1941) assumed that the earlier view of Annau *et al.* (1935) was in part correct, and that the differences between the oxidation of the two substrates could probably be explained by the co-existence of two different paths of fumarate breakdown, the first occurring via malate, the second leading directly to oxaloacetate through a phosphorylated derivative of fumarate. This hypothesis is supported by the observation of Lipmann (1942) that, in a similar manner, the transformation of crotonate to acetoacetate occurred in rabbit kidney without intermediate formation of β-hydroxybutyrate.

Clifton (1942), studying the fermentation of $C_4$-dicarboxylic acids by washed suspensions of *Clostridium tetani*, found that fumarate is partly fermented to butyrate and acetate and partly to lactate, malate and ethanol. Malate and succinate were not fermented. Pickett (1943), with the same organism, showed that if incubation is continued for 4 days, malate is slowly fermented with production of acetate and butyrate, ethanol, carbon dioxide and succinate. The latter was not fermented. Cohen, Nisman & Cohen-Bazire (1948) have shown that citrate and α-ketoglutarate are slowly fermented to butyrate and acetate by strain GR 4 of *Cl. saccharobutyricum*. It is very unlikely that these two compounds are broken down via the $C_4$-dicarboxylic acids system, as it will be shown that succinate is not fermented by this organism.