PRO LABORATORIO

Group Separation of Amino Acids by Adsorption Analysis

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After the pioneer work of E. FISCHER[1] and H. D. DAKIN[2] on methods of protein analysis, little advance was made during the years 1920–1940, when electrodialysis[3] was really the only method to be developed. During the current decade, however, several methods embodying new principles have been described. The most important of these are the microbiological assay[4], the application of the principles of isotope dilution[5] and of solubility product[6], and adsorption analysis[7]. Of these, the first two require special biological or technical equipment, and as adsorption has been intensively studied in this Institute, we decided to explore the latter method[8].

The first article dealing especially with the conditions for the adsorption of amino acids[9] was published at the time that DAKIN'S method of analysis became known, but several years elapsed before the results were applied to protein analysis. Around 1940, however, several series of articles began to appear, some in Germany and some in England and America. The German investigators[10] have merely developed the classical method of TSWETT, but the others[11] have also evolved and applied quite new methods in which adsorption has been replaced by partition between two liquids phases. Another important recent advance is the replacement of some of the usual adsorbents by ion exchange resins[12]. As no attempts will be made in this paper to enumerate all the papers published in the field, the reader is referred to some recent reviews and monographs[1].

The procedure we first adopted was that described by SCHRAMM and PRIMOSIGH[2], but we soon found that their method could be improved in several particulars; first by the use of better adsorbents, and secondly by avoiding many of the evaporations, which are both time-consuming and liable to introduce errors. The evaporations are now reduced by running some of the adsorptions in series, i. e. as in the separation of leaf pigments[3], but in a quantitative manner.

The method is based on the following considerations[4]:

(1) Only the aromatic amino acids are adsorbed on active charcoal[5].
(2) From the filtrate, the basic amino acids are selectively adsorbed on Wofatit C[6].
(3) The remaining amino acids are all adsorbed on Wofatit KS[7].
(4) Of the amino acids in group 3, only the acid ones are retained by Amberlite IR-4[8].

By "aromatic" amino acids are meant those containing a benzene ring, i. e. phenylalanine, tyrosine and tryptophane; by "basic", those with an isoelectric point above 7, i. e. arginine, lysine and histidine; by "acid", those with an isoelectric point under 5, i.e. aspartic and glutamic acids; and by "neutral", those with the isoelectric point at about 6, i. e. those with one carboxyl and one amino group, excepting the aromatic ones, but including proline and hydroxyproline.

Method

In the following description of our analytical procedure, we refer to fig. 1 with accompanying explanations. For convenience it is assumed that a total of about 50 mg of amino acids is to be analyzed.

[1] E. FISCHER, Z. physiol. Chem. *33*, 151 (1901).
[2] H. D. DAKIN, Bioch. J. *12*, 290 (1918).
[3] G. L. FOSTER, C. L. A. SCHMIDT, J. biol. Chem. *56*, 545 (1923). – A. A. ALBANESE, J. biol. Chem. *134*, 467 (1940). – H. THEORELL, Å. ÅKESSON, Ark. Kem. Min. Geol. *16A*, No. 8, 1 (1942). – A. H. GORDON, A. J. P. MARTIN, R. L. M. SYNGE, Bioch. J. *35*, 1369 (1941).
[4] Many references by: E. E. SNELL, Adv. in Protein Chem. *2*, 85 (1945).
[5] D. RITTENBERG, G. L. FOSTER, J. biol. Chem. *133*, 737 (1940).
[6] M. BERGMANN, W. H. STEIN, J. biol. Chem. *128*, 217 (1939).
[7] See notes 10, 11, 12 below, and 1 on the following column.
[8] Preliminary results by: A. TISELIUS, Ark. Kem. Min. Geol. *15B*, No. 6, 1 (1941). – A. TISELIUS, The Svedberg 1884–1944, Almkvist och Wiksell, Uppsala 1944, page 370.
[9] E. ABDERHALDEN, A. FODOR, Fermentforsch. *2*, 74 (1919).
[10] K. FELIX, A. LANG, Z. physiol. Chem. *182*, 125 (1929). – K. FREUDENBERG, H. WALCH, H. MOLTER, Naturwiss. *30*, 87 (1942). – W. KOSCHARA, Z. physiol. Chem. *280*, 55 (1944). – G. SCHRAMM, J. PRIMOSIGH, Ber. dtsch. chem. Ges. *76*, 373 (1943); *77*, 417 (1944). – F. TURBA *et al.*, B. *74*, 1829 (1941); *75*, 340 (1942); Naturwiss. *31*, 508 (1943). – T. WIELAND *et al.*, Z. physiol. Chem. *273*, 24 (1942); Naturwiss. *30*, 374 (1942); B. *76*, 823 (1943).
[11] A. J. P. MARTIN, R. L. M. SYNGE, Bioch. J. *35:2*, 1358 (1941). – R. J. BLOCK, J. exper. Biol. Med. *51*, 252 (1942). – R. K. CANNAN, J. biol. Chem. *152*, 401 (1944). – D. T. ENGLIS, H. A. FIESS, Ind. Eng. Chem. *36*, 604 (1944). – H. A. GORDON, A. J. P. MARTIN, R. L. M. SYNGE, Bioch. J. *38*, 65 (1944). – C. S. CLEAVER, R. A. HARDY, H. G. CASSIDY, J. amer. chem. Soc. *67*, 1343 (1945).
[12] R. J. MYERS, J. W. EASTES, F. J. MYERS, Ind. Eng. Chem. *33*, 697 (1941). – R. J. BLOCK, J. exper. Biol. Med. *51*, 252 (1942). – R. K. CANNAN, J. biol. Chem. *152*, 401 (1944).

[1] T. WIELAND, Die Chemie *56*, 213 (1943). – A. J. P. MARTIN, R. L. M. SYNGE, Adv. in Protein Chem. *2*, 1 (1945). – S. CLAESSON (Diss.), Ark. Kem. Min. Geol. *23A*, No. 1, 1 (1946). – A. TISELIUS, Adv. in Protein Chem. *3* (1946), in press.
[2] G. SCHRAMM, J. PRIMOSIGH, Ber. dtsch. chem. Ges. *77*, 417 (1944).
[3] L. GATTERMANN, Die Praxis d. org. Chemikers, 24th ed., page 402, Berlin 1936.
[4] The behaviour of cysteine and cystine is somewhat doubtful, but the matter is still being studied.
[5] G. SCHRAMM, J. PRIMOSIGH, Ber. dtsch. chem. Ges. *76*, 373 (1943). – See also note 8 on the preceding column.
[6] T. WIELAND, Ber. dtsch. chem. Ges. *77*, 539 (1944).
[7] K. FREUDENBERG, H. WALCH, H. MOLTER, Naturwiss. *30*, 87 (1942).
[8] R. K. CANNAN, J. biol. Chem. *152*, 401 (1944). – C. S. CLEAVER, R. HARDY, H. G. CASSIDY, J. amer. chem. Soc. *67*, 1343 (1945).

First three filters containing charcoal, Wofatit C and Wofatit KS respectively are connected in such a manner that the eluate from the first filter must flow through the second, and the eluate from the second through the third. The amino acid solution in about 10 ml 5% acetic acid is forced through the filters, beginning with the top filter. To make sure that all acids other than the aromatic ones have been washed from the charcoal, an additional 50 ml 5% acetic acid are forced through[1] (fig. 1; op. 1a).

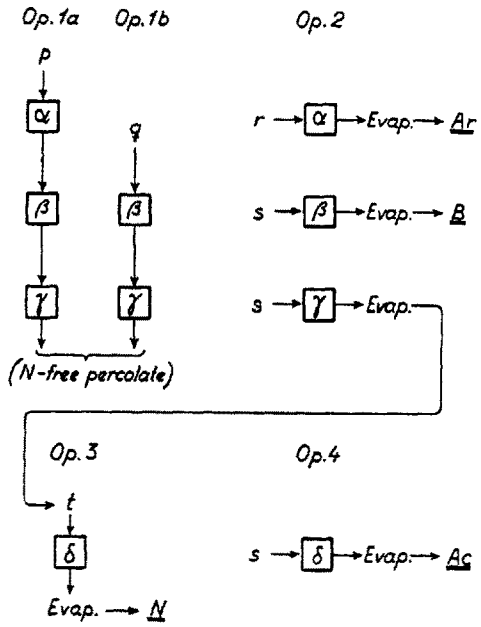

Fig. 1. *Diagram of the analysis procedure.*
The different filters are indicated by squares, imagined in five phases of the analysis.
α = Carbo activatus, β = Wofatit C, γ = Wofatit KS, δ = Amberlite IR-4. *Op.* = operation No. *Evap.* = evaporation.
The different groups of amino acids have been denoted by: *Ar* = aromatic, *B* = basic, *Ac* = acid, *N* = neutral. – The small letters denote the eluents: *p* = 5% acetic acid, *q* = 20% acetic acid, *r* = 5% phenol in 20% acetic acid, *s* = N HCl, *t* = distilled water.

At this stage the top filter, which contains only aromatic amino acids, is removed and through the remaining two filters is passed a quantity of 20% acetic acid. The purpose of this is to wash the Wofatit C free from neutral and acid amino acids, among which the leucines are most strongly retarded (fig. 1; op. 1b). The expression "retarded" is used here to distinguish the behaviour of these amino acids from that of the basic ones, which must be retained. It can be mentioned here, that the solution to be analyzed may have a content of HCl not exceeding N/100. With higher concentrations the histidine is liable to pass the Wofatit C filter.

[1] T. WIELAND, Die Chemie *56*, 213 (1943). – A. J. P. MARTIN, R. L. M. SYNGE, Adv. in Protein Chem. *2*, 1 (1945). – S. CLAESSON (Diss.); Ark. Kem. Min. Geol. *23A*, No. 1, 1 (1946). – A. TISELIUS, Adv. in Protein Chem. *3* (1946), in press.

The quantity of solution used must be determined by the adsorption capacity of the actual sample of Wofatit C. Thus in the case of a particular preparation which has been used and regenerated many times, we have found that 1 liter 20% acetic acid will elute leucine quantitatively without eluting any histidine. But in the case of the same sample that had been used only a few times, is was possible to elute the leucine by the use of much smaller quantities of acetic acid. Evidently, the adsorption capacity of Wofatit C increases with use, but as this applies equally to all amino acids, it raises no difficulties, because it is only necessary to increase the volume of fluid.

When the filters have been washed as described above, they are very slowly eluted with the following solutions: for the charcoal 300 ml 5% phenol in 20% acetic acid, and for the Wofatit filters 500 ml and 750 ml respectively N HCl. The two latter eluates are evaporated on a water bath, so that the amino acids are left as solids, only contaminated with small amounts of impurities, which do not disturb the subsequent analysis. Unfortunately this simple evaporation is not satisfactory for the first eluate, but a suitable alternative procedure is described in the experimental part. In this way the aromatic and basic amino acids are obtained as separate evaporation residues, while the acid and neutral ones are obtained together (fig. 1; op. 2).

The latter residue is dissolved in about 10 ml water and the solution is poured through Amberlite IR-4 in filter δ. After washing with a further 100 ml water the neutral amino acids will have passed the filter, while the acid ones will be retained and can then be eluted by 250 ml N HCl. The two eluates are evaporated separately on a water bath and the two groups of amino acids are obtained in pure form in the respective residues (fig. 1; op. 3 and 4).

In order to make the method more convenient we have also tried metal filters, goldplated internally, of the type described by TISELIUS and CLAESSON[1]. As our filters were intended for running in series, they were slightly modified and some coupling devices were designed[2] (fig. 2). With a suitable set of interchangeable filters and connections the whole analysis may be performed very conveniently, and it is also possible to perfuse each filter either in the direction used originally, or in the reverse direction if this latter alternative is thought to be the better.

[1] A. TISELIUS, S. CLAESSON, Ark. Kem. Min. Geol. *15B*, No. 18, 1 (1942). – See also note 1, page 23, 2nd column.

[2] It is impossible to pack an adsorption column homogeneously, therefore the front of the solution will always be more or less irregular. By the use of coupling pieces with a quite narrow channel, a good turbulent mixing of the liquid flowing between two filters is afforded, and the front entering the second filter is made horizontal. In the near future the role of such coupling pieces will be treated in detail by one of us. (See also Ark. Kem. Min. Geol. *24A*, No. 16).

For satisfactory results, with 50 mg of mixed amino acids, the filters should have the following approximate sizes:

Filter α CA, III, HAc [1]	$1000\,\pi$ mm³
Filter β W, C, HAc	$2000\,\pi$ mm³
Filter γ W, KS, HAc	$8000\,\pi$ mm³
Filter δ IR-4, HCl, aq	$4000\,\pi$ mm³

These will cover all types of amino acid mixtures, obtainable from proteins, even those having unusually high or low concentration of certain groups. The only exception is the case of the protamines, which contain about 90% arginine. If only an aliquot of the eluate from W, KS, HAc is used for the separation of acid and neutral amino acids, a smaller filter with IR-4 should be used, e.g. for two fifths of the total a filter of $2000\,\pi$ mm³ should be used.

The capacities of the filters used normally (i.e. with about 50 mg of material) are, as mentioned above, 1000, 2000, 8000 and $4000\,\pi$ mm³ respectively. From

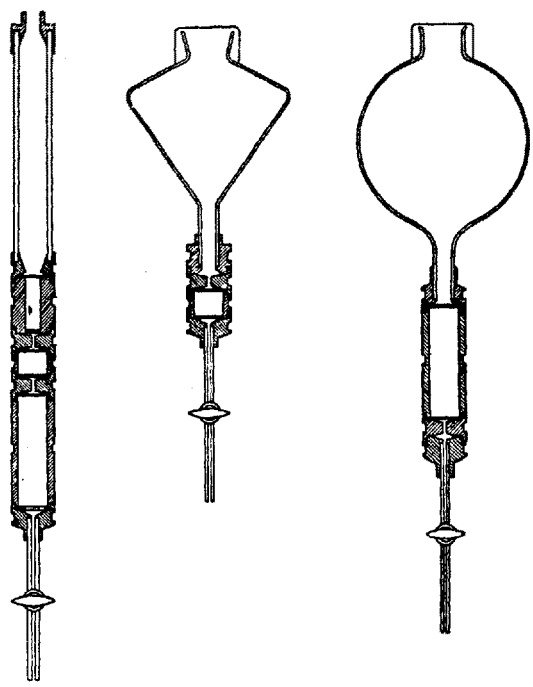

Fig. 2. *Sectional drawing*, showing some metal filters coupled together by the aid of connection pieces and provided with containers for the eluent. The operations here illustrated are from left to right No. 1α, 2β and 2γ (in the last case the filter is placed in the reversed position).

a set of metal filters whose capacities form a 2-power series, it is thus possible to select the four which are most suitable for analysis of any given amount of sample. In this way a considerable range can be covered with a minimum number of metal filters.

Experimental part

The *amino acids* we have used have all been of the highest purity obtainable, usually *pro analysi* or C. P. Those whose adsorption properties have been most studied are:

Aromatic: Phenylalanine, and to a lesser extent tyrosine and tryptophane.
Basic: Arginine, histidine hydrochloride, and to a lesser extent lysine dihydrochloride.
Acid: Aspartic acid and glutamic acid (the latter sometimes as hydrochloride).
Neutral: Glycine and leucine, and to a lesser extent cystein hydrochloride, proline and hydroxyproline.

These amino acids have been intentionally chosen as being the most unsuitable for series analysis, because it is only in this way that a reliable method can be elaborated. Thus we have chosen phenylalanine because it is, of the aromatic amino acids, the most easily eluted from charcoal, and so on.

The *adsorbents* that have been used are:

(1) *Carbo Activatus* (Merck, Darmstadt), which had been washed once a day for two months with distilled water and afterwards dried. Small parts of this large batch (number III of CLAESSON[1] was used for this work) are boiled in 20% acetic acid, then washed in distilled water many times and finally stored in 5% acetic acid. Every filter is, after filling and before the adsorption, washed through with about 100 ml 5% acetic acid[2]. This adsorbent is called CA, III, HAc. Because of the finely divided state of the charcoal, air pressure must be applied in order to force liquids through the filter, but in the case of other adsorbents, pressure need only exceptionally be applied.

2 and 3. *Wofatit* C *and Wofatit* KS (IG. Farbenindustrie) are two ion exchange resins, which together with some Amberlites we have obtained from "Nordiska Armaturfabriken", Linköping. The first resin contains COOH-groups and the latter SO_3H-groups.

Wofatit C is ground to a particle size of about 0·1 mm and put through 10–15 "cycles" with N HCl—water—N/2 NaOH—water—N HCl and so on, after which it is put through some cycles with N/10 NaOH instead of N/2 NaOH. This Wofatit is stored in N HCl until use and then it is first washed with water—N/10 NaOH—water—N HCl—water—20% acetic acid, after which it is kept in glacial or 20% acetic acid overnight, and is further washed with 20% acetic acid just before the experiment. This adsorbent is called W, C, HAc.

Especially during the first 10 washings the NaOH extracts great quantities of coloured substances, but towards the end of the whole procedure the washings become progressively lighter in colour. When using this Wofatit in acid or neutral solutions, no colour at all is extracted. A greater drawback of Wofatit C is, that it swells strongly in alkaline media and also, but to a much smaller extent, in acid solution. As this adsorb-

[1] Explications in the experimental part.

[1] S. CLAESSON (Diss.), Ark. Kem. Min. Geol. *23A*, No. 1, 1 (1946).
[2] G. SCHRAMM, J. PRIMOSIGH, Ber. dtsch. chem. Ges. *77*, 417 (1944).

ent in our method is only used in acid or neutral solution, this phenomenon is without significance when glass filters are used. The metal filters, however, must be packed with just the correct amount, so that the resin neither swells too much nor forms cracks or loose pockets.

Wofatit KS is prepared in the same manner as Wofatit C. It is more stable, does not give up such great quantities of colour and does not swell so markedly. This adsorbent is called W, KS, HAc.

(4) *Amberlite IR-4* (The Resinous Products and Chemical Company, Philadelphia) is an ion exchange resin of the alkylene polyamine formaldehyde type. It was ground to a particle size of about 0·1 mm and during several days washed alternately with N HCl and water—at least 25 times with each. This is done most conveniently by pouring the liquids through a column of the adsorbant, e.g. in 250 ml portions for 50 g substance. The preparation is stored in water and is before use washed with further quantities of water. This adsorbent that extracts only negligible quantities of nitrogen, is called IR-4, HCl, aq.

The great advantage with the Wofatits, Amberlites and other organic ion exchangers over the usual adsorbents is that they can easily be regenerated[1]. Below is given a description of our methods for doing this for the different exchangers.

Wofatit C (about $2000\,\pi$ mm^3) is after the experiments washed with 300 ml N HCl and then with 300 ml 20% acetic acid. Both solutions are passed very slowly through the filters. Wofatit KS (about $8000\,\pi$ mm^3) is washed in the same manner but with somewhat greater volumes. Amberlite IR-4 (2000–$4000\,\pi$ mm^3) is washed with 300 ml N HCl and then with nearly 1 l water. In all these cases the substance need not be removed from the filter which, once packed, can be used many times.

Because of the great quantities of *eluents* that are used, it has been hardly possible to free them from nitrogen. Our reagents have however been very pure, and we have simply subtracted their blank from our analysis values.

With the phenol we have had some trouble. A preparation that we first used was found to destroy phenylalanine during evaporation in a beaker on a water bath. For this reason we tried instead freshly distilled phenol, which at first seemed to be better, but it was soon found that it too destroyed some of the phenylalanine. Vacuum evaporation could not be applied, as we could not control the splashing of the 5% phenol in 20% acetic acid, and for that reason we tried the following method.

The eluate is collected in a 500 ml Erlenmeyer flask, which after the elution is closed by a ground-in wash bottle head, the normal outlet of which is connected to the air pressure line. The head has been slightly modified so that an oblique jet of air is directed on the surface of the liquid with a swirling motion. The flask is heated on a boiling water bath and evaporation is almost complete in two hours. When only a few ml of the liquid are left, about 20 ml of water or acetic acid are added and evaporation is continued with heating and blowing to complete dryness (about 15 minutes). The residue contains only traces of phenol but 99–100% of the phenylalanine.

As a quantitative *test of the distribution of nitrogen* we have throughout used the micro-Kjeldahl method. Our modification ($CuSO_4$, K_2SO_4, H_2O_2) has not given theoretical values for the basic amino acids, but we have applied an empirical correction factor, and we hope to find a more satisfactory modification.

For qualitative tests of amino acid distribution in the elaboration of the method we used the ninhydrin reaction. 1 ml sample was boiled for 1–2 minutes with 0·5 ml M phosphate buffer p_H 7 and two drops 1% aqueous ninhydrin. A violet-blue (or in some cases red) colour indicated the presence of some tens of γ of amino acid. When only smaller quantities were available, we boiled together for 1 minute in a dwarf test tube 2 drops of sample (about 0·1 ml), 1 drop 2 M phosphate buffer p_H 7 and 1 drop 2% aqueous ninhydrin. A weak but distinct colour was thus obtained with quantities as small as about 1 γ of glycine or 5 γ of phenylalanine.

Below we give some experimental results from *series analyses of amino acid mixtures*, containing about 45 mg of free amino acids. The calculated and the obtained values for two different solutions and the compositions of these are tabulated. The amounts of adsorbents used are for CA, III, HAc 1400—$1200\,\pi$ mm^3, for W, C, HAc 2100—$2000\,\pi$ mm^3, for W, KS, HAc 8500—$8000\,\pi$ mm^3 and for IR-4, HCl, aq (sol. 2; exper. 4 and 5) 4000 to $2000\,\pi$ mm^3. Metal filters have been used in exper. 1, 2 and 4, and glass filters in exper. 3 and 5. As to solution No. 1 no H_2S has been used, but in exper. 4 and 5 both solution No. 2 and the 5% and 20% acetic acid were treated with this reagent, corresponding to the descriptions of SCHRAMM and PRIMOSIGH[1].

Solution No. 1 and 2. Composition per sample (5 ml).

Group	Amino acids	Solution No. 1		Solution No. 2	
		mg	γ N	mg	γ N
Ar	Phenylalanine	5·0	410	3·92	531
	Tryptophane			1·96	
B	Arginine	7·5	2815	1·96	1361
	Histidine-HCl	2·5		3·92	
Ac	Aspartic acid			2·35	981
	Glutamic acid-HCl	7·5	575	9·80	
N	Glycine	15·0	4030	5·61	2520
	Leucine	12·5		9·36	
	Cysteine-HCl			1·87	
	Hydroxyproline			3·74	
	Total	50·0	7830	44·5	5393

[1] R. J. MYERS, J. W. EASTES, F. J. MYERS, Ind. Eng. Chem. *33*, 697 (1941).

[1] G. SCHRAMM, J. PRIMOSIGH, Ber. dtsch. chem. Ges. *77*, 417 (1944).

Solution No. 1. Nitrogen found in amino acid groups, as % of total calculated nitrogen

Exper. No.	Arom.	Bas.	Acid + neutr.	Total
1	5·5	32·5	62·1	100·2
2	5·5	30·9	62·0	98·3
3	5·3	31·1	62·7	99·1
Mean	5·4	31·5	62·3	99·2
Calc.	5·5	32·5	62·1	100

Solution No. 2. Nitrogen found in amino acid groups, as % of total calculated nitrogen

Exper. No.	Arom.	Bas.	Acid + neutral	Neutral	Acid	Total
4	10·7	22·6	67·0	47·2	18·2	99–100
5	10·5	24·8	64·0	46·3	17·3	99
Mean	10·6	23·7	(65·5)	46·8	17·8	99–100
Calc.	9·9	24·8	65·3	47·0	18·3	100

As can be seen from the preceding tables, the results are not as good as could be desired. On the other hand they are of the same accuracy as those of SCHRAMM-PRIMOSIGH[1] and of THEORELL-ÅKESSON[2]. The low values for the basic amino acids probably depend on some destruction during the evaporation of the eluate from W, C, HAc, and the disproportion between aromatic and basic amino acids in exper. 4 and 5 probably depends on the fact that arginine is to some small extent retained on charcoal, when the eluting 5% acetic acid is saturated with H_2S. These errors in the analysis are still being studied, and there is reason to hope that they may soon be eliminated. In that event the method should be accurate to within 0·5% of the total N.

The following details of actual experiments give an idea of the relatively short time required for an analysis. Op. 1a may take 1 hour (i.e. the rate of percolation should be about 1 ml per minute). Op. 1b may go faster, 1 l being passed through in about 2 hours. On the other hand, op. 2 must procede slowly, and may conveniently be made overnight. As a rule we try to elute the filters β and γ the same day that the analysis is begun, taking at least 4 hours for this, after which the evaporations are performed on a water bath during the night.

The next morning, when eluates $2\,\beta$ and γ have evaporated, the residue γ is dissolved in water and poured through the δ filter. The elution of this with water and with N HCl may take one half and one hour respectively, and these eluates are evaporated on a water bath during the next night.

The whole analysis is thus performed during two working-days, while the Kjeldahl analyses will take the same time as in other methods. The preparation of the filters the day before the analysis does not take long, and during the analysis itself a great deal of time is left for other work, e. g. a parallel analysis.

Discussion

The method is founded on the idea of former investigators, especially SCHRAMM-PRIMOSIGH[1], of separating an amino acid mixture into groups by a combination of chromatographic procedures. In addition to this we have also adopted the principle used in the qualitative separation of the leaf pigments[2], namely the use of a series of adsorbents one after another in the same operation. What is new in our method is the coupling together in series of independent filters, which can be detached and separately eluted, so that a quantitative separation of amino acids into groups is possible. In addition some facts upon which the method is based are new, namely the adsorption of amino acids from 20% acetic acid on Wofatit C and KS and the subsequent elution from these adsorbents by N HCl.

A great advantage of the method is that the reagents used are all easily removed by evaporation, thus avoiding the accumulation of salts in some fractions, as occurs in some other procedures for adsorption analysis[3]. In addition any salts that may originally be present in the amino acid mixture are automatically removed (fig. 1; op. 1a and b). Also the use of pyridine as eluent (frequently used in earlier works) has been totally avoided. A serious drawback at present is, however, the great difficulty of obtaining Wofatit C, but we have heard that attempts are being made in America to produce an adsorbent with similar properties.

As yet we have obtained only four groups against SCHRAMM-PRIMOSIGH's five[1], but we plan to separate the neutral group into two parts, using some ion exchange resin (in place of the alumina) together with formaldehyde, ethanol, acetone or some other substance, that changes the electrical properties of the amino acids.

Some drawbacks with the electrodialytic method are that it does not accomplish a separation of the aromatic from the neutral amino acids and that some operations must be performed twice[4]. On the other

[1] G. SCHRAMM, J. PRIMOSIGH, Ber. dtsch. chem. Ges. *77*, 417 (1944).
[2] H. THEORELL, Å. ÅKESSON, Ark. Kem. Min. Geol. *16A*, No. 8, 1 (1942).

[1] G. SCHRAMM, J. PRIMOSIGH, Ber. dtsch. chem. Ges. *77*, 417 (1944).
[2] L. GATTERMANN, Die Praxis d. org. Chemikers, 24th ed., page 402, Berlin 1936.
[3] J. W. DUBNOFF, J. biol. Chem. *141*, 711 (1941). – F. TURBA, Ber. dtsch. chem. Ges. *74B*, 1829 (1941).
[4] H. THEORELL, Å. ÅKESSON, Ark. Kem. Min. Geol. *16A*, No. 8, 1 (1942).

hand it permits the determination of ammonia nitrogen, which we have not yet studied but hope soon to explore.

Since we hope to use the method for analysis of virus proteins, we plan on the one hand to adapt it for use on protein hydrolysates, and on the other to decrease the amount of material required. The accuracy of the method even with very small amounts should be as high as is attained by the usual analytical methods for amino acids. Naturally we also hope to apply the method for the estimation of as many individual amino acids as possible, using colorimetric or other methods, where no specific adsorption procedures are available.

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Zusammenfassung

Es wird eine leicht durchführbare Methode für die Trennung von Aminosäuregemischen in die vier natürlichen Gruppen – aromatische, basische, neutrale und saure – beschrieben. Die Methode beruht auf der Verwendung von vier Absorptionsfiltern, wovon drei hintereinandergeschaltet sind und die separat eluiert werden. Die Genauigkeit der Methode ist die bei der Aminosäureanalyse übliche; die für die Fraktionierung benötigte Zeit ist die gleiche wie diejenige der schnellsten Methoden, die bis jetzt für solche Zwecke benutzt wurden. Der große Vorteil der neuen Methode besteht darin, daß jede Trennungsreaktion nur einmal durchgeführt werden muß und daß alle Fraktionen völlig salzfrei erhalten werden.

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Variation de la chaleur spécifique du caoutchouc en fonction de l'élongation

La théorie développée par VOGT[1] prévoit que la chaleur spécifique du caoutchouc étiré doit être indépendante de l'élongation (à condition que la tension, à longueur constante, varie linéairement avec la température). HAMILL, MROWCA et ANTHONY[2] ont montré que la théorie de VOGT cesse d'être applicable lorsque l'extension du caoutchouc est accompagnée de la formation d'une seconde phase.

D'après les mesures de ORNSTEIN, WOUDA et EYMERS[3], la chaleur spécifique du caoutchouc vulcanisé varierait considérablement avec l'étirement, passant par un minimum (65% seulement de sa valeur initiale) pour une extension de l'ordre de 100%. Il s'agissait de mesures de la chaleur spécifique moyenne entre 80°C et la température ordinaire. Avec un dispositif permettant d'évaluer la chaleur spécifique vraie à la température ordinaire, BOISSONNAS[4] a répété ces mesures et obtenu des résultats très différents de ceux d'ORNSTEIN. Pour un échantillon de caoutchouc non vulcanisé, la chaleur spécifique ne varie pas plus de 3% entre 0 et 200% d'élongation; pour un échantillon de caoutchouc vulcanisé, elle varie au plus de 10% entre 0 et 400%.

Il nous a paru intéressant de reprendre ces dernières mesures en augmentant leur précision. Le calorimètre employé, dérivé de celui qui a été utilisé par BOISSONNAS, est du type Nernst-Eucken. Sur un cylindre de «Dellite»[1] (diamètre 15 mm, épaisseur 0,4 mm, longueur 90 mm), sont enroulés un fil de platine (thermomètre) et un fil de constantan (chauffage). Un fil de caoutchouc (2 m environ, section carrée 3–4 mm²) est bobiné sur ce support à l'élongation désirée; le tout est suspendu dans un récipient dans lequel on fait le vide. L'ensemble est plongé dans un thermostat. L'énergie fournie par le courant de chauffe produit une élévation de la température du caoutchouc de 1°C environ.

En raison des grandes pertes thermiques dues à la faible capacité et la grande surface de l'équipage, la courbe température–temps s'écartait trop de la ligne droite pendant le refroidissement (phase finale de la mesure), pour permettre de calculer la correction au moyen de la classique formule de Regnault-Pfaundler. Nous avons employé un procédé très simple, applicable à toute mesure calorimétrique dans laquelle la marche de la température est nettement exponentielle. On établit un graphique sur papier logarithmique (échelle linéaire en abscisse pour le temps, échelle logarithmique en ordonnée pour la température); le coefficient angulaire de la droite obtenue donne alors le coefficient de la formule de Newton. La reproductibilité de nos mesures atteint ainsi ± 0,2%, bien que la correction de la fuite thermique soit de l'ordre de 8%.

Les mesures faites avec un échantillon de caoutchouc non vulcanisé (fil découpé dans de la feuille anglaise), à 25°C, ne montrent pas de variation de la chaleur spécifique avec l'étirement, pour des élongations comprises entre 0 et 300% (voir tracé I). Pour les caoutchoucs vul-

[1] W. W. VOGT, dans «Chemistry and Technology of Rubber», p. 375-76, Reinhold Publ. Corp., New York (1937).

[2] W. H. HAMILL, B. A. MROWCA et R. L. ANTHONY, Ind. eng. Chem. *38*, 106 (1946).

[3] L. S. ORNSTEIN, J. WOUDA et J. EYMERS, Proc. Acad. Sci. Amsterdam *33*, 273 (1930).

[4] C. G. BOISSONNAS, Ind. eng. Chem. *31*, 761 (1939).

[1] Papier bakélité, produit par la Fabrique suisse d'Isolants, Bretonbac.