DISCUSSION

Polylysine has been shown to possess various biological activities. It is bacteriostatic (Katchalski, Bichowski-Slomnitzki & Volcani, 1953) and virostatic (Stahmann, Graf, Patterson, Walker & Watson, 1951); it inhibits thromboplastic activity (de Vries *et al.* 1951), fibrinolysis (Ginsburg, de Vries & Katchalski, 1952), the proteolytic activity of pepsin (Katchalski, Berger & Neumann, 1954) and the clearing action of heparin on alimentary lipemia (Stein, de Vries, Wislicki & Katchalski, 1954).

In all the above-mentioned cases polylysine acts as a retarder of biological and enzymic processes. The effect reported here on the staphylocoagulase clotting reaction demonstrates that polylysine can also have an accelerating action in a specific biological system. This accelerating action of the basic peptide has been shown to affect the second stage of the staphylocoagulase clotting system, i.e. the clotting of fibrinogen by coagulase thrombin. In preliminary experiments it has also been observed that polylysine accelerates the clotting of fibrinogen by thrombin.

The mechanism of this acceleration is unknown. The following possibilities should be considered: (a) polylysine inactivates a coagulase thrombin inhibitor, (b) as a result of the interaction between polylysine and fibrinogen, a complex is formed which is attacked by the clotting agent more readily than fibrinogen itself, (c) the clotting time is shortened as the result of the ability of the highly charged threadlike polylysine molecules to form a link between the clotting agent and the fibrinogen; the local concentration of the clotting agent surrounding the fibrinogen molecules is thus increased and the clotting reaction enhanced.

The fact that polylysine and protamine exert an accelerating activity on the staphylocoagulase clotting system, whereas polyaspartic acid shows no effect, suggests that this ability is associated with the polycationic nature of the effective peptides.

SUMMARY

1. Poly-L-lysine accelerates the conversion of fibrinogen into fibrin in the simultaneous presence of staphylocoagulase and coagulase-reacting factor.

2. The clot-promoting activity of polylysine in the above system is due to the accelerating action of polylysine on the clotting of fibrinogen by coagulase thrombin.

REFERENCES

- Berger, A. & Katchalski, E. (1951). *J. Amer. chem. Soc.* **73**, 4084.
 Duthie, E. S. & Lorenz, L. L. (1952). *J. gen. Microbiol.* **6**, 95.
 Ginsburg, I., Vries, A. de & Katchalski, E. (1952). *Science*, **116**, 15.
 Kaplan, M. H. & Spink, W. W. (1948). *Blood*, **3**, 573.
 Katchalski, E., Berger, A. & Neumann, H. (1954). *Nature, Lond.*, **173**, 998.
 Katchalski, E., Bichowski-Slomnitzki, L. & Volcani, B. E. (1953). *Biochem. J.* **55**, 671.
 Katchalski, E., Grossfeld, I. & Frankel, M. (1948). *J. Amer. chem. Soc.* **70**, 2094.
 Laki, K. (1951). *Trans. 4th Conference*, p. 223, Josiah Macy Jr. Foundation.
 Miale, J. B. (1949). *Blood*, **4**, 1039.
 Stahmann, M. A., Graf, L. H., Patterson, E. L., Walker, J. C. & Watson, D. W. (1951). *J. biol. Chem.* **189**, 45.
 Stein, Y., Vries, A. de, Wislicki, L. & Katchalski, E. (1954). *Metabolism* (in the Press).
 Tager, M. (1948). *Yale J. Biol. Med.* **20**, 369.
 Vries, A. de, Schwager, A. & Katchalski, E. (1951). *Biochem. J.* **49**, 10.

Glucuronide Metabolism in Plants

2. THE ISOLATION OF FLAVONE GLUCOSIDURONIC ACIDS FROM PLANTS*

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The flavone glucosiduronic acids baicalin and scutellarin are known to occur in plants of the genus *Scutellaria*. Mölich & Goldschmiedt (1901) suggested, on qualitative histological evidence, that scutellarin, or a very similar compound, exists in plants of other genera. Baicalin has been isolated

from the root of *Scutellaria baicalensis* (Shibata, Iwata & Nakamura, 1923), where it forms a considerable proportion (10–20%) of the dry weight of tissue. It was also reported to occur in the foliage of *Scutellaria columnae* (Charaux & Rabaté, 1940b). Scutellarin was originally isolated from the leaves of *Scutellaria altissima* (Mölich & Goldschmiedt, 1901) and has since been prepared from the foliage

* For Paper 1 of this series, see Levvy (1954).

of several other species, e.g. *S. baicalensis* and *Scutellaria indica* (Shibata *et al.* 1923), *Scutellaria scordifolia* (Miwa, 1932) and from the leaves of *Centaurea scabiosa* ('greater knapweed') (Charaux & Rabaté, 1940a).

The aglycones, baicalein and scutellarein, of these glucosiduronic acids are closely related, and were found to be identical with 5:6:7-trihydroxyflavone and 5:6:7:4'-tetrahydroxyflavone, respectively (Shibata *et al.* 1923; Bargellini, 1915). The proposed structures of these flavones, originally synthesized by Bargellini (1915, 1919), were subsequently questioned (for review, see Plant, 1931), but have now been confirmed by less equivocal syntheses (Sastri & Seshadri, 1946). From indirect evidence, Shibata & Hattori (1930) have suggested that the point of conjugation is at the hydroxy group in position C-7 of the flavone nucleus in each case.

The purpose of this investigation was to isolate baicalin and scutellarin as possible substrates for the enzymes baicalinase (Miwa, 1932) and animal-tissue β -glucuronidase, and for comparison with other glucosiduronic acids which might later be isolated from field crops (see Marsh & Levvy, 1953). *S. baicalensis* is a native of the Far East, where its root has, or had, some use in pharmacy, but it cannot readily be cultivated in quantity in this country. An examination of *Scutellaria galericulata* ('common skullcap'), indigenous to Britain, was therefore made; its foliage yielded a previously unknown glucosiduronic acid of chrysin (5:7-dihydroxyflavone), which closely resembled baicalin in many respects. The acid in this compound was identified after enzymic hydrolysis with animal-tissue β -glucuronidase, and the aglycone after acid hydrolysis. From *S. columnae*, which has established itself locally in England, was isolated not baicalin (see above) but scutellarin, which was also prepared from *C. scabiosa*, and from *Scutellaria tournefortii*, a new source. This last plant is easy to cultivate in Britain.

EXPERIMENTAL AND RESULTS

Materials. *S. galericulata* and *C. scabiosa* were collected in Bedfordshire during the summer of 1953 by Dr J. G. Dony. *S. columnae* was collected in Somerset in May 1954 by Mr I. W. Evans. *S. tournefortii* was cultivated in Aberdeenshire from seed presented by the Royal Botanic Gardens, Kew. Authentic samples of baicalin, baicalein and chrysin were given by Professor S. Hattori, Tokio University.

Methods. The mouse-liver β -glucuronidase used for the enzymic hydrolysis of chrysin glucosiduronic acid was prepared according to the procedure of Levvy & Marsh (1952) for use in reducing-sugar measurements. Determinations of C, H, and of the optical rotation of chrysin glucosiduronic acid were made by Weiler and Strauss, Oxford. Spectral absorption was measured with a Beckman Model DU quartz spectrophotometer. All melting points are corrected, unless otherwise stated.

Isolation of chrysin glucosiduronic acid and scutellarin

The method of extraction was in general similar to those employed by previous workers for the isolation of flavone glucosiduronic acids from plants. The fresh or air-dried material was boiled under reflux with water (10 ml./g. fresh or dried leaf) for 2 hr., then strained through surgical gauze. Smaller particles were removed by centrifuging at 1500 g for 15 min. After the supernatant had been concentrated *in vacuo* to a suitable volume, conc. HCl (0.1 vol.) was added to the hot solution to precipitate the crude flavone glycosides, and after 12 hr. at 0° these were filtered off and washed with water. The crude products were then powdered under cold methanol and filtered; this removed much colouring matter. The residues were extracted with methanol in a 'hot Soxhlet' apparatus for up to 48 hr. until no further solid was extracted. During this period the glucosiduronic acids crystallized from the boiling solvent. The products were filtered off after 24 hr. at 0° and recrystallized several times from 95% (v/v) aqueous methanol.

Chrysin glucosiduronic acid

From the fresh leaf of *S. galericulata*, chrysin glucosiduronic acid was isolated (2.6% of the dry weight of leaf, after one recrystallization from 95% aqueous methanol), but no scutellarin, the presence of which had been suggested by Mölich & Goldschmiedt (1901) from histological observations. (Found on product dried at 140° *in vacuo*: C, 58.6; H, 4.2. $C_{21}H_{18}O_{10}$ requires C, 58.6; H, 4.2%.) The identification of chrysin and glucuronic acid in this compound is described below.

Properties. Chrysin glucosiduronic acid crystallized from aqueous methanol in fine yellow needles, m.p. 225–226° (decomp.). The loss in weight on drying at 140° *in vacuo* was 3.1%; the anhydrous product had $[\alpha]_D^{25} - 112 \pm 2^\circ$ in 50% (v/v) aqueous pyridine (c, 1.2). It was quite soluble in hot acetic acid, difficultly soluble in boiling methanol, ethanol and acetone (about 0.1%), and almost insoluble in water, ether and benzene. It was easily soluble in aqueous alkalis (saturated solution about 0.05M), giving yellow solutions which became greenish on standing.

The addition of $FeCl_3$ solution to an ethanolic solution gave a greenish coloration, red by transmitted light. The addition of lead acetate solution to an aqueous pyridine solution gave a yellow precipitate of the lead salt; addition of a solution of barium acetate gave a yellow colour, and calcium chloride an orange colour, but no precipitates, as distinct from the properties of baicalin as described by Shibata *et al.* (1923). The reduction of an aqueous ethanolic solution with magnesium and HCl gave a deep yellow coloration, but no precipitate.

Chrysin glucosiduronic acid slowly reduced Fehling's solution, but rapidly reduced it after hydrolysis with 2N-HCl at 100° for 30 min. A strongly positive reaction for uronic acid was obtained with Tollens' naphthoresorcinol reagent.

The general properties of chrysin glucosiduronic acid closely resembled those of baicalin, as described by Shibata *et al.* (1923), and, indeed, no apparent depression of the melting point was observed on admixture with an authentic sample of baicalin, m.p. 222°, prepared from *S. baicalensis* root. Chrysin glucosiduronic acid and baicalin can be discriminated by their u.v. absorption spectra, as described below, provided both compounds are available.

Identification of glucuronic acid after enzymic hydrolysis of chrysin glucosiduronic acid

A sample of chrysin glucosiduronic acid was supplied to Dr G. A. Levvy and was found to be readily hydrolysed by mouse-liver β -glucuronidase (Levy, 1954). From a previous investigation of the specificity of this enzyme (Levy & Marsh, 1952) it could be concluded that the compound obtained from *S. galepiculata* leaf was therefore a β -D-glucopyranosiduronic acid, since the enzyme is specific for the hydrolysis of this group. It was desirable to verify this assumption, however, by isolation of derivatives of glucuronic acid after the enzymic hydrolysis. The method employed was similar to that of Levy (1948).

An aqueous solution of chrysin glucosiduronic acid (0.05M; 20 ml.) in 0.1M acetic acid—NaOH buffer at pH 5.2 was incubated with mouse-liver β -glucuronidase (5 ml.) containing a total of 4500 'glucuronidase units' (G.U.) where 1 G.U. liberates 1 μ g. phenolphthalein in 1 hr. at 38° from 1.25 mM phenolphthalein glucuronide, in the same buffer.

After incubation at 38° for 48 hr., a yellow precipitate (chrysin) had formed and was removed by centrifuging at 1500 g for 15 min. The supernatant was then dialysed into water (200 ml.) at 3° for 48 hr. Acidification of the dialysate with 3N- H_2SO_4 to pH 2 precipitated unchanged substrate and chrysin, which were removed by filtration. Final traces of flavone compounds were then removed by extracting the filtrate with ethyl acetate (5 $\times$ 30 ml.), in which freshly acidified glucuronic acid has a negligible solubility. The aqueous solution was then shaken with $BaCO_3$ to remove sulphate, and filtered. A small sample of the filtrate was removed, and the free glucuronic acid content determined by titration with ceric sulphate, according to the procedure of Levy (1946). When corrected for the free glucuronic acid remaining in the dialysis sac, it was calculated that 75 mg. glucuronic acid were liberated, estimated as reducing sugar. This corresponded to 39% hydrolysis of the total substrate. A control experiment showed that in the absence of enzyme no free-reducing sugar was liberated.

The aqueous solution, calculated to contain 63 mg. glucuronic acid, was evaporated *in vacuo* to about 2 ml. and oxidized to D-glucosaccharic acid, followed by coupling with *o*-phenylenediamine, according to the procedure of Lohmar, Dimler, Moore & Link (1942) as modified by Levy (1948). This yielded 28 mg. of the dibenziminazole derivative (24% of theoretical from the glucuronic acid content), m.p. 236–237° (decomp.). From this was prepared the dihydrochloride, m.p. 265–267° (decomp.) and dipicrate, m.p. 210–211° (decomp.) with change of form at 140–142°. Authentic specimens of these compounds were prepared from D-glucurone (180 mg.) by oxidation and subsequent coupling using the same procedure. This yielded 110 mg. (30% of theoretical) of the dibenziminazole derivative of D-glucosaccharic acid, m.p. 240° (decomp.), from which were obtained the dihydrochloride, m.p. 267–268° (decomp.), $[\alpha]_D^{21} + 52.0 \pm 0.5^\circ$ in water (c, 2), and the dipicrate m.p. 212° (decomp.) with change of form at 142°. These data are in good agreement with those obtained by Levy (1948).

None of the derivatives obtained from the products of enzymic hydrolysis when admixed with the corresponding authentic specimens produced any depression of the melting points.

The washed and dried flavone precipitates obtained in this experiment were combined and boiled under reflux with ether (200 ml.). The mixture was filtered and the filtrate evaporated to dryness and recrystallized from 80% (v/v) aqueous ethanol to yield chrysin (58 mg.; 59% of theoretical), m.p. 270–272° (uncorr.). The ether-insoluble residue was recrystallized from 95% (v/v) aqueous methanol, yielding chrysin glucosiduronic acid (128 mg.; 30% of starting material), m.p. 224° (decomp.).

This experiment gave satisfactory proof that the sugar liberated by hydrolysis of the glycoside from *S. galepiculata* leaf was D-glucuronic acid.

Scutellarin

From *C. scabiosa*. Scutellarin was isolated from the foliage (2.4% of the dry weight of leaf, after two recrystallizations). The product, even when crude, had no melting point below 300°, but darkened slowly above 230°, whereas Charaux & Rabaté (1940a) stated that when isolated from this source it had m.p. 205–230°. The present finding agrees with the earlier workers (Mölich & Goldschmidt, 1901; Shibata *et al.* 1923), who obtained no definite melting point below 300°. (Found on product dried at 130° *in vacuo*: C, 54.4; H, 4.5. Calc. for $C_{21}H_{18}O_{12}$: C, 54.5; H, 3.9%.)

From *S. tournefortii*. The freshly cut leaf yielded scutellarin (3.3% of the dry weight of leaf, after one recrystallization). The impure product melted at about 195°, above which temperature it apparently resolidified; this effect was due to the presence of a more soluble impurity, for after several further recrystallizations the product darkened slowly above 230°, but did not melt below 300° (see above). (Found on product dried at 130° *in vacuo*: C, 54.2; H, 4.1. Calc. for $C_{21}H_{18}O_{12}$: C, 54.5; H, 3.9%.)

From *S. columnae*. The foliage of this plant yielded scutellarin (4.5% of the dry weight of leaf), but no baicalin (cf. Charaux & Rabaté, 1940b). The product slowly darkened above 220°, but did not melt below 300°.

Preparation of the aglycones by acid hydrolysis of the glucosiduronic acids

The finely powdered anhydrous glucosiduronic acids (150 mg.) were hydrolysed by boiling with a mixture of acetic acid (15 ml.) and dil. H_2SO_4 (10%, w/v; 15 ml.) under reflux for 5 hr. (Charaux & Rabaté, 1940a). The glycosides slowly passed into solution as the reaction proceeded. The solutions were then concentrated *in vacuo* at 45° to remove acetic acid, and diluted with water (40 ml.) to precipitate the aglycones. The mixtures were then extracted thrice with ether (total 60 ml.) and the ethereal solutions washed with water and dried over anhydrous Na_2SO_4 . After evaporation of the solvent, the residues were recrystallized from aqueous ethanol (80%, v/v). The yields of the crude products were almost quantitative.

The acetyl derivatives of the aglycones were prepared by heating with anhydrous sodium acetate and acetic anhydride in the usual way, and were recrystallized from ethanol.

Chrysin. Hydrolysis of the glucosiduronic acid isolated from *S. galepiculata* yielded chrysin, m.p. 268–269° (uncorr.). (Found on product dried at 130°: C, 70.4; H, 4.7. Calc. for $C_{15}H_{10}O_4$: C, 70.8; H, 4.0%.) There was no depression in the melting point on admixture with an authentic sample of chrysin, m.p. 275° (uncorr.), nor, however, with an authentic

Fig. 1. The ultraviolet absorption spectra of 0.05 mm chrysin glucosiduronic acid (—), baicalin (---) and scutellarin (.....) in ethanol.

Fig. 2. The ultraviolet absorption spectra of 0.05 mm chrysin, baicalein and scutellarein in ethanol; —, chrysin from *S. galericulata*; ×—×, authentic chrysin; ---, baicalein; ..., scutellarein.

sample of baicalein, m.p. 262° (uncorr.) (decomp.) derived from *S. baicalensis* root. These flavones could, however, be distinguished by their u.v. absorption spectra (see below) when both were available.

Acetylation of chrysin (from *S. galericulata*) yielded chrysin diacetate as colourless needles, m.p. 190–192°. (Found on product dried at 130°: C, 66.8; H, 4.2; CH₃CO, 24.8. Calc. for C₁₉H₁₄O₆: C, 67.4; H, 4.1; CH₃CO; 25.4%). There was no depression of the melting point on admixture with an authentic sample of chrysin diacetate, m.p. 189–191°, but when mixed with an authentic sample of baicalein triacetate, m.p. 188–190°, the melting point was 163–168°. The acetates of the aglycones provide the most satisfactory method for distinguishing chrysin glucosiduronic acid from baicalin.

Scutellarein. The products obtained on hydrolysis of scutellarin from *C. scabiosa*, *S. tournefortii* and *S. columnae*

Table 1. Ultraviolet absorption maxima and minima of ethanolic solutions of chrysin glucosiduronic acid, baicalin, scutellarin and their aglycones

~ Denotes an inflexion.

Compound	$\lambda_{\max.}$ (m μ .)	$\epsilon_{\max.}$	$\lambda_{\min.}$ (m μ .)	$\epsilon_{\min.}$
Chrysin glucosiduronic acid	270 ~ 306	29 600 14 400	— 235	— 10 300
Baicalin	246 279 314	11 800 30 000 16 400	237 250 305	10 900 11 400 15 900
Scutellarin	285 335	20 000 26 100	252 305	6 400 12 300
Chrysin	270 318	28 500 11 900	235 302	10 400 10 600
Baicalein	276 324	26 600 15 100	238 302	11 700 11 000
Scutellarein	286 339	16 600 18 300	252 310	6 300 10 700

did not melt below 300°. Acetylation of scutellarein gave the tetraacetate as colourless prisms. The acetate derived from *C. scabiosa* had m.p. 237° (found on product dried at 120°: C, 60.6; H, 4.1; CH₃CO, 37.9%), that from *S. tournefortii* had m.p. 235–237° (found on product dried at 120°: C, 60.9; H, 4.2; CH₃CO, 38.7. Calc. for C₁₉H₁₄O₁₀: C, 60.8; H, 4.0; CH₃CO, 37.9%), and that from *S. columnae* had m.p. 235°. There was no depression of the melting points on admixture of any of the scutellarein acetates from the different sources.

Ultraviolet absorption spectra

A sensitive method of distinguishing between chemically similar flavonoid compounds is by a comparison of their absorptions in the ultraviolet (see Hattori, 1932). The spectra of ethanolic solutions of chrysin glucosiduronic acid, baicalin and scutellarin are shown in Fig. 1, and those of chrysin, baicalein and scutellarein in Fig. 2. The spectrum of chrysin obtained from *S. galericulata* corresponded closely with that of an authentic specimen of chrysin, and the spectra of specimens of scutellarin and scutellarein from *C. scabiosa*, *S. tournefortii* and *S. columnae* were found to be identical.

The absorption maxima and minima of these compounds are given in Table 1. An increase in the number of hydroxyl groups on the flavone nucleus caused a general bathochromic shift of the spectra of both the glucosiduronic acids and of the aglycones; the absorption maximum at the lower wavelength was simultaneously weakened, and that at the higher wavelength strengthened. These general observations confirm the work of Hattori (1932).

SUMMARY

1. Chrysin glucosiduronic acid was isolated from the leaves of *Scutellaria galericulata*, the glucuronic acid residue being identified after enzymic hydrolysis, and the aglycone after acid hydrolysis.

2. Scutellarin was isolated from the foliage of *S. tournefortii* and *S. columnae*, the products being identical with that obtained from the foliage of *Centaurea scabiosa*.

3. The ultraviolet absorption spectra of the flavone glucosiduronic acids and of their aglycones have been compared.

The author wishes to thank Dr G. A. Levvy for his interest in this work, and Dr J. G. Dony and Mr I. W. Evans for their willing co-operation in obtaining plant material. He is indebted to the Royal Botanic Gardens, Kew, for presenting seeds of *S. tournefortii*.

REFERENCES

- Bargellini, G. (1915). *Gazz. chim. ital.* **45** I, 69.
 Bargellini, G. (1919). *Gazz. chim. ital.* **49** II, 47.
 Charaux, C. & Rabaté, J. (1940a). *J. Pharm. Chim., Paris*, **9**, 155.
 Charaux, C. & Rabaté, J. (1940b). *J. Pharm. Chim., Paris*, **9**, 401.
 Hattori, S. (1932). *Acta phytochim., Tokyo*, **6**, 131.
 Levvy, G. A. (1946). *Biochem. J.* **40**, 396.
 Levvy, G. A. (1948). *Biochem. J.* **42**, 2.
 Levvy, G. A. (1954). *Biochem. J.* **58**, 462.
 Levvy, G. A. & Marsh, C. A. (1952). *Biochem. J.* **52**, 690.
 Lohmar, R., Dimler, R. J., Moore, S. & Link, K. P. (1942). *J. biol. Chem.* **143**, 551.
 Marsh, C. A. & Levvy, G. A. (1953). *Biochem. J.* **53**, xxxix.
 Miwa, T. (1932). *Acta phytochim., Tokio*, **6**, 154.
 Mölsch, H. & Goldschmiedt, G. (1901). *Mh. Chem.* **22**, 679.
 Plant, S. G. P. (1931). *Rep. Progr. Chem.* **28**, 147.
 Sastri, V. D. N. & Seshadri, T. R. (1946). *Proc. Indian Acad. Sci.* **23** A, 262.
 Shibata, K. & Hattori, S. (1930). *Acta phytochim., Tokyo*, **5**, 117.
 Shibata, K., Iwata, S. & Nakamura, M. (1923). *Acta phytochim., Tokyo*, **1**, 105.

The Amino Acid Composition of Wool

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When the present work was started early in 1953 no complete amino acid analysis of a single wool sample had been published, although Graham, Waitkoff & Hier (1949) had estimated fourteen amino acid constituents of a wool hydrolysate, chiefly by microbiological methods. While this paper was in preparation, however, our attention was drawn to a paper by Simmonds (1954), who has completed the amino acid analysis of an Australian 64's quality wool. The wool sample whose analysis is reported here was of the same quality, and it is of interest, therefore, to compare the two analyses. For a survey of amino acid analyses of wool, previously published in the literature, reference should be made to Simmonds (1954).

EXPERIMENTAL

Chromatographic separations and estimations of amino acids

All the columns used for starch chromatography were of 0.9 cm. diameter and 30 cm. length and were prepared according to the directions of Stein & Moore (1948) from starch manufactured by Gordon Slater Ltd., Manchester. Six different types of chromatograms were employed in the analysis of wool hydrolysates. Leucine, isoleucine, phenylalanine and methionine were separated on starch columns eluted with benzyl alcohol-*n*-butanol-water (1:1:0.280, by vol.). The volume of water in the mixture was smaller than that used by Stein & Moore (1948) because the columns were run at 18°. The columns were loaded with 3.5 mg. of

hydrolysate and 0.5 ml. fractions were collected for the estimations of leucine, phenylalanine and isoleucine, but for the accurate estimation of methionine, which occurs to the extent of 0.5% in the hydrolysate, 10 mg. loads were applied and 1.5 ml. fractions collected. Thiodiglycol was added to the eluting solvent for the methionine estimations. This chromatogram is illustrated in Fig. 1 and referred to hereafter as starch I. Valine and tyrosine were separated from 2.5 mg. of wool hydrolysate on starch columns eluted with 17% (v/v) aqueous 0.57N-HCl in *n*-butanol; 0.5 ml. fractions were collected (Stein & Moore, 1948). The valine peak coincided with that of methionine, and the methionine contribution to the combined peak was deducted to give the

Fig. 1. Elution of amino acids from chromatogram starch I (Nin.) (see text). A, 3.5 mg. of wool hydrolysate for estimations of phenylalanine, leucine and isoleucine (0.5 ml. fractions); B, 10 mg. of wool hydrolysate for estimation of methionine only (1.5 ml. fractions).