570. *The Action of Formaldehyde on Proteins. Part III.* The Hydrolysis of Formaldehyde-hardened Proteins by Enzymes.*

By R. D. HAWORTH, R. MACGILLIVRAY, and D. H. PEACOCK.

It has been found that certain proteins treated with formaldehyde under acid conditions can be hydrolysed by papain and the products further hydrolysed by trypsin. From the hydrolysate from "Ardil" protein fibre, six formaldehyde-containing fragments have been isolated by adsorption on charcoal, Zeocarb-215, and cellulose, and analysed by paper chromatography. Acid hydrolysis has shown that aspartic and glutamic acid and at least three other amino-acids are associated with these six formaldehyde containing fragments. The results suggest (1) that *N*-methylenebisamide derivatives of aspartic and glutamic acid play a considerable part and (2) that lysine and arginine play little, if any, part in the reaction of proteins and formaldehyde under acid conditions.

IN Part I (*J.*, 1950, 1493) of this series evidence was presented that under alkaline conditions formaldehyde acted on the amide groups in proteins and converted them into *N*-hydroxymethylamide groups. It was also noted that proteins treated with formaldehyde at pH 0 contained only a very low proportion of groups which reacted with 6-bromo-2-naphthol and it was regarded as probable that *N*-methylenebisamide groups were predominant, although cross linkages between amide and other groups were not excluded. Proteins insolubilised by formaldehyde under acidic conditions contain formaldehyde which falls into three different categories with regard to ease of removal. Some of the formaldehyde, including that combined in *N*-hydroxymethylamide groups, is removed by boiling water, part (including that united as *N*-methylenebisamide group) is split off by acids, and part is not recovered by acid hydrolysis but remains combined with tyrosine and possibly other amino-acids. While it is conceivable that regulated acid hydrolysis might degrade an insolubilised protein to more manageable fragments still containing methylene bridges it is certain that during the acid treatment some of the less stable methylene-bridged compounds would be broken down, and that new compounds would be formed to an extent depending on the conditions of hydrolysis. Preliminary experiments indicated that alkaline hydrolysis presented similar difficulties and in these circumstances recourse was had to enzymic hydrolysis. It was found that ground-nut protein, soya-bean protein, and casein, after acid-insolubilising treatment, were not attacked by papain, pepsin, or trypsin, but when the loosely bound formaldehyde was removed by boiling water the remaining protein was attacked by papain, particularly if the enzyme was activated by hydrogen cyanide at pH 8—9. Hydrogen sulphide, sodium hydrogen sulphite, and cysteine were much less effective than hydrogen cyanide. The hydrolyses were followed by formaldehyde titration and the results are summarised in Table 1. The percentage of peptide groups hydrolysed was calculated by using the value 0·007 mole of amino-group per g. of protein derived from Chibnall's analytical figures (Traill, *J. Soc. Dyers Col.*, 1945, **61**, 150); the acid hydrolysis of ground-nut protein gave a Sörensen figure of 0·0066 mole per g. of protein, in reasonable agreement with the above. The high formaldehyde content of the proteins treated with formaldehyde under alkaline conditions is probably due to reaction with arginine, as it is found that arginine, ground-nut protein, and acid–formaldehyde-insolubilised ground-nut protein after treatment with alkaline formaldehyde no longer respond to the Sakaguchi test for arginine.

After these preliminary experiments attention was concentrated upon "Ardil" fibre made by Imperial Chemical Industries Limited by the action of formaldehyde on ground-nut protein (Traill, *loc. cit.*), and Table 2 summarises the results obtained when the fibre was boiled with water to remove the labile formaldehyde and then digested with cyanide-activated papain.

* Part II, preceding paper.

TABLE 1. *Papain digestion of formaldehyde-treated proteins after removal of labile formaldehyde.*

Protein (1·0 g.) suspended in water (50 c.c.) containing papain (0·5 g.) and potassium cyanide (0·2 g.).

Protein	CH_2O treat-ment	CH_2O (%) left in protein after water-treatment	Time of digestion (days)	Sörensen titration; 25 c.c. of solution, 0·05N-NaOH	Sörensen titration, control	Hydro-lysis (%)
Ground-nut ...	none	none	0·25	16·2	1·2	22
,, ...	pH 0	1·6	3	15·5	,,	21
,, ...	pH 3	0·9	,,	14·0	1·3	20
,, ...	5% K_2CO_3	2·0	,,	18·1	1·2	26
,, ...	10% K_2CO_3	2·8	,,	16·1	,,	22
Casein	none	none	0·25	16·5	,,	22
,, ...	pH 0	1·7	3	16·2	,,	22
Soya bean	none	none	0·25	17·1	,,	
,, ...	pH 0	1·8	3	16·9	,,	

TABLE 2. *Hydrolysis of "Ardil" fibre with cyanide-activated papain at* 40°.

The fibre (1 g.) was suspended in water (50 c.c.) containing papain (0·5 g.) and potassium cyanide (0·2 g.).

Time of digestion (days)	Undissolved protein (g.)	Sörensen titration of solution	Sörensen titration of control	Amino-groups set free (mole per 1 g. of protein)	Hydrolysis of peptide links (%)
1·5	0·15	3·3	0·4	0·0004	9·5
3·25	0·05	6·2	0·9	0·0015	20
4·5	,,	8·1	,,	0·0018	24
5·5	,,	8·2	,,	0·0018	24
7·5	,,	8·3	,,	0·0019	26

It was possible that the insolubilising process might have produced a non-homogeneous material with a higher formaldehyde content on the outer layer of the fibre than in the core. In order to test this the formaldehyde content of the original fibres and of the residues obtained after varying periods of digestion were determined. The results summarised in Table 3 indicate that the fibre is homogeneous after being insolubilised. By using

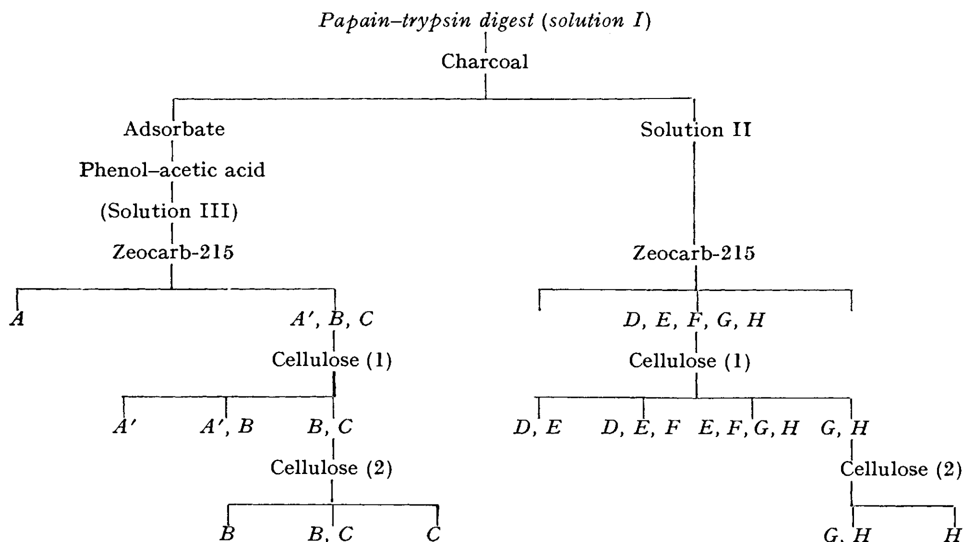
TABLE 3. *Formaldehyde in undissolved fractions of "Ardil" fibres after papain digestion.*

Time of digestion (hours)	Ardil undissolved (g.)	CH_2O in undissolved material (%)
Control fibre	5·0	1·2, 1·2
4	3·1	1·1, 1·2
6	2·6	1·2, 1·2
9	1·3	1·1, 1·1
10	1·1	1·2, 1·3

the same sample of fibre it was found that the degree of hydrolysis after 4—5 days' digestion varied from 25% to 40% with different samples of commercial papain. The degree of hydrolysis, 25%, obtained with papain indicated that the average molecular size of the polypeptide in the digest solution corresponded to four amino-acids and as the object of this work was to obtain the methylene-bridged amino-acids corresponding to the methylene bridges in the protein it was decided to hydrolyse further the papain digest solution. Although pepsin and trypsin did not attack the water-boiled insolubilised protein, it was found that they brought about a further hydrolysis of the papain digest solution. Trypsin gave the better result and took the hydrolysis to 70—80%. The resulting solution when tested with dimedone showed no free formaldehyde, and after acid hydrolysis and distillation the formaldehyde recovered corresponded to 95% of the formaldehyde taken up by the fibre. It is improbable that the formaldehyde has been liberated during the digestion and has subsequently combined with amino-acids present, because the addition of free formaldehyde to a digest solution stopped the hydrolysis. We consider that the digest solution contains at this stage not only the methylene-linked polypeptides and amino-acids corresponding to the methylene bridges in the original insolubilised proteins, but also formaldehyde-free amino-acids and polypeptides. For the immediate object of this work, only the

compounds containing methylene bridges were of interest, and attention was focused on those compounds from which formaldehyde was liberated by acid hydrolysis; compounds containing formaldehyde linked to aromatic nuclei are not considered at this stage. The further hydrolysis by trypsin of the papain digest solution gave variable results depending on the nature of the fibre, the extent to which the papain digestion had been carried, and the previous history of the trypsin. The results described in this paper were obtained with selected digest solutions in which the total hydrolysis by the two enzymes had proceeded to 70—80%. When the hydrolysis was stopped at an earlier stage the polypeptides obtained were naturally different, but two-dimensional paper chromatograms showed similar characteristics. The less hydrolysed solutions gave diffuse areas which on further hydrolysis could be resolved into spots of increasing definition.

The papain–trypsin digest (solution I) obtained in the manner described above gave twenty-four sharply defined spots on a two-dimensional paper chromatogram and several diffuse areas of colour, after development with ninhydrin. Solution I was treated with charcoal and filtered, and the adsorbate was eluted with a solution of phenol and acetic acid to give solution III. A sample was evaporated to dryness under reduced pressure. The solid residue contained 7—9% of formaldehyde determined by acid hydrolysis; the amount of formaldehyde in the charcoal adsorbate was less when the hydrolysis had not been carried so far. As " Ardil " fibre, after being boiled with water, contained 1·7% of formaldehyde (Tetlow, *J. Sci. Food Agric.*, 1950, **1**, 193) a considerable concentration of hydrolysable methylene-bridged material had been effected. Solution III gave three ninhydrin-reacting spots *A*, *B*, *C* on a two-dimensional paper chromatogram; *A* was subsequently split into *A* and a similar material *A'* by the use of cellulose columns. All four compounds contained formaldehyde which was set free by acid hydrolysis. The filtrate from the charcoal, solution II, was separated by a column of Zeocarb-215 into a number of fractions from which only one group contained acid-hydrolysable formaldehyde. This group of fractions was mixed and a sample on a two-dimensional paper chromatogram gave five ninhydrin-reacting spots, *D*—*H*. The solution of these substances was evaporated to dryness under reduced pressure, dissolved in butanol–acetic acid–water, and put through a cellulose column. The fractions were examined on two-dimensional paper chromatograms which showed a partial separation into *D* and *E*; *D*, *E*, and *F*; *E*, *F*, *G*, and *H*; and *G* and *H*; but only the fractions containing *G* and *H* liberated formaldehyde on acid hydrolysis. The solution of *G* and *H* was evaporated to dryness, dissolved in butanol–diethylamine–water, and put through a cellulose column, and *H* was isolated. The process is represented in the annexed scheme.

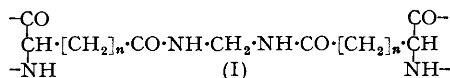


The substances *A*, *A'*, *B*, *C*, and *H*, and the mixture of *G* and *H*, were hydrolysed by acid, and the hydrolysates analysed by two-dimensional paper chromatograms; two chromatograms were made in each case, with phenol and butanol–acetic acid, and phenol and butanol–propanol–diethylamine as the pairs of solvents. The results are summarised in Table 4.

TABLE 4.

Acid	*A*	*A'*	*B*	*C*	*H*	*G–H*
Aspartic	+	+	+	+	+	+
Glutamic	+	+	+	+	+	+
Leucine	+	+		+	+	+
Valine	+		+	+		+
Alanine	+	+			+	+
Serine		+	+	+	+	+
Proline		+	+	+		
Tyrosine			+	+		
Phenylalanine			+	+		
Cystine						+

The results described represent the first step towards the isolation of the methylene-bridged units present in insolubilised ground-nut protein. Numerous suggestions have been made concerning the nature of these bonds (French and Edsall, *Adv. Protein Chem.*, 1945, **2**, 305; Traill, *Chem. and Ind.*, 1950, 23), but the absence of lysine and arginine from the formaldehyde-containing fragments, also observed by Waldschmidt-Leitz (*Zellwolle, Kunstseide, Seide*, 1941, **46**, 444) for hardened casein, indicates that the frequent suggestion that these amino-acids play an important part in acid–formaldehyde reactions is not in agreement with these experimental observations. On the other hand all the formaldehyde-containing fragments yield the dibasic, aspartic and glutamic acid together with at least three other amino-acids, and the suggestion that the amide groups of asparagine and glutamine participate in the insolubilising process is strongly supported by our observations. A methylenebisamide structure of type (I) would probably be readily formed under the



acid-insolubilising conditions and a considerable degree of stability towards acids would be expected by analogy with the properties of model compounds (see Part I). Such a structure would also be consistent with the observations that the acid-hydrolysable formaldehyde content and the amide value of substance *A* were in the ratio of 1 : 1·8, but in other fractions it is possible that serine-*O*- or cysteine-*S*-derivatives of *N*-hydroxymethylamide compounds also contribute.

Further evidence in support of the view that the amide groups are largely concerned in the formation of methylene bridges comes from the action of formaldehyde in alkaline solution on acid-insolubilised proteins. When ground-nut protein is treated with formaldehyde in 10% potassium carbonate solution and then condensed with 6-bromo-2-naphthol, 2·5% of bromine is taken up, corresponding to combination with 35 amide groups/10^5 g. of protein or 27% of the total amide groups (Part I). If the protein is first insolubilised in acid solution and then submitted to this treatment the bromine uptake is only 0·5—0·6% corresponding to seven amide groups/10^5 g. of protein.

EXPERIMENTAL

Papain Hydrolysis of Formaldehyde-treated Proteins.—Samples (10—15 g.) of ground-nut protein, casein, and soya-bean protein were treated with formaldehyde at pH 0 and, in the case of the first-named protein, at pH 3, and in presence of 5 and 10% potassium carbonate solution (Part I). The treated protein (10 g.) was heated with water (500 c.c.) for 30 minutes at 100°. The water was decanted and the process repeated. The protein was then filtered off, washed, and dried at 100° for 2 hours. Papain (0·5 g.) was mixed with water (30 c.c.), left for 0·5 hour, and then filtered off. The filtrate was made up to 50 c.c., the formaldehyde-treated and boiled protein (1 g.) and potassium cyanide (0·2 g.) were added, and the mixture was incubated at 40°. The pH was initially 8—9 and at the end of digestion about 8. Control experiments with the

enzyme alone were carried out at the same concentration. Samples (10 c.c.) were withdrawn at the intervals stated in Table 1 and titrated by the Sörensen method.

Enzymic Hydrolysis of " Ardil " Fibre.—The fibre was supplied by Imperial Chemical Industries Limited, Nobel Division, and had been prepared as described by Traill (*J. Soc. Dyers Col.*, 1945, **61**, 150). The formaldehyde content was 2·8%. " Ardil " fibre (50 g.) was heated with water as described above and dried; the formaldehyde content was then 1·6%. Papain (2·5 g.) was extracted with water (150 c.c.), and the filtrate made up to 250 c.c. 25 C.c. of this solution gave a Sörensen titration of 1·1 c.c. with 0·05N-sodium hydroxide. Boiled " Ardil " fibre (5·0 g.) and potassium cyanide (2·0 g.) were added to the papain solution (250 c.c.), and the mixture was incubated at 40°. The pH at the start was 8·6. After 7 days the protein solution, was filtered from the insoluble residue (0·1 g.); 25 c.c. of this solution gave a Sörensen titration of 15·1 c.c. (0·1N-sodium hydroxide), and the control 0·8 c.c. To the light brown solution, 15% trichloroacetic acid solution (80 c.c.) was added and the precipitate (0·9 g.; formaldehyde, 0·05%) collected. The excess of trichloroacetic acid was extracted with ether (3 × 120 c.c.), and the solution then aerated for 30 minutes to remove ether; the volume obtained was 320 c.c., and 10 c.c. gave a Sörensen titration of 5·6 c.c. (0·1N-sodium hydroxide). The solution was adjusted to pH 8·5 by addition of 2N-sodium hydroxide (about 8·0 c.c.), and trypsin (2 g.) added; 10 c.c. of this solution then gave a Sörensen titration of 6·6 c.c. (0·1N-sodium hydroxide). Thymol was added as a preservative and the solution incubated for 5 days at 40°, 2N-sodium hydroxide solution being added from time to time to keep the solution just alkaline to phenolphthalein. Buffered solutions were not used because of the subsequent difficulties in chromatography.

Time of digestion (days)	0	2	3	4	5	6
Sörensen titration of 10 c.c.; c.c. 0·1N-NaOH	6·6	8·8	9·2	9·8	9·8	9·8
Sörensen titration of 10 c.c. of control papain–trypsin solution	1·1	1·2	1·3	1·4	1·4	1·4

A sample of this solution was made faintly acid with acetic acid and mixed with dimedone solution. No precipitate was formed overnight, showing the absence of any quantity of formaldehyde. Another sample of the solution was hydrolysed in 6N-sulphuric acid, and the formaldehyde formed distilled into a solution of dimedone. The weight of the precipitate corresponded, to formaldehyde (55 mg.) in the total digest solution. " Ardil " fibre (5 g.), hydrolysed similarly gave a precipitate corresponding to 60 mg. of formaldehyde.

Charcoal Adsorption.—Charcoal (30 g.) was stirred for 1 hour with 5% acetic acid, filtered off, and washed with distilled water. The wet charcoal was then added to the digest solution (I) (620 c.c.) prepared from " Ardil " fibre (10 g.), and the mixture stirred for 1 hour. The charcoal was then filtered from the pale yellow solution (II), and stirred for 1 hour with a solution of phenol (20 g.) in 20% acetic acid (400 c.c.). The charcoal was collected and the filtrate concentrated at reduced pressure and below 50° to a thick syrup. Addition of ether or ethanol precipitated a pale yellow amorphous powder (III) which was washed with ether and dried (yield, 0·8 g.; formaldehyde content, 8·2%). A two-dimensional paper chromatogram by the descending method (Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224), with (i) butanol–acetic acid (Partridge, *Biochem. J.*, 1948, **42**, 238) and (ii) 80% phenol or 80% phenol containing 1% of ammonia gave, on development with ninhydrin at 100°, three spots, *A*, *B*, and *C*. R_F values in the order of the solvents are: *A*, purple, 0·13, 0·46, 0·76; *B*, blue, 0·44, 0·64, 0·56; *C*, purple, 0·58, 0·86, 0·78. In subsequent experiments a solvent was used containing *n*-butanol (70 c.c.), *n*-propanol (28 c.c.), water (25 c.c.), and diethylamine (2 c.c.), instead of phenol–ammonia.

Fractionation on Zeocarb-215.—A Zeocarb-215 column (15 × 2·5 cm.) containing Zeocarb (15 g.; 60—80 B.S.S. mesh) was prepared as described by Partridge and Westall (*Biochem. J.*, 1949, **44**, 418). An aqueous solution of the mixed peptides (III) (1·0 g.) was run on to this column and, after being washed with water until the washings were neutral, the peptides were eluted with 0·15N-ammonia until the ammonia began to come through the column. The eluate was collected in 45 fractions (each 15 c.c.). Fractions 1—20 inclusive and 25 and 26 contained no substances reacting with ninhydrin. Fractions 21—24 were each put on a one-dimensional paper chromatogram with 80% phenol as solvent. Fraction 21 contained apparently a single peptide (*A*) and fractions 22—24 mixtures of *A*, *B*, and *C*. 1 C.c. of *A* was hydrolysed with 1 c.c. of concentrated hydrochloric acid under reflux for 40 hours. The bulk of the hydrochloric acid was then removed by evaporation under reduced pressure and the volume made up to 1·0 c.c. with distilled water. Two-dimensional chromatograms, using 80% phenol, butanol–acetic acid, and butanol–propanol–diethylamine, showed the presence of five amino-acids in the hydrolysate. The R_F values, in the order of the solvents, are: aspartic acid, weak spot, 0·18,

0·22, 0·04; glutamic acid, very strong spot, 0·31, 0·25, 0·05; alanine, weak spot, 0·56, 0·30, 0·20; valine, weak spot, 0·82, 0·49, 0·40; leucine, strong spot, 0·88, 0·67, 0·54. These R_F values are within ±0·02 of the values found for the pure amino-acids singly. 7 C.c. of fraction 21 * after hydrolysis with 6N-sulphuric acid and distillation into potassium cyanide (Walker, " Formaldehyde," Reinhold Publ. Corp., p. 263) gave 9·5 mg. of formaldehyde. Determination of ammonia by hydrolysis (Wormall and Kaye, *J. Soc. Chem. Ind.*, 1945, **64**, 75) gave 17·5 mg. of amide group in 7·0 c.c. of the same solution.

Fractionation on a Cellulose Column.—A column (20·0 × 2·0 cm.) of powdered cellulose (10 g.; Whatman, Ashless) was prepared with the butanol–acetic acid mixture. The mixed peptides III (0·5 g.) dissolved in the butanol–acetic acid mixture were brought on to the column which was then extracted with the same solvent and the eluate collected in 20 fractions (each 10 c.c.). One-dimensional paper chromatograms with 80% phenol on each fraction showed that fractions 4 and 5 contained peptides, *B* and *C* fractions 6 contained *B* and a peptide *A'*, resembling *A*, and fractions 7—11 contained *A'*. The other fractions contained no peptides or amino-acids. All peptide-containing fractions also contained formaldehyde, detected by hydrolysis with sulphuric acid (1·5 c.c. of 9N to 1·0 c.c. of solution), distillation, and addition of chromotropic acid (McFadyen, *J. Biol. Chem.*, 1945, **158**, 107). Fraction 10 was evaporated to dryness under reduced pressure and hydrolysed with 5N-hydrochloric acid. Two-dimensional paper chromatograms of the hydrolysate showed the presence of six amino-acids, whose R_F values for the same solvents as before and in the same order were: aspartic acid, 0·16, 0·18, 0·04; glutamic acid, 0·32, 0·27, 0·06; serine, 0·34, 0·20, 0·13; alanine, 0·56, 0·29, 0·20; proline, 0·90, 0·32, 0·22; leucine, 0·88, 0·68, 0·54. Fractions 4 and 5, containing the mixed peptides *B* and *C*, were evaporated to dryness and the solid (80 mg.) was dissolved in a small volume of the butanol–propanol–diethylamine mixture. This solution was brought on to a cellulose column (10 g.; Whatman, Ashless), prepared with the above solvent, and then developed with the same solvent. The eluate was collected in 20 fractions (each 4 c.c.). Fractions 8 and 9 contained peptide *C* only, 10 and 11 contained *B* and *C*, and 12—15 *B* only. The other fractions contained no substances reacting with ninhydrin. Peptides *B* and *C* both contained formaldehyde liberated on hydrolysis with acid. Fraction 8, peptide *C*, was evaporated to dryness and the residue (9 mg.) hydrolysed with 5N-hydrochloric acid. Two-dimensional paper chromatograms with the same solvents as before showed the presence of eight amino-acids: aspartic acid, R_F 0·17, 0·18, 0·13; glutamic acid, 0·33, 0·26, 0·05; serine, 0·34, 0·19, 0·13; proline, 0·91, 0·32, 0·23; tyrosine, 0·56, 0·43; valine, 0·79, 0·48, 0·40; leucine, 0·84, 0·66, 0·54; phenylalanine, 0·85, 0·62, 0·56. Fraction 13, peptide *B*, was evaporated to dryness and the residue (12 mg.) hydrolysed. Two-dimensional paper chromatograms showed the presence of seven amino-acids, the same solvents as before being employed: aspartic acid, R_F 0·18, 0·19, 0·05; glutamic acid, 0·32, 0·26, 0·07; serine, 0·34, 0·20, 0·14; proline, 0·90, 0·32, 0·24; tyrosine, 0·57, 0·44, 0·26; valine, 0·77, 0·50, 0·41; phenylalanine, 0·84, 0·62, 0·58.

Solution II, the Charcoal Filtrate.—Fractionation on Zeocarb-215. The column was constructed in three sections: (*a*) 15 × 2·5 cm. (15 g.), (*b*) 10 × 1·8 cm. (10 g.), and (*c*) 6 × 1·5 cm. (7·5 g.) (Partridge, *loc. cit.*). The charcoal filtrate, solution II, was run through the column, and the peptides and amino-acids eluted with 0·15N-ammonia, and collected in forty fractions (each 25 c.c.). Fractions 21—25 contained acid-hydrolysable formaldehyde, and fractions 2—32 contained substances reacting with ninhydrin. Fractions 21—25 were mixed and evaporated to dryness under reduced pressure below 50°. Two-dimensional paper chromatograms with 80% phenol and butanol–propanol–diethylamine gave five spots: D, R_F 0·63, 0·21; E, 0·49, 0·31; F, 0·28, 0·50; G, 0·26, 0·66; H, 0·18, 0·70.

Fractionation on cellulose. A column of powdered cellulose (15 × 7 cm.; Whatman, Ashless) was prepared with butanol–acetic acid. The mixed peptides *D*—*H* were dissolved in the same solvent (15 c.c.) and put on the column, which was eluted with butanol–acetic acid, and the eluate collected in 35 fractions (each 15 c.c.). Fractions 17—33 contained peptides or amino-acids, fractions 23—33 contained acid-hydrolysable formaldehyde, fractions 17—20 contained *D* and *E*, fraction 21 *D*, *E*, and *F*, fraction 22 *E*, *F*, *G*. and *H*, and fractions 23—33 *G* and *H*. Fractions 23—33 were mixed, evaporated to dryness under reduced pressure and the solid dissolved in butanol–propanol–diethylamine. This solution was put on a cellulose column (10 g.) which was then developed with the same solvent, and the eluate collected in twenty-five fractions (each 15 c.c.); fractions 3—6 contained *G* and *H* with traces of *D*, *E*, and *F*; fractions 7—13 contained *G* and *H*; and fractions 15—23 *H* only. All fractions contained acid-hydrolysable

* In the case of polypeptides other than *A*, mentioned in Table 4, formaldehyde was detected qualitatively only; quantitative work is in progress.

formaldehyde. The last-mentioned fractions 15—23 were evaporated to dryness under reduced pressure and the solid hydrolysed with 5N-hydrochloric acid (5 c.c.) for 30 hours. After removal of the hydrochloric acid by evaporation under reduced pressure the residue was dissolved in water (2 c.c.) and put on two-dimensional paper chromatograms with phenol, butanol–acetic acid, and butanol–propanol–diethylamine as solvents. The amino-acids found, with R_F values in the order of the solvents were: aspartic, 0·17, 0·18, 0·04, weak; glutamic, 0·32, 0·28, 0·05, strong; serine, 0·35, 0·20, 0·12, very strong; alanine, 0·54, 0·29, 0·20, strong; leucine, 0·85, 0·67, 0·54, strong.

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