THE EXAMINATION OF A BACTERIOLOGICAL PEPTONE

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Paper chromatography has been used for the qualitative examination of the peptides in three batches of "Oxoid" bacteriological peptone. Batches A, B and C have shown in addition to the free amino acids 54, 49 and 50 peptides respectively. Variation occurs in the peptide content of the three batches of peptone examined.

IN a previous paper[1] the separation of the free amino acids and those in a peptide form in "Oxoid" bacteriological peptone were reported. In the present paper the qualitative identification of the constituent peptides by paper chromatography is described.

EXPERIMENTAL

Three batches were subjected to separation into basic, acidic, neutral and aromatic groups as previously described using the method of Fromageot, Justiz and Lederer[2]. Each was examined by placing 0·003 ml. at 1·5 cm. distance on a line drawn 2 cm. from the bottom of a Whatman No. 1 paper $11\frac{1}{4}$ in. $\times$ $18\frac{1}{2}$ in. The basic, neutral and aromatic groups were developed three times with a butanol—acetic acid—water system[3], This multiple development has been used for sugars[4] and amino acids[5], and was used here satisfactorily with peptides, the spots being more compact and the separation more complete. For the acidic group a butanol—acetic acid—water system gave trailing spots but it was found that a benzyl alcohol—acetic acid—water system, 4:1:5 by volume, gave better results.

After development the paper was dried for 30 minutes at 60°. Two strips were cut from either end of the paper sheet and the presence of the different fractions revealed by the ninhydrin colour reaction. Each group gave rise to several fractions, each representing either a single amino acid or a mixture of amino acids and peptides. Using the two strips as guides, horizontal strips were cut from the remnants of the paper and each fraction eluted with water[6]. Each eluate was evaporated to dryness at room temperature in a vacuum desiccator over silica gel.

The residue was dissolved in 0·08 ml. 10 per cent *iso*propanol and 0·003 ml. samples were subjected to chromatography with firstly butanol —acetic acid—water, secondly phenol saturated with buffer pH 6·2, the paper being buffered at pH 6·2 and thirdly a *m*-cresol—ammonia[7] 0·03 per cent system to examine its homogeneity. Buffered paper gave good separation but the resulting eluate contained salts which resulted in unsatisfactory chromatograms, and extraction of the dried eluate with acetone containing 1 per cent v/v concentrated hydrochloric acid[8] failed to extract

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the peptides completely. The *m*-cresol—ammonia 0·03 per cent system was used for separative chromatography in place of phenol buffered paper. Finally each fraction was developed using a solvent system that produced the best separation into subfractions. Each subfraction was completely hydrolysed with concentrated hydrochloric acid and subjected to two dimensional paper chromatography.

Table I shows the number of fractions, subfractions and peptides present in each group from the three batches of bacteriological peptone.

TABLE I

ANALYSIS OF A BACTERIOLOGICAL PEPTONE

Batch	Number of Fractions			Number of Subfractions			Number of Peptides		
	A	B	C	A	B	C	A	B	C
Basic Group	10	8	7	19	14	17	15	12	11
Acidic Group	6	4	4	10	13	13	8	12	13
Neutral Group ..	9	7	9	29	30	24	22	17	15
Aromatic Group ..	7	7	8	10	8	17	9	8	11
TOTAL	32	26	28	68	65	71	54	49	50

RESULTS

Tables II-V show the amino acid content of the peptides separated from the acidic, basic, neutral and aromatic groups respectively of batch A. Arbitrary figures ranging from 1–10 indicate the relative amounts of amino acid on the chromatogram judged from the size of the spot and the intensity of the colour; "trace" represents a very weak spot. Some subfractions show richness in a particular amino acid which is most probably due to its presence in the free state but contaminated with a peptide. Some peptides occur in different subfractions, this may be the result of their forming trails due to their length or to their having very close R_F values.

The three batches of peptone showed the same free amino acids, but very few similar peptides like those in the fractions F1–1 of the basic group (see subscript of Table II), F1 and F3–1 of the acidic group, F1 of the neutral group and F1–1 and F1–2 of the aromatic group. The majority of the peptides in batch B and C differ from those of batch A; in some cases the variation is slight. Batches A and B are poor in ornithine while rich in arginine; the reverse is true for batch C.

DISCUSSION

The method of Fromageot and his colleagues did not effect complete separation of the peptides into groups. Silica gel satisfactorily separated the basic peptides in the three batches of peptone, shown by the preponderance of the basic amino acid in their hydrolysates, while acid alumina adsorbed only acidic peptides yet separated them incompletely. Charcoal separated the majority of peptides containing aromatic amino acids. The non-specific adsorption of peptides on the different adsorbents may be due to the complexity of the mixture and to the length of

TABLE II

THE HYRDOLYSATES OF FRACTIONS* OBTAINED FROM ACIDIC GROUP—(BATCH A)

Fraction	R_F	Asp	Glu	Orn	Lys	Arg	His	Gly	Ala	Val	Met	Leu	Ser	Thr	α-Amino butyric acid	Tyr	Phe	Pro	Hypro
F1	0·16–0·24(b)	2	2	—	2	1	—	2	1–2	1	—	1	2	1	—	—	—	—	—
F2–1	0·16–0·26(b)	2	2	—	2	1	—	2	2	1	—	1	2	1	—	—	—	—	—
F3–1	0·09–0·18(b)	1	2	—	1	1	—	2	2	1	—	1	2	1	—	—	—	—	—
F4–1	0·11–0·17(b)	1	—	—	—	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F4–2	0·17–0·29(b)	2	4	—	—	—	—	2	2	1	1	1	2	1	—	—	—	1	1
F5–1	0·11(b)	trace	1	—	trace	—	—	trace	trace	—	—	—	trace	—	—	—	—	—	—
F5–2	0·17(b)	1	(6)†	—	—	—	—	1	1	—	—	—	1	1	—	—	—	—	—
F5–3	0·19–0·36(b)	1	5	—	1	1	—	2	1	1	1	1	2	1	—	—	—	—	—
F6–1	0·15–0·26(b)	2	3	—	—	1	—	2	2	1	—	1	2	—	—	1	1	1	1
F6–2	0·26–0·59(b)	1	2	—	1	—	—	2	2	2	1	2	2	1	—	trace	—	—	—

* In the fractions shown, the first figure indicates the number of the fraction and the second the number of the subfraction.
† The number in parentheses indicates that the amino acid is most likely to be present in the free state.
(b) R_F in butanol—acetic acid—water.
(c) R_F in *m*-cresol—ammonia.

TABLE III

THE HYDROLYSATES OF FRACTIONS* OBTAINED FROM BASIC GROUP—(BATCH A)

Fraction	R_F	Asp	Glu	Orn	Lys	Arg	His	Gly	Ala	Val	Met	Leu	Ser	Thr	α-Amino butyric acid	Tyr	Phe	Pro	Hypro
F1–1	0–0·14(b)	1	2	—	4	2	—	2	2	1	—	1	1	1	—	trace	—	1	1
F1–2	0·2(b)	—	2	—	2	1	—	1	1	1	—	1	1	—	—	—	—	—	—
F2–1	0·29–0·4(c)	—	—	(2)	(4)	1	—	—	—	—	—	—	—	—	—	—	—	—	—
F2–2	0·4–0·51(c)	—	1	1	(5)	1	1	1	1	trace	—	trace	1	trace	—	—	—	—	—
F2–3	0·51–0·91(c)	—	1	trace	3	1	1	1	1–2	trace	—	trace	1	trace	—	—	—	—	—
F3–1	0·55–0·71(c)	—	1	1	1	(4)	1	1	1–2	trace	—	trace	1	trace	—	—	—	1	1
F3–2	0·71–0·91(c)	—	1	1	3	4	2	1	4	1	—	1	1	1	—	—	—	1	1
F4–1	0·53–0·71(c)	—	1	—	1	2	—	1	1	—	—	—	1	—	—	—	—	—	—
F4–2	0·71–0·97(c)	—	1	1	2	3	1	1	3	1	—	1	1	trace	—	—	—	—	—
F5	0·13–0·2(b)	1	1	—	3–4	2	1	2	2	2	—	1	1	1	—	—	—	—	—
F6	0·13–0·2(b)	1	1	—	3–4	2	1	3	2	2	—	1	2	1	—	—	—	—	—
F7–1	0·2(b)	—	—	—	1	—	—	1	2	1	—	1	1	—	—	—	—	—	—
F7–2	0·23–0·31(b)	1	—	—	2	1	1	1	2	1	—	1	1	—	—	—	—	—	—
F8–1	0·14–0·23(b)	—	—	—	3	1	—	1	1	—	—	—	1	—	—	—	—	—	—
F8–2	0·3(b)	—	1	—	4	2	1	2	2	1	—	2	2	1	—	—	—	1	1
F9–1	0·19(b)	—	—	—	3	1	—	1	1	—	—	—	1	—	—	—	—	—	—
F9–2	0·3–0·46(b)	—	1	1	1	1	—	1	1	1	—	2	1	—	—	—	—	—	—
F10–1	0·15(b)	—	—	—	1	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F10–2	0·34–0·52(b)	—	1	—	1	1	—	1	1	1	1	1	1	trace	—	—	1	1	1

* See footnote to Table II.

TABLE IV

THE HYDROLYSATE OF FRACTIONS* OBTAINED FROM NEUTRAL GROUP—(BATCH A)

Fraction	R_F	Asp	Glu	Orn	Lys	Arg	His	Gly	Ala	Val	Met	Leu	Ser	Thr	α-Amino butyric acid	Tyr	Phe	Pro	Hypro
F1	0–0·11(b)	1	2	1	2	1	—	3	2	1	—	1	2	1	—	—	—	—	—
F2	0–0·12(b)	1	2	1	2	1	—	1	1	—	—	—	1	—	—	—	1	—	—
F3–1	0·13(c)	2	3	trace	1	1	—	1	1–2	—	—	—	1–2	1	—	—	—	—	—
F3–2	0·13–0·25(c)	1	2	—	2	1	—	1	2	1	—	1	1	1	—	—	—	—	—
F3–3	0·25–0·69(c)	1	2	trace	2	1	—	2	2	1	—	1	1	1	—	—	—	—	—
F4–1	origin(c)	1	3	—	1	—	—	1	1	—	—	—	2	1	—	—	—	—	—
F4–2	0·02–0·07(c)	trace	2	—	1	—	—	(4)	2	1	—	1	(4)	1	—	—	—	—	—
F4–3	0·07–0·13(c)	trace	trace	—	—	—	—	(4)	—	—	—	—	(4)	—	—	—	—	—	—
F4–4	0·13–0·34(c)	—	2	1	1	—	—	1	2	1	—	—	1	—	—	—	—	—	—
F4–5	0·4–0·57(c)	trace	2	—	1	trace	—	1	1	1	—	1	1	—	—	—	—	—	—
F5–1	origin(c)	1	4	1	1	1	—	1	2	trace	—	trace	2	1	—	—	—	—	—
F5–2	0·03–0·11(c)	trace	1	—	trace	—	—	1	2	trace	—	trace	1	(3)	—	—	—	—	—
F6–1	origin(c)	1	4	—	1	trace	—	1	4	1	—	1	2	1	—	—	—	—	—
F6–2	0·03–0·14(c)	trace	2	—	—	1	—	2	(5)	1	—	1	2	1	—	—	—	—	—
F6–3	0·14–0·28(c)	trace	2	—	1	1	—	1	1–2	1	—	1	1	1	—	—	—	1	1
F6–4	0·28–0·77(c)	trace	2	—	1	1	—	1	2	1	—	1	1	1	—	—	—	1	1
F7	0·15–0·29(c)	—	2	trace	1	1	—	2	2	1	1	1	2	1	—	1	—	—	—
F8–1	0·18(b)	—	1	—	1	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F8–2	0·28(b)	—	1	—	1	—	—	1	3	1	—	1	1	1	1	—	—	—	—
F8–3	0·34(b)	1	3	—	1	1	—	1	2	1	—	1	1	trace	1	—	—	—	—
F9–1	0·37(b)	1	1	—	1	1	—	1	1	1	—	1	1	—	(4)	—	—	—	—
F10–1	0·16(b)	—	1	—	1	—	—	1	1	—	—	trace	1	—	—	—	—	—	—
F10–2	0·33–0·37(b)	—	—	—	—	—	—	1	—	1	—	—	1	—	1	—	—	—	—
F10–3	0·37–0·47(b)	1	2	—	1	trace	—	2	2	(7)	(3)	2	1	1	1	—	—	—	—
F11–1	0·15(b)	—	1	—	1	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F11–2	0·38(b)	—	2	—	trace	trace	—	1	1–2	1	1	1	1	1	—	—	—	—	—
F11–3	0·43–0·6(b)	trace	2	—	1	1	—	1–2	3	2	—	(8)	1	1	—	trace	1	1	1
F11–4	0·6–0·71(b)	trace	2	—	1	1	—	1–2	3	2	—	3	1	1	—	trace	trace	1	1

* See footnote to Table II.

TABLE V

THE HYDROLYSATES OF FRACTIONS* OBTAINED FROM AROMATIC GROUP—(BATCH A)

Fraction	R_F	Asp	Glu	Orn	Lys	Arg	His	Gly	Ala	Val	Met	Leu	Ser	Thr	α-Amino butyric acid	Tyr	Phe	Pro	Hypro
F1–1	0–0·12(b)	1	2	—	2	1	—	2	2	1	—	1	2	1	—	1	1	1	1
F1–2	0·12–0·15(b)	1	2	—	1–2	1	—	1–2	2	1	—	1	1	1	—	1	1	1	1
F1–3	0·15–0·3(b)	1	2	trace	2	1	—	2	2	2	1	2	1–2	1	—	1	1	1	1
F2	0·17(b)	1	2	trace	2	1	—	2	2	2	1	2	1–2	1	—	1	1	1	1
F3	0·21–0·31(b)	1	2	trace	1–2	1	—	1	2	2	—	2	1	1	—	1	1	1	1
F4	0·27–0·41(b)	1	2–3	1	2	1	—	1–2	2	2	1	2–3	1	1	—	(3)	1	1	1
F5–1	0·16(b)	—	—	—	—	—	—	1	1	—	—	—	1	—	—	—	—	—	—
F5–2	0·33–0·53(b)	1	2	trace	2	1	—	1	2	2	1	3	1	1	—	2	1–2	1	1
F6	0·5–0·63(b)	1	2	—	1	1	—	1–2	2	2	1	3	1–2	1	—	trace	(3)	1	1
F7	0·52–0·8(b)	1	2	1	1	1	—	1–2	2	3	1	4	1–2	1	—	1–2	2	1	1

* See footnote to Table II.

the peptide chain involved. But this method of separation is suitable for a preliminary group separation of such a complex mixture of peptides.

Chromatography using a multiple development technique resulted in the separation of each group into several fractions that were subsequently separated into their constituent peptides.

Batches A, B and C showed respectively 54, 49 and 50 peptides; few were identical while the remainder showed some variation either in their amino acid content or in the relative strength of some amino acids. This variation may be due to slight differences in the conditions under which the peptone was prepared or to the synthesis of some new peptide bonds during the enzymatic hydrolysis of the blend of proteins used in the manufacture of the peptone.

The manufacturers consider that ornithine present is most likely produced from arginine as one part of the hydrolysis takes place under alkaline conditions. Similarly α-amino butyric acid may be produced from threonine as there is no satisfactory evidence that it occurs in any native protein.

The unknown ninhydrin positive spots previously reported[1] were not found.

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