A STUDY OF BACTERIOLOGICAL MEDIA
THE EXAMINATION OF PROTEOSE-PEPTONE*

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The free and total amino acid content of three batches of Proteose-Peptone is presented. The presence of hydroxyproline and the high content of glycine and alanine suggest that a part of the protein used in the manufacture belongs to collagen. The three batches have shown similar strepogenin activity.

IN previous papers "Oxoid" peptone[1,2], Bacto-Casitone[3,4] and Casamin E[5] were examined for their constituent amino acids and peptides. This communication describes the quantitative estimation of the free and total amino acids in three batches of Proteose-Peptone (B120)[6], which is particularly adapted for use for the production of various bacterial toxins[6-8].

EXPERIMENTAL AND RESULTS

Three batches of Proteose-Peptone designated A, B and C were examined for the free and total amino acids and for their strepogenin activity using methods previously described[3,5]. The results are given in Tables I and II.

TABLE I

THE QUANTITATIVE ESTIMATION OF THE FREE AND TOTAL AMINO ACIDS OF PROTEOSE-PEPTONE

Amino acid	Free amino acid g./100 g.			Total amino acids g./100 g.		
	A	B	C	A	B	C
Gly	0·36	0·29	0·32	10·6	11·1	11·1
Ala	0·64	0·51	0·57	6·4	6·5	6·1
Val	0·48	0·38	0·48	4·3	4·1	4·2
Leu's	1·99	1·67	1·93	9·4	8·8	8·8
Ser	0·63	0·50	0·56	3·4	3·4	3·4
Thr	0·59	0·44	0·53	4·3	4·3	4·2
Tyr	0·64	0·47	0·7	1·9	1·8	1·9
Phe	1·41	1·22	1·46	3·6	3·2	3·4
Met	0·56	0·77	0·93	1·6	1·6	1·5
Arg	0·34	0·33	0·43	6·7	6·8	6·7
His	0·30	0·17	0·14	2·0	2·0	2·0
Cys	—	—	—	0·45	0·5	0·54
Lys	0·36	0·30	0·33	5·3	5·0	5·4
Pro	0·37	0·25	0·29	5·4	5·4	5·3
Asp, Glu	2·34	2·05	2·15	18·2	18·0	18·5
Try	0·23	0·15	0·23	—	—	—
Hypro	—	—	—	3·9	4·3	3·8
Total	11·24	9·50	11·06			

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TABLE II

THE EFFECT OF PROTEOSE-PEPTONE ON THE GROWTH OF *L. CASEI*

Time hours	Control	Batch A mg.			Batch B mg.			Batch C mg.			Wilson Liver L, mg.		
		0·1	0·5	1·0	0·1	0·5	1·0	0·1	0·5	1·0	0·1	0·5	1·0
		A– Medium of Steele and others*											
24	0	16	23	63	10	22	63	12	23	52	10	15	20
48	5	83	182	232	68	167	239	82	191	232	33	78	143
72	204	129	238	269	122	221	279	132	241	266	124	132	205
		Acid Production**											
48	9	0·95	3·05	4·75	0·95	3·25	4·70	1·05	3·55	4·85	0·6	1·35	2·8
72	4·15	3·35	7·15	9·35	3·05	6·40	8·75	3·30	7·55	8·25	1·95	3·3	5·35
		B– Medium of Kodicek and Mistry*											
24	54	90	169	210	81	164	191	102	159	210	63	105	143
48	272	278	303	325	280	302	320	280	297	318	266	285	295

* Scale reading of Klett-Summerson colorimeter.
** ml. of 0·077 N sodium hydroxide.

The chromatograms of DNP-amino acids and peptides were similar to those obtained with Bacto-Casitone[3] and Casamin E[5] with the exception of a spot that appeared on standing of the ether extract and seemed to be an artifact. The peptide spots were found to consist of a mixture of peptides as evidenced by *N*-terminal amino acid analyses.

DISCUSSION

Proteose-Peptone is recommended as an ingredient of bacteriological media for the production of toxins. It has been examined to see whether it has any characteristic features to distinguish it from the bacteriological media previously examined[1–5]. From Table I it is seen that there exists some variation in the individual free amino acids among the three batches examined. The total free amino acids varies from 9·5 to 11·2 per cent, this is lower than that for Bacto-Casitone 15·9 to 17·5 per cent and Casamin E 24·7 to 41·8 per cent. Proteose-peptone thus contains a slightly higher per cent of peptides than the other media reported. The total amino acids were similar in the three batches showing that the proteins used for their manufacture are identical. The high glycine, alanine and arginine and the presence of hydroxyproline may reflect the features of the protein used for the preparation of Proteose-Peptone. As the collagen exhibits the characteristic feature of containing hydroxyproline and having a high proportion of glycine[9], it seems likely that a part of the protein used in the manufacture belongs to this group. It is seen also from Table I that the pattern in which the free amino acids exists in Proteose-Peptone is different from Bacto-Casitone[3] and Casamin E[5]. In the latter two, pancreatic digestion was used for their preparation. Bovine pancreatic juice[10] is composed of trypsin, chymotrypsin and two carboxypeptidases. From the specificity of trypsin and chymotrypsin it is anticipated that peptides containing arginine and lysine in the *C*-terminal position are liberated by the action of the former while peptides with aromatic amino acids in the *C*-terminal position are produced by the action of the latter. The resulting peptides are further acted upon by carboxypeptidases resulting in the liberation of the basic and aromatic amino acids. As a result of the action of the pancreatic enzymes there is a high proportion of the free

aromatic and basic amino acids and a low content of proline. This was found to be the case in Bacto-Casitone and Casamin E. In the case of Proteose-Peptone the liberation of arginine and lysine is about one-tenth that in Bacto-Casitone and Casamin E. This suggests that a different procedure of enzymatic digestion has been used for its preparation.

The spots examined were found to be a mixture of peptides and there appears to be some variation in the peptide constituents in corresponding spots.

The stimulatory effect on the growth and lactic acid production of *L. casei* was similar in magnitude in the three batches as seen in Table II. The pattern of stimulation in the two basal media was similar to results obtained previously with Bacto-Casitone and Casamin E.

There was no significant difference between the strepogenin activity of Proteose-Peptone and that of Bacto-Casitone and Casamin E. The strepogenin effect can be evoked by peptides which differ considerably in their sequence structures as well as their amino acid content[1]. This may explain the similarity of the strepogenin effect of Bacto-Casitone, Casamin E and Proteose-Peptone which differed markedly in the amino acid content of their peptides. Various peptones[7,8] including a casein hydrolysate, were examined as constituents of a medium for toxin production of *Corynebacterium diphtheriae* and it was found that Proteose-Peptone was the most satisfactory. It seems most likely that the toxin factor is more specific than strepogenin, and that the peptides responsible for the toxin production may differ from those with strepogenin effect. Peptides with strepogenin activity were found not to enhance the multiplication of cultured appendix cells[12].

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