LETTERS TO THE EDITORS

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Nature of Peptones

IN a letter published in NATURE[1], a method was described by us whereby the ratio

$$\frac{\text{N-total cleavable}}{\text{N-split hydrolytically}}$$

may be easily computed, and hence the extent of cleavage which has been performed by one or several proteinases acting on a given protein or peptone determined. Since then, still more proteins and peptones have been investigated and it seems desirable to give a brief account of the results achieved. It should be emphasized beforehand, however, that the proteins investigated by us generally showed the same behaviour as regards their cleavability by the proteinases employed irrespective of their physical-chemical properties, chemical composition and source of occurrence, as indicated in Table 1 below.

(1) The ratio $\frac{\text{N-total cleavable}}{\text{N-split hydrolytically}}$ as obtained by exhaustive cleavage with pepsin-hydrochloric acid was in all cases equivalent to a Z value of 4 with the sole exception of casein (Table 1).

TABLE 1.

Substrate	Cleaved exhaustively by	N-total cleavable / N-split hydrolytically
Ovalbumin-pepsin peptone	Pepsin-hydrochloric acid	4·02
,, ,, ,,	Pancreatic proteinase	4·02
Pancreatin proteinase peptone from ovalbumin (cleaved exhaustively)	—	3·96
Fibrin pepsin peptone	Pepsin-hydrochloric acid	4·10
Casein pepsin peptone	,, ,, ,,	4·82
,, ,, ,,	Pancreatic proteinase	3·93
Pancreatic proteinase-peptone from casein	Pancreatic proteinase	3·82

(2) All peptones obtained from proteins by incomplete splitting with pepsin-hydrochloric acid (thus leaving a substantial margin for further action by additional enzyme) are split by the succeeding action of purified pancreatic proteinase (tested as to its freedom from protaminase and polypeptidases) to the above ratio of 4 (Table 1).

(3) Exhaustive cleavage by pepsin-hydrochloric acid followed by exhaustive cleavage by pancreatic proteinase reduced the above ratio in all cases to a value of 3 (Table 2).

TABLE 2.

Substrate	Cleaved exhaustively by	N-total cleavable / N-split hydrolytically
Ovalbumin pepsin peptone	Pepsin-hydrochloric acid followed by exhaustive cleavage with pancreatic proteinase	3·18
Casein pepsin peptone	,, ,, ,,	3·10
Fibrin pepsin peptone	,, ,, ,,	3·09

(4) Proteins split by the action of pancreatic proteinase alone were found to give the same nitrogen ratio as obtained by the action of pepsin-hydrochloric acid, namely, 4 (Table 1).

These results are summarized in the accompanying tables.

The peptones prepared from proteins by cleavage with pepsin-hydrochloric acid as shown in Table 1 were not subjected to exhaustive splitting during the process of preparation, since this would involve the addition of substantial amounts of enzyme carrier protein which might cause aberrations from the real N-values, etc., of the substrates investigated. The further degree of cleavage which might be performed by this enzyme or any other proteinase employed in succession had always been observed in enzyme tests and added to the N-value corresponding to the free (NH_2 + NH)-groups found in the peptone (see ref. 1).

A detailed report will be given elsewhere.

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Growth Stimulation of *L. casei* E. by Pyrimidines

PURINE and pyrimidine derivatives have been shown to be growth stimulators for a number of organisms; uracil has been shown necessary for *L. arabinosus* and *Leuconostic mesenteroides*[1], and the anaerobic growth of *Staph. aureus*[2], thymine for *S. Lactis*[1], adenine for *L. arab.*, *L. pentosus*[1], and adenine and guanine for *L. plantarum*[3]. Adenine, guanine, uracil and xanthine have been included in media for the growth of *L. casei*[4,5], and Feeney and Strong[6] have shown adenine and guanine to be stimulatory for *L. casei* E. under certain conditions.

During work on the purification of unknown factors present in liver and required for the growth of *L. casei* E., it was found that the active material displayed properties which suggested the presence of purine or pyrimidine derivatives, and accordingly a number of synthetic compounds were tested for growth-promoting activity. Of those tried only one was found to give any response, namely, orotic acid (uracil 4-carboxylic acid).

A casein hydrolysate basal medium was used, the same as that described by Chattaway, Happold and Sandford[7] with the inclusion of riboflavin (40 μgm./l.) and biotin (5 μgm./l.) and without the addition of a liver eluate. The inoculation was a loopful of a faintly opalescent suspension of bacterial cells in sterile water, and growth was estimated by titration of the lactic acid produced after 72 hr. incubation at 37° C., using brom-thymol blue as indicator. Titrations from 0·5–2 c.c. $N/10$ lactic acid have been repeatedly obtained with the derivative mentioned in a minimum concentration of 0·01 μgm./ml. medium, with no growth in control flasks. The response has varied from time to time and on a few occasions there has been no response, and this is ascribed to variations in the casein hydrolysate used, since the concentration of unknown factors in the hydrolysate may vary. Substances which have been found not to