CCXXXVI. THE HYDROLYSIS OF CASEINOGEN BY PEPSIN AND BY TRYPSIN-KINASE.

BY JOHN DEMPSTER STIRLING
AND GEORGE MACFEAT WISHART.

*From the Hannah Dairy Research Institute, Kirkhill, Ayr,
and the Institute of Physiology, Glasgow University.*

(Received October 21st, 1932.)

DURING the past few years the existence of an integral molecule of caseinogen has been questioned. Linderstrøm-Lang [1928] has extracted caseinogen with a variety of warm (60°) solvents (chiefly 60 % alcohol with or without $N/1000$ HCl and dilute HCl-NaCl solutions) and has been able to separate the protein into various fractions which differ in a number of respects, notably in their phosphorus contents. The interpretation of these experiments is difficult, for no information has, as yet, been obtained as to whether the reaction between solvent and substrate is physical or chemical. Linderstrøm-Lang, however, believes that caseinogen is heteromolecular, that it is in reality a loose physical association of several proteins of different phosphorus contents which, in all the processes to which caseinogen is ordinarily subjected, form a co-precipitation system.

Whatever interpretation be adopted, whether the various fractions are to be considered as existing chemically bound to give an integral caseinogen molecule, or whether they are held together in a species of loose physical association, it would render untenable the idea that all the phosphorus of caseinogen is concentrated in one "prosthetic" group. This is difficult to reconcile with the finding of Rimington [1927, 1] that, by the action of dilute alkali on caseinogen, he could obtain a protein containing 11 to 13 % nitrogen and only slight traces of phosphorus. Rimington [1927, 2] was also able to isolate from tryptic digests of caseinogen an enneapeptide triphosphoric ester (phosphopeptone) in amount representing about 50 % of the original caseinogen phosphorus. In terms of the Linderstrøm-Lang hypothesis, caseinogen must then be a loose physical association of large molecules, which, when subjected to the disruptive action of alkali, loses its phosphorus and still remains a co-precipitation system—the "dephosphorised caseinogen" of Rimington. Such a requirement imposes a considerable strain on the "loose association" hypothesis.

Rimington and Kay [1926] have investigated the action of pepsin and trypsin¹

¹ Trypsin, in our paper, is used to denote the mixture of enzymes usually found in pancreatic extracts. This consists, according to the nomenclature of Waldschmidt-Leitz [1929], of trypsin (inactivated) trypsin-kinase (activated) and erepsin. Of these only trypsin-kinase has any action on caseinogen, although the others can hydrolyse its lower degradation products.

on caseinogen. They confirmed the findings of previous workers that during peptic digestion an insoluble product is formed (para- or pseudonuclein) which contains a large proportion of the caseinogen phosphorus. They also showed that, at the end of 9 days' digestion with pepsin, no measurable amount of free phosphate was obtained, though all the caseinogen phosphorus was now in a form not precipitated by trichloroacetic acid. With trypsin, it was found that there was a fairly rapid liberation of organically-bound acid-soluble phosphorus with accompanying slow liberation of inorganic phosphorus. The liberation of amino-acid nitrogen was followed simultaneously with that of acid-soluble phosphorus but no information was obtained regarding the partition of phosphorus between the various products formed at different stages of the digestion. Linderstrøm-Lang and his co-workers have recently [Holter, Linderstrøm-Lang and Funder, 1932] studied, in some detail, the peptic digestion of caseinogen and of the fractions obtained by Linderstrøm-Lang [1928]. They found that the precipitates (paranuclein) formed in each case had almost identical N/P ratios but that these values were quite different from that obtained by Rimington for his phosphopeptone. Linderstrøm-Lang suggests that paranuclein may be formed during tryptic digestion and that it may be the precursor of Rimington's phosphopeptone. In this connection it must be remembered that the solubility of paranuclein may be a function of p_H and its failure to precipitate during tryptic digestion may be due to the alkaline reaction of the digestion solution. None of the work quoted can therefore be taken to indicate that the action of pepsin on caseinogen is fundamentally different from that of trypsin. Waldschmidt-Leitz and Simons [1926] have demonstrated that both pepsin and trypsin-kinase act on caseinogen by the rupture of amide-linkages, *i.e.* for every amino-group set free a carboxyl is liberated. To demonstrate whether or not the same amide-linkage of caseinogen is attacked by both enzymes requires other methods of investigation than those already reviewed.

With regard to the initial attack of trypsin on caseinogen Northrop [1924] has made certain assumptions on which he has built a method for investigating the kinetics of the action of the enzyme. Northrop wished to study the effect of increase of caseinogen concentration on the rate of disappearance of caseinogen and he was forced to employ a method which, at any instant, would separate the protein from its cleavage products and so allow the amount of the former to be estimated. To this end he added trichloroacetic acid to the digestion solution at successive intervals of time and assumed that the precipitate represented unchanged caseinogen. He makes the statement that the first step in the action of trypsin, *i.e.* the hydrolysis of the caseinogen molecule itself, results in the formation of products completely soluble in trichloroacetic acid. If Northrop's conception of the initial step of the hydrolysis of caseinogen be correct, we should expect that, at any instant during digestion, the N/P ratio of a trichloroacetic acid filtrate should be identical with that of the original caseinogen. Preliminary experiments carried out by

us showed that this was by no means the case. Moreover we have found significant differences between the behaviour of pepsin and trypsin so far as the course of the acid-soluble N/P ratio is concerned, differences which appeared to indicate distinct differences in the mode of attack of the two enzymes. Accordingly, in the present paper, we report experiments designed to follow the liberation from caseinogen by the action of pepsin and of trypsin-kinase of nitrogen and phosphorus compounds soluble in trichloroacetic acid.

It would appear that no disadvantage is attached to the use of commercial pepsin preparations in view of their freedom from other proteolytic enzymes and accordingly we have employed B.D.H. pepsin (granular) in these studies. The use of commercial trypsin, however, appears to require some justification. Willstätter *et al.* [1926] have shown that, of the pancreatic proteases, only trypsin-kinase has any action on caseinogen. The others have no action on any molecule of magnitude greater than polypeptides and the latter being soluble in trichloroacetic acid the action of the peptidases will be confined entirely to the acid-soluble material resulting from the hydrolysis of caseinogen and its acid-insoluble degradation products. The change from acid-insoluble to acid-soluble will therefore be due entirely to the action of trypsin-kinase. We have used, in these experiments, an Allen and Hanbury's trypsin preparation without further purification, and we shall refer to the action of this preparation as being that of trypsin-kinase.

In view of the doubt expressed regarding the constancy of the composition of caseinogen, it is well to refer to the source of our specimen. It was prepared from milk by the improved electrodialysis method of Van Slyke [1923] and was a pure white product of N/P ratio 20.0 (caseinogen "nach Hammarsten" has N/P 18.6 while the purest specimen obtained by Van Slyke had N/P 22.2). It contained a negligible quantity of acid-soluble nitrogen and no acid-soluble phosphorus.

EXPERIMENTAL.

Hydrolysis by trypsin-kinase.

Caseinogen solution. This was made up fresh before each experiment and of twice the concentration desired in the digestion solution. The caseinogen was rubbed to a paste with distilled water in a mortar and dilute NaOH added slowly with stirring (0.8 cc. *N* NaOH per g. caseinogen). When all the alkali had been added the liquid was centrifuged and the clear solution removed from a slight sediment and diluted to 100 cc. with distilled water. In this way a solution of p_H slightly greater than 7.0 was obtained.

Enzyme solution. This was a 1 % filtered solution of Allen and Hanbury's trypsin and was made up fresh for each experiment. The amount of enzyme required to achieve the degree of hydrolysis desired in a given time was determined by preliminary experiment and was obtained by dilution of the 1 % solution.

Buffer solution. The optimum range of trypsin activity is p_H 7.8–8.7 [Waldschmidt-Leitz, 1923–24]. During digestion of caseinogen there is a very considerable shift of p_H towards the acid side and with the higher protein concentrations buffering is a matter of some difficulty. In our experiments the p_H has been followed throughout the course of the digestion (hydrogen electrode) and the maximum shift found was about 0.3 p_H . As the digestion solutions were always initially buffered to a p_H near to the upper limit of the optimum range it can be said that at no time, during the course of the digestion, was the enzyme subjected to a p_H below the lower limit of the optimum. The buffer used was the KCl-borate mixture (p_H 8.8) of Clark and Lubs [Clark, 1928] and was made up in a concentrated solution. (Clark and Lubs buffer was obtained by diluting 4 cc. of this concentrated solution to 10 cc.)

Procedure. A series of stoppered tubes was made up, each containing 5 cc. caseinogen solution and 4 cc. concentrated buffer solution. The tubes were placed in the thermostat and after they had attained the desired temperature (37°) 1 cc. diluted enzyme solution was added to each. At 10 or 15 minute intervals a tube was withdrawn and 10 cc. 10% trichloroacetic acid were added. After shaking, the contents were immediately filtered and acid-soluble nitrogen (micro-Kjeldahl) and phosphorus [Fiske and Subbarow, 1925] determined on aliquot portions of the filtrate. The total nitrogen and phosphorus and initial acid-soluble nitrogen and phosphorus contents were determined on enzyme-containing solutions which had not been placed in the thermostat. Controls were run by incubating tubes containing distilled water instead of enzyme solution. The changes followed by us were found to be due wholly to the presence of the enzyme as the buffer alone did not produce any hydrolysis.

Results. The amounts of acid-soluble nitrogen and phosphorus liberated at successive intervals of time with four different concentrations of caseinogen

Table I A. *Trypsin-kinase (0.5 mg. trypsin/10 cc. solution).*

Exp. No. } Fig. No. }		1		2		3		4	
Total N (mg. in 10 cc. solution)		8.77		19.60		25.81		44.71	
Total P (mg. in 10 cc. solution)		0.460		0.937		1.350		2.250	
(Time mins.)		N		N		N		N	
		P		P		P		P	
10	15*	4.66	0.334	3.08	0.384	3.43	0.203	4.41	0.25
20	30	6.50	0.421	6.37	0.609	5.74	0.397	8.02	0.48
30	45	7.00	0.460	8.99	0.745	8.16	0.570	10.33	0.66
40	60	7.66	0.461	10.82	0.857	10.37	0.740	13.30	0.99
50	75	8.18	0.463	12.49	0.916	12.01	0.850	16.69	1.20
60	90	8.60	0.463	14.06	0.930	13.62	0.991	18.45	1.36
70	105	8.54	—	14.81	—	14.14	1.00	21.60	1.60
80	120	—	—	15.29	—	14.70	1.01	21.81	1.66
90	135	—	—	15.65	—	15.48	1.04	24.50	1.73
100	150	—	—	16.35	—	16.28	1.11	26.08	1.84
110	165	—	—	16.45	—	17.85	1.14	20.83	1.87
120	180	—	—	—	—	17.92	—	27.55	1.93

* 15 minute intervals refer only to Exp. 4.

N = mg. acid-soluble N per 10 cc. solution.

P = mg. acid-soluble P per 10 cc. solution.

are shown in Table I A and curves showing the changes graphed as percentages of the total nitrogen and phosphorus are given in Figs. 1-4. The N/P ratios of the trichloroacetic acid precipitate at successive intervals of time were obtained by difference and are shown in Table II.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Hydrolysis by pepsin.

Caseinogen solution. This was made up in a manner similar to that for trypsin-kinase except that dilute HCl was used instead of alkali.

Enzyme solution. This was a 1 % filtered solution of granular pepsin (B.D.H.) the appropriate quantity of enzyme being obtained by dilution.

 Table I B. *Pepsin (15 mg./10 cc. solution).*

Exp. No. } Fig. No. }	5		6		7		8	
Total N (mg. in 10 cc. solution)	9.66		12.36		18.34		22.72	
Total P (mg. in 10 cc. solution)	0.515		0.652		0.959		1.23	
(Time mins.)	N	P	N	P	N	P	N	P
10	4.38	0.095	5.14	0.082	4.52	0.071	4.34	0.071
20	5.67	0.155	6.72	0.168	6.51	0.129	6.62	0.095
30	6.20	0.177	7.56	0.215	7.95	0.186	7.14	0.176
40	6.23	0.194	7.81	0.239	8.82	0.233	9.10	0.231
50	6.27	0.192	8.16	0.257	9.27	0.254	9.00	0.255
60	6.37	—	7.91	0.262	9.62	0.275	9.87	0.274
70	—	—	—	—	10.08	0.284	10.50	0.301
80	—	—	—	—	10.85	0.288	—	—
90	—	—	—	—	10.92	0.298	—	—
100	—	—	—	—	10.85	0.306	—	—
110	—	—	—	—	10.96	0.312	—	—

N = mg. acid-soluble N per 10 cc. solution.

P = mg. acid-soluble P per 10 cc. solution.

Buffer solution. The KCl-HCl mixture (p_H 1.8) of Clark and Lubs was used. The p_H optimum for pepsin as given by Northrop [1922] is 1.5–2.5 and our solutions throughout the course of the digestions were within this range.

Procedure: as for trypsin-kinase with the addition of one experiment in which six tubes containing the same amount of caseinogen but varying amounts of enzyme were incubated for 90 minutes. At the end of that time the amounts of acid-soluble nitrogen and phosphorus were estimated. This constituted Exp. 9.

Results. These are shown in Tables I B and II and in Figs. 5–8. The results of Exp. 9 are given in Table III.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Table II. *N/P ratios of trichloroacetic acid precipitates.*

Exp. No. Time (mins.)	Trypsin-kinase				Pepsin			
	1	2	3	4	5	6	7	8
10 15*	32.6	29.9	19.5	20.2	12.6	13.1	15.6	15.8
20 30	58.2	40.3	21.1	20.8	11.1	12.2	14.3	14.1
30 45	∞	55.3	22.6	21.7	10.3	11.6	13.4	13.7
40 60	—	109.7	25.3	24.9	10.7	11.7	13.1	13.6
50 75	—	338.6	27.6	26.7	10.5	11.4	12.9	14.0
60 90	—	791.4	34.0	29.6	—	12.1	12.7	13.4
70 105	—	—	33.3	35.6	—	—	12.2	13.2
80 120	—	—	32.7	38.8	—	—	11.2	—
90 135	—	—	—	39.6	—	—	11.2	—
100 150	—	—	35.5	46.1	—	—	11.3	—
110 165	—	—	37.9	—	—	—	11.3	—
120 180	—	—	—	54.5	—	—	—	—

* 15 minute intervals refer only to Exp. No. 4.

Table III. *Exp. 9.*

Total N (10 cc. solution) 15.40 mg. Total P (10 cc. solution) 0.770 mg.
Each tube incubated for 90 minutes.

Pepsin (mg. per 10 cc. solution)	Acid-soluble N		Acid-soluble P		N/P ratio of trichloroacetic acid ppt.
	(mg. per 10 cc. solution)	Percentage total N	(mg. per 10 cc. solution)	Percentage total P	
5	6.70	43.5	0.107	14.0	13.12
10	7.96	51.7	0.184	24.0	12.70
15	8.85	57.5	0.248	32.0	12.54
20	8.94	58.1	0.276	36.0	13.08
25	9.47	61.5	0.306	40.0	12.77
30	9.60	62.3	0.315	41.0	12.75

DISCUSSION.

The graphs show the relative rates of liberation of acid-soluble nitrogen and phosphorus during the digestion of caseinogen. In no case, either with trypsin-kinase or with pepsin, is it found that acid-soluble nitrogen and phosphorus are produced at the same rate, and it is therefore apparent that the first step in the degradation of caseinogen by proteolytic enzymes does not result in products which are completely soluble in trichloroacetic acid. This is quite contrary to the assumption made by Northrop [1924]. The fact that Northrop's assumption is unjustified is shown more clearly by Table II, in which are summarised the N/P ratios of trichloroacetic acid precipitates at successive intervals of time. It is quite apparent from these ratios that solubility in trichloroacetic acid is no index of the first stage of the degradation of caseinogen either by trypsin-kinase or pepsin. The caseinogen molecule can undergo very considerable disruption before becoming completely soluble in trichloroacetic acid and Northrop's work on the kinetics of trypsin action will have to be reconsidered from this new point of view.

Haldane [1930], commenting on Northrop's work, draws attention to his finding that the effect of substrate concentration is quite different according as the total action is measured by change in conductivity or production of amino-nitrogen, or by the actual destruction of caseinogen. Haldane suggests that this result may be due to the fact that Northrop's trypsin solutions contained carboxypolypeptidase and aminopolypeptidase (erepsin). These enzymes do not attack molecules of magnitude greater than polypeptides and would therefore play no part in the change from acid-insoluble to acid-soluble. A second alternative is suggested by Haldane, namely that trypsin has a greater affinity for the degradation products of caseinogen than for the protein itself. Such a hypothesis would be necessary to explain Northrop's finding that the amount of substance rendered soluble in trichloroacetic acid was almost directly proportional to the concentration of caseinogen between 1.5 and 5 % if the substance precipitated by trichloroacetic acid were wholly caseinogen. In our experiments this was certainly not the case and moreover we have found that the rate of liberation of acid-soluble nitrogen falls off

slightly with increase in caseinogen concentration. From the point of view of enzyme kinetics it is necessary that Northrop's work should be repeated using a method which will separate caseinogen completely from its cleavage products.

It will be seen from Figs. 1 to 8 that with trypsin-kinase acid-soluble nitrogen is liberated more slowly than acid-soluble phosphorus while, with pepsin, the reverse is true. This appears to indicate that the action of the two enzymes is fundamentally different, evidence for which can best be obtained by treating the data for each enzyme separately.

Trichloroacetic acid appears to separate peptic digests of caseinogen, in the initial stages of the hydrolysis of the protein, into two fractions: (1) insoluble in trichloroacetic acid and relatively rich in phosphorus (N/P ratio less than that of caseinogen), (2) soluble in trichloroacetic acid and relatively poor in phosphorus (N/P ratio greater than that of caseinogen). It is known that, at any one instant, several reactions are proceeding side by side during proteolysis and it might be expected that both the acid-soluble and acid-insoluble fractions would consist of mixtures of substances at various stages of degradation. That this is not the case, so far as the acid-insoluble material is concerned, may be ascertained from Table III. Here it is seen that, although the acid-soluble phosphorus has increased from 14 to 41 % of the total and the acid-soluble nitrogen from 44 to 62 %, the N/P ratio of the acid-insoluble material has remained practically constant. It is to be concluded therefore that, from the stage represented by tube No. 1 to that represented by tube No. 6 only one acid-insoluble substance was present and that the increase in acid-soluble material between these two stages resulted from the splitting of the acid-insoluble substance into two or more substances completely soluble in trichloroacetic acid.

Thus at least 86 % of the phosphorus of caseinogen can be split off in the form of a body insoluble in trichloroacetic acid and having N/P ratio of 12.8. From the fact that the precipitate from tube No. 1 had a N/P ratio of 13.1 we may infer that very little, if any, caseinogen still remained unattacked by the enzyme, and it is to be presumed that, in the 90 minutes which had elapsed since the beginning of the digestion, part of the non-caseinogen acid-insoluble material had acted as substrate and had contributed to the formation of acid-soluble phosphorus. It seems reasonable to conclude, therefore, that the initial action of pepsin on caseinogen is to split the protein into substances one of which is insoluble in trichloroacetic acid and contains all the phosphorus, while the other is soluble in trichloroacetic acid and contains no phosphorus. Although Exp. 9 demonstrates the cleavage of this phosphorus-containing acid-insoluble substance (N/P ratio 12.8) into fractions which are completely soluble in trichloroacetic acid it appears that this is not the only mode of degradation. This is shown by our data on the time-course of the N/P ratio of the trichloroacetic acid precipitate with varying concentrations of caseinogen as summarised in Table II. It will be seen that, in Exps. 5 and 6, the only acid-insoluble material present after 10 minutes' digestion is the

substance of N/P ratio 12.8 already mentioned. In Exps. 7 and 8 some substance of higher N/P ratio (probably unchanged caseinogen) is still present even after 10 minutes. Every experiment, however, shows a slow change in N/P ratio with time, a fact which indicates that here we have to deal with the cleavage of the acid-insoluble material (N/P ratio 12.8) into two fractions, (1) insoluble in trichloroacetic acid and low N/P ratio, (2) soluble in trichloroacetic acid and high N/P ratio. On the basis of viscosity measurements Linderstrøm-Lang [Holter *et al.*, 1932] has suggested that the mode of attack of the enzyme on its substrate may be different at different stages of digestion and our work would appear to confirm this. It may also be noted that a similar slow breakdown is shown in Table XII of Linderstrøm-Lang's paper. The phosphopeptone (paranuclein) investigated by Linderstrøm-Lang had a N/P ratio 8 and would appear to be a later stage of digestion than our acid-insoluble material of ratio 12.8. It was noted from Table II that the N/P ratio of the acid-insoluble material was tending towards the value obtained by Linderstrøm-Lang and it is possible that paranuclein is derived from our acid-insoluble substance by the second mode of breakdown suggested. It has to be remembered that Linderstrøm-Lang's material was characterised by being insoluble at acid reaction in the absence of trichloroacetic acid and that in his experiments the p_H of the system was not kept constant throughout the digestion. This may explain the fact that his phosphopeptone accounted for about 70 % of the total protein-phosphorus while in our Exp. 9 less than 60 % of the phosphorus is acid-insoluble and it is still in the form of the substance with a N/P ratio 12.8. The mode of breakdown seems therefore to depend on the composition of the system.

On the basis of these findings a provisional scheme for the hydrolysis of caseinogen by pepsin might be suggested as below:

(1) and (2) represent simultaneous modes of breakdown which may proceed at different relative rates according to conditions.

The course of the digestion of caseinogen with trypsin-kinase seems to be altogether different from that with pepsin. It will be seen from Figs. 1 to 4 that, with the former, the rate of liberation of acid-soluble phosphorus is greater than that of acid-soluble nitrogen. The action of the enzyme on caseinogen and on its immediate degradation products is characterised by the production of two fractions (1) insoluble in trichloroacetic acid and poor in phosphorus, (2) soluble in trichloroacetic acid and rich in phosphorus. As will be seen from Table II the N/P ratio of the trichloroacetic acid precipitate is increasing rapidly in the case of trypsin-kinase while it is decreasing slowly with pepsin. Since solubility in trichloroacetic acid may be taken as a rough guide to molecular size, it would appear that with trypsin-kinase phosphorus is liberated in comparatively small molecules but, with pepsin, in the form of large molecules. The differences in behaviour of the two enzymes can best be explained by the assumption that all the phosphorus of caseinogen is concentrated in one relatively small group. The phosphorus linkages would therefore appear to direct to some extent the action of proteolytic enzymes—trypsin-kinase effecting rupture of peptide linkages in proximity to phosphorus atoms and so confining the phosphorus to the cleavage product of lower molecular weight; the action of pepsin being relegated to peptide linkages distant from phosphorus atoms and giving rise to phosphorus-containing substances of high molecular weight. That groups within the molecule can exert a controlling influence over the action of enzymes is shown by the necessity for a free carboxyl or a free amino-group for the action of the polypeptidases [Grassmann and Dyckerhoff, 1928].

The further course of the hydrolysis with trypsin may be ascertained from Exps. 1 and 2 in which it was found possible to obtain all the phosphorus in an acid-soluble condition while about 20 % of the nitrogen remained insoluble. It is possible that the first action of trypsin-kinase on caseinogen results in the formation of two fractions, one acid-soluble and containing all the phosphorus, the other acid-insoluble and containing no phosphorus, the acid-insoluble phosphorus of the successive intervals of Exps. 1 to 4 being ascribed to unchanged caseinogen. The phosphorus-containing fraction would approximate to the size of Rimington's phosphopeptone which, it will be remembered, was isolated in amount representing over 50 % of the original caseinogen phosphorus. Rimington's compound is more likely to be a primary cleavage product of the caseinogen molecule than a secondary product resulting from the hydrolysis of a paranuclein as suggested by Linderstrøm-Lang [Holter *et al.*, 1932].

It is difficult to see how our results can be interpreted otherwise than as indicating that all the phosphorus is concentrated in one relatively small group in caseinogen, whether the latter is a single molecule or an association of molecules. If, as Linderstrøm-Lang suggests, caseinogen is an association of various proteins containing different amounts of phosphorus, it is impossible to account for the formation of one acid-insoluble substance containing over

86 % of the total phosphorus during peptic digestion. The possibility must therefore be seriously entertained that Linderstrøm-Lang's alcoholic-HCl has a chemical effect on caseinogen, and that his various fractions are not homogeneous.

SUMMARY.

The action of trypsin-kinase and pepsin on the caseinogen molecule results in the formation of products which are not completely soluble in trichloro-acetic acid.

With pepsin the rate of liberation of acid-soluble nitrogen is greater than that of acid-soluble phosphorus while with trypsin-kinase the opposite is true.

These results are explained on the basis of the existence of a caseinogen molecule with all the phosphorus concentrated in one comparatively small group.

The action of trypsin-kinase is considered to be on a peptide linkage in proximity to the phosphorus atoms thus confining all the phosphorus to the cleavage product of lower molecular weight. The action of pepsin is on a peptide linkage distant from the phosphorus atoms giving rise to a phosphorus-containing substance of high molecular weight.

The formation of Rimington's phosphopeptone is considered to be specific for the action of trypsin-kinase and that of paranuclein for the action of pepsin.

We have to thank Dr N. C. Wright for his interest in this work and for supplying us with caseinogen.

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