CLXXXIV. THE NATURE OF PARANUCLEIN

III. THE INTERRELATIONSHIP OF ITS COMPONENT FRACTIONS

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IN two previous communications [Herd, 1936; 1937] have been recorded the results of investigation of the pseudonucleins which precipitate when phosphoproteins are digested with pepsin. These materials have been shown to be not single substances, but mixtures of at least two fractions, only one of which is soluble in trichloroacetic acid. No adequate explanation could be offered, however, as to why certain of the protein degradation products should combine and precipitate in this manner. As a means of investigating this problem, it was decided to study the interrelationship of the component fractions under different conditions of digestion and with protein precipitants other than trichloroacetic acid.

It was stated by the early workers, chiefly Salkowski [1899], that it is possible to vary the amount of paranuclein produced on digestion of caseinogen by varying (*a*) the relative concentrations of enzyme and substrate, and (*b*) the temperature of reaction. In each case, the amount of paranuclein formed was increased as the rate of digestion was decreased. Experiments were accordingly undertaken to determine whether this variation might have some effect on the composition of the paranuclein formed.

Digestion of caseinogen at different pH values

Three experiments were carried out in which portions of a caseinogen solution were digested at five different *pH* values. The paranucleins formed were centrifuged off, washed and analysed for N and P; they were also examined with regard to their behaviour to trichloroacetic acid. N and P are expressed throughout as % of the totals present.

The concentration of caseinogen (Merck, *nach Hammarsten*) in the final digest liquor was 2 %, that of pepsin (B.D.H.) was 0.4 %.

In each case, digestion was continued to the point of flocculation of the precipitating paranuclein. This criterion, quite satisfactory below *pH* 2.5, was somewhat arbitrary at *pH* 3 and 4, since at these values the original caseinogen was not fully in solution, and one suspension was thus merely replacing another. The results, however, indicate that the paranuclein obtained was in reality paranuclein, not undigested caseinogen.

The results are shown in Table I. For the sake of comparison, the three experiments are grouped together; the top line of each group represents Exp. 1, the second Exp. 2, the bottom line Exp. 3.

The results of analysis of the paranucleins obtained are detailed in Table II; a weakly alkaline solution of each paranuclein was prepared and an aliquot portion precipitated with trichloroacetic acid.

Table I

N/P caseinogens = 15.2, 14.6, 15.2, respectively

pH	Paranuclein (indirect)			Supernatant liquor		
	N%	P%	N/P	N%	P%	N/P
1	22.2	36.7	8.4	77.8	63.3	19.0
	17.4	32.1	7.9	82.6	67.9	17.6
	20.5	25.6	12.8	79.5	74.4	16.5
2	15.1	29.9	7.6	84.9	70.1	18.4
	15.3	26.1	8.5	84.7	73.9	16.8
	12.0	22.5	8.1	88.0	77.5	17.3
2.5	13.8	27.6	7.6	86.2	72.4	18.0
	14.1	23.8	8.6	85.9	76.2	16.4
	19.8	27.9	10.8	80.2	72.1	16.9
3	24.2	38.2	9.6	75.8	61.8	18.6
	29.0	40.4	10.5	79.1	59.6	17.4
	32.7	39.7	12.5	67.3	60.3	17.0
4	49.8	59.0	13.1	50.2	41.0	18.6
	40.6	48.1	12.3	59.4	51.9	16.7
	51.8	71.3	11.1	48.2	28.7	25.6

Table II

pH	Paranuclein (direct) N/P	Acid-soluble fraction			Acid-insoluble fraction		
		N%	P%	N/P	N%	P%	N/P
1	7.3	37.9	23.6	11.6	62.1	76.4	5.9
	8.2	48.9	34.0	10.4	51.1	66.0	5.6
	8.3	58.2	35.2	13.7	41.8	64.8	5.3
2	6.8	49.5	32.3	9.8	50.5	67.7	5.2
	7.5	48.8	30.8	11.9	51.2	69.2	5.6
	8.4	56.4	34.8	13.6	43.6	65.2	5.6
2.5	6.2	37.5	26.5	8.7	62.5	73.5	5.2
	6.5	44.5	27.5	10.4	55.5	72.5	5.0
	7.6	48.2	29.1	12.6	51.8	70.9	5.6
3	6.5	36.1	24.2	8.6	63.9	75.8	5.6
	8.6	35.9	29.4	10.5	64.1	70.6	7.8
	8.5	44.9	31.0	12.1	55.5	69.0	6.8
4	8.8	24.2	18.5	11.6	75.8	81.5	8.2
	9.1	30.5	26.4	10.4	69.5	31.5	8.1
	9.5	28.1	24.2	11.1	71.9	75.8	9.0

It will be seen from Table I that there is a striking increase in the amount of paranuclein produced when the pH diverges from the optimum for digestion, particularly at pH 4, and that the increase in amount of paranuclein produced is associated in general with a rise in N/P ratio. That this increase in quantity is not due to adsorbed, undigested caseinogen is shown by the analyses of the paranucleins, since the acid-insoluble fractions (in which caseinogen would be found) have a fairly low constant value throughout.

Digestion of caseinogen at different temperatures

An experiment was also undertaken to determine the effect of digesting caseinogen at different temperatures. Three portions of a caseinogen-pepsin digest mixture were incubated at 21°, 37.5° and 55° respectively, and the paranucleins centrifuged off after flocculation for analysis in the usual manner. The results are shown in Tables III and IV.

Table III

N/P caseinogen = 16.8

Temp. ° C.	Paranuclein (indirect)			Supernatant liquor		
	N%	P%	N/P	N%	P%	N/P
21	12.4	40.3	5.2	87.6	59.7	24.6
37.5	10.8	35.4	5.1	89.2	64.6	23.2
55	9.9	27.7	6.0	90.1	72.3	21.2

Table IV

Temp. ° C.	Paranuclein (direct) N/P	Acid-soluble fraction			Acid-insoluble fraction		
		N%	P%	N/P	N%	P%	N/P
21	8.5	63.2	40.8	13.2	36.8	59.2	5.3
37.5	7.7	59.0	35.5	12.9	41.0	64.5	4.9
55	7.3	48.3	34.9	10.3	51.7	65.1	5.7

It will be seen from the above results that the quantity of paranuclein which precipitates is but slightly affected by the temperature of digestion, contrary to the statements of Salkowski. As with the results obtained by digestion at different *pH* values, the N/P ratios of the paranucleins formed appear to be slightly higher the greater the total quantity precipitating.

The fractionation of paranuclein by tannic and phosphotungstic acids

The use of trichloroacetic acid discloses the existence in paranuclein of a fraction soluble in this acid, containing some 40 % of the total N and 30 % of the total P. Table V shows comparative results obtained with tannic and phosphotungstic acids as precipitants.

Table V

Para- nuclein N/P	Trichloroacetic acid-soluble			Tannic acid-soluble			Phosphotungstic acid-soluble		
	N%	P%	N/P	N%	P%	N/P	N%	P%	N/P
7.4	48.1	30.5	11.7	43.2	31.3	10.1	14.8	—	—

Tannic acid is thus clearly similar in action to trichloroacetic acid; with phosphotungstic acid, unfortunately, no P estimations can be made, but the N estimation shows clearly that its effect is different from those of the others, since it is capable of precipitating 85 % of the paranuclein N.

These results suggest that the soluble portion of paranuclein is not itself an individual.

DISCUSSION

Although the results obtained confirm the original suggestion that alteration of the conditions of digestion causes an alteration in the relationship of the component fractions of paranuclein, they indicate definitely that there is no one constant fraction. It appears, rather, that the material may differ in composition without change in appearance. A certain constancy is obtained for the N/P ratio of the acid-insoluble fraction of the paranuclein, if the values for *pH* 4 be excepted, but the absolute amounts formed show considerable variation. Thus, correlating the % acid-insoluble N in the paranuclein with the % N from caseinogen precipitated in the paranuclein as a whole, we find for the first of the three experiments that the amount of caseinogen N in the acid-insoluble portion may vary from 62.1 % of 22.2 % = 13.8 % at *pH* 1, to 50.5 % of 15.1 % = 7.6 % at *pH* 2, and 63.9 % of 24.2 % = 16.5 % at *pH* 3.

It may be asked, however, what the factor is which is responsible for the loose combination of certain digestion products to form an insoluble product. To this no answer can as yet be given. It may be suggested that since the products which precipitate are all highly polar, they are united in a loose salt-like combination; and that the precipitation of this complex is a question simply of the physical conditions. In support of this, reference may be made to the observation by v. Moraczewski [1895] that in presence of ovalbumin, the amount of paranuclein which precipitates is increased; the author [Herd, 1936] has found that the fraction of paranuclein which is soluble in trichloroacetic acid is reduced from 40 to 20 % in the presence of a high crude pepsin concentration, the latter acting presumably as a protein.

SUMMARY

Alterations in the conditions of digestion of caseinogen may cause a considerable variation in the total amount of paranuclein produced, the quantity being greatly increased by digestion at a *pH* away from the optimum for digestion. Alteration of temperature has, however, little effect. There is no evidence of any one constant fraction.

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