XXVIII. SOME PHOSPHORUS COMPOUNDS OF MILK. III.

DEPHOSPHORISED CASEINOGEN. THE ACTION OF ALKALI UPON CASEINOGEN.

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THE name "dephosphorised caseinogen" was given by Rimington and Kay [1926] to the protein produced from caseinogen by the action of 0.25 *N* sodium hydroxide for 24 hours at 37°. This substance contains only minimal amounts of phosphorus but in other respects does not differ greatly from caseinogen. Preliminary experiments indicated, however, that dephosphorised caseinogen possesses a low nitrogen content, values lying between 11.1 % and 13.1 % being found for samples prepared from different brands of commercial "casein," the total nitrogen of caseinogen itself being 15.65 %.

During the reaction with sodium hydroxide a considerable evolution of ammonia occurs and it was with the object of ascertaining the source of this ammonia that the following investigations were undertaken.

Dephosphorised caseinogen, obtained from a specimen of caseinogen prepared from separated milk, was analysed by the nitrogen distribution method of Van Slyke, employing the modifications recently suggested by Plimmer and Rosedale [1925]. The analyses were performed in duplicate. In Table I are

Table I. *Distribution of nitrogen in dephosphorised caseinogen.*

Total nitrogen = 11.06 %, total phosphorus = 0.009 %.

	Error of the method (Van Slyke) % of total nitrogen	Caseinogen				Caseinogen Mean of last three columns	Dephos- phorised caseinogen Mean of two analyses % of total nitrogen	Dephos- phorised caseinogen as percentage of total N-amide N	Caseinogen as percentage of total N-amide N
		Crowther and Raistrick % of total nitrogen	Plimmer and Rosedale % of total nitrogen	Van Slyke % of total nitrogen					
Amide-N	0.37	10.25	10.63	10.35	10.41	4.35	—	—	—
Humin-N	0.39	1.26	1.08	2.35	1.56	1.80	1.88	1.74	—
Total N of bases	—	26.62	22.68	22.85	24.05	27.25	28.49	26.84	—
Arginine-N	1.27	9.31	7.74	7.46	8.17	6.82	7.13	9.12	—
Histidine-N	0.93	6.55	5.54	5.23	5.77	7.03	7.35	6.44	—
Lysine- + cystine-N	1.23	10.76	9.41	10.16	10.11	13.39	14.01	11.28	—
Total N of filtrate	1.50	62.31	62.58	63.75	62.88	64.13	67.05	70.16	—
Amino-N of filtrate	1.10	55.44	51.93	55.43	54.27	60.21	62.95	60.56	—
Non-amino-N of filtrate	1.20	6.87	10.66	8.32	8.62	3.92	4.10	9.60	—

recorded the mean results expressed as percentages of total nitrogen, together with some previous analyses of caseinogen to be found in the literature. The figures given by Plimmer and Rosedale have been recalculated for expression as percentages of total nitrogen. In order further to facilitate the comparison of dephosphorised caseinogen with caseinogen, the nitrogen distribution in these two proteins has also been calculated in such a way that the nitrogen of each fraction is expressed as a percentage of the total nitrogen of the protein less the amide nitrogen.

From these figures it will be seen that the amide nitrogen of dephosphorised caseinogen is distinctly lower than that of caseinogen. There is also some decrease in the arginine nitrogen accompanied by an increase in the lysine + cystine fraction of the former. Arginine, in the free state, is known to be attacked by hot sodium hydroxide with the liberation of ornithine and ammonia and it is highly probable that a similar change has here taken place. The resulting ammonia would be evolved along with that derived from the amide nitrogen groups whilst the other product, ornithine, would become included in the figure for lysine + cystine nitrogen.

The nature of the groups giving rise to what is termed amide nitrogen on acid hydrolysis is not known with certainty, although there is evidence in support of the view put forward by Osborne that this nitrogen is present in the protein in the form of amides of the dicarboxylic acids; and in the particular case of caseinogen, Luck [1924] has succeeded in isolating a dipeptide of lysine and glutamine in which this linkage is present. It must be admitted however that the substance isolated by Luck represents only a portion of the groups capable of yielding ammonia when caseinogen is subjected to hydrolysis.

The ease with which a portion of the amide nitrogen fraction of caseinogen is converted into ammonia by the action of relatively weak alkali at 37° is a point of some interest and is in keeping with the supposition that acid amide groups exist in the protein.

It will be seen however that there still remains some 4 % of the total nitrogen of dephosphorised caseinogen in the form of amide nitrogen and in order to ascertain whether this also could be removed by the prolonged action of sodium hydroxide, of the same strength as previously employed, the following experiments were performed in which the rate of ammonia liberation was followed.

Equal volumes of a 4 % solution of caseinogen and of 0.5 *N* sodium hydroxide, both previously warmed to 37°, were mixed and a sample at once taken for total nitrogen determination, the remainder being transferred to a well-stoppered bottle, held in a thermostat at 37° and containing a little paraffin to prevent foaming. A rapid stream of air was then drawn through the solution and the liberated ammonia collected in standard acid, the excess of acid being titrated at suitable intervals and the ammonia determined by difference.

The result of such an experiment is shown in Fig. 1.

Fig. 1. Liberation of ammonia from caseinogen by 0.25 *N* sodium hydroxide at 37°.

There is a rapid liberation of ammonia during the first 30 hours, at the end of which time approximately 50 % of the total amide nitrogen has been eliminated. During this initial stage the velocity of ammonia production remains constant but subsequently falls. At the end of 70 hours the reaction has almost ceased, there being still however a very gradual liberation of ammonia not amounting to more than 0.5 mg. in 24 hours.

The total ammonia production up to this point is equivalent to about 65 % of the total amide nitrogen. The remainder appears to be comparatively stable towards alkali of the strength employed in these experiments.

Dephosphorised caseinogen gives all the colour tests which are given by caseinogen. It is insoluble in water but soluble in sodium hydroxide, requiring approximately the same quantity of alkali as caseinogen to effect complete solution. It is only very slowly attacked by proteolytic enzymes, a circumstance which may be due to some racemisation having taken place during its preparation by action of the alkali upon the protein. It is not coagulated by rennin.

SUMMARY.

1. The low nitrogen content of dephosphorised caseinogen in comparison with caseinogen is accounted for by its much lower content in amide nitrogen.

2. During the action of sodium hydroxide upon caseinogen, ammonia is evolved, derived chiefly from the groups giving rise to amide nitrogen on acid hydrolysis, but also to some extent as a result of the decomposition of arginine.

3. Dephosphorised caseinogen is only slowly attacked by proteolytic enzymes. It is not coagulated by rennin.

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