III. CASEINOGEN AND CASEIN.

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The question of the chemical identity of caseinogen and casein and with it, of the nature of rennet action, still remains open in spite of many attempts at a solution. Hammarsten suggested that by the action of rennet, caseinogen was hydrolysed to form two new proteins, casein and whey-protein. Köster [1881] analysed these proteins and found that casein contained somewhat more nitrogen (15·84 %) than caseinogen and whey-protein much less (13·1–13·6 %). On the other hand it has been suggested that rennet action is either purely physical or is only concerned with the inorganic constituents of milk and that casein is chemically identical with caseinogen. During the progress of this work a new suggestion has been put forward by van Slyke and Bosworth [1913], according to whom caseinogen is split by rennet into two molecules of casein.

If Hammarsten's theory is correct, it should be possible to find some chemical differences between caseinogen and casein. Elementary analyses have yielded the following results:—

Caseinogen	C	H	N	S	P
Makris [1876]	53·02	7·42	14·20	—	—
Hammarsten [1883–1885]	52·96	7·05	15·65	0·758	0·347
Chittenden & Painter [1887]	53·3	7·07	15·91	0·82	0·84–0·89
Lehmann & Hempel [1894]	54·0	7·04	15·6	0·771	0·847
Ellenberger [1902]	53·07	7·13	15·64	0·76	0·8
Laqueur & Sackur [1903]	—	—	15·45	0·757	0·772
Burow [1905]	52·825	7·095	15·64	0·725	0·808
Tangl [1908]	52·69	6·81	15·65	0·832	0·877
van Slyke & Bosworth [1913]	53·50	7·13	15·80	0·72	0·71
Mean	53·17	7·09	15·67	0·768	0·82
Casein					
Köster [1881]	—	—	15·84	—	—
Rose & Schulze [1885]	53·94	7·14	15·14	1·01	—
Raudnitz [1904]	—	—	15·5	—	0·7–0·88
Kikkoji [1909]	—	—	—	—	0·85–0·87
van Slyke & Bosworth [1913]	53·50	7·26	15·80	0·72	0·71
Mean	53·72	7·20	15·57	0·87	0·79

The only reliable analysis of casein is that of van Slyke and Bosworth [1913] who obtained identical results for caseinogen and casein. They have also obtained somewhat more sulphur than phosphorus, which is in agreement with the supposition that there are equal numbers of atoms of these two elements in the molecules of these proteins.

The object of the following investigation was to determine the difference, if any, between caseinogen and casein both in elementary composition and in Hausmann numbers.

Most of the nitrogen estimations quoted above have been carried out by Kjeldahl's method and are thus inclined to be too low. The author has made estimations both by Kjeldahl's and by Dumas' method. Sulphur estimations are usually carried out by one of the various fusion methods described in the literature. These all lead to the precipitation of barium sulphate in the presence of comparatively excessive amounts of alkali salts, usually chlorides. It has however been shown by Allen and Johnston [1910] and Johnston and Adams [1911] that the presence of even relatively small amounts of such salts destroys the accuracy of sulphate estimations. This source of error has been avoided in the analyses described below by adopting a slight modification of Carius' method.

The following mean results were obtained for caseinogen and casein :—

	Caseinogen %	Casein %
C	53.20	53.05
H	7.09	7.03
N (Dumas)	15.63	15.81
N (Kjeldahl)	15.61	15.62
S	1.015	1.009
P	0.731	0.809

The results for sulphur are higher than have been previously obtained for caseinogen. It will be seen above that Rose and Schulze [1885] obtained 1.01 % sulphur in casein in agreement with the author's result. The sulphur contents of caseinogen and casein appear to be identical, but casein apparently contains more phosphorus than caseinogen. The difference is however not sufficient to warrant the supposition that the two proteins are chemically different.

As will be seen from the following figures the Hausmann numbers for the two proteins are also too close to establish any definite difference.

	Caseinogen % total N	Casein % total N
Ammoniacal N	10.23	10.31
Melanin N	1.53	1.66
Diamino N	22.94	24.03
Monamino N	65.31	63.90
	<hr/> 100.01	<hr/> 99.90

Osborne and Harris [1903] obtained from caseinogen

	% total N
Ammoniacal N	10.31
Melanin N	1.34
Diamino N	22.34
Monamino N	66.01
	<hr/> 100.00

with which the author's results are in substantial agreement.

EXPERIMENTAL PART.

ELEMENTARY ANALYSIS OF CASEINOGEN AND CASEIN.

The caseinogen used was Kahlbaum's "Casein nach Hammarsten" carefully freed from fat by prolonged extraction in a Soxhlet apparatus with ether.

The casein was prepared from milk by the action of rennet and purified by Hammarsten's method. The last traces of fat were removed as above. Two specimens were separately prepared and analysed.

Before analysis the samples were allowed to stand at least 16 hours exposed to the air of the balance room, and when each portion was weighed out for an estimation a second portion was taken and dried to constant weight at 40° in vacuum over P_2O_5 and the results corrected for the percentage of water thus found. It was found impossible to handle the very hygroscopic dry proteins with any certainty of accuracy. The ash was also estimated and allowed for. All the results are calculated for the ash-free dry proteins.

Carbon and hydrogen estimations were carried out in an ordinary combustion tube filled with alternate layers of copper oxide and lead chromate. The substance in the boat was covered with a mixture of lead chromate and potassium bichromate.

Nitrogen estimations were carried out both by Kjeldahl's and by Dumas' method.

Sulphur was estimated by the following modification of the Carius method. About 0.5 grm. of the protein was heated in a sealed tube with 7-8 cc. fuming nitric acid for two days at 300°. After opening the tube the contents were diluted and filtered and the filtrate treated with a slight excess of barium chloride over that required to combine with the sulphuric and phosphoric acids. No precipitate was obtained. The solution was evaporated to dryness, a few cc. of concentrated hydrochloric acid added and again evaporated to dryness completely to remove the nitric acid. The residue

was taken up with 50 cc. of water and 2·2 or 4·5 cc. of dilute (6·5%) hydrochloric acid added. The barium sulphate was thus obtained in a granular condition. It was collected on a layer of "BaSO₄ asbestos" in a Gooch crucible, washed and dried in an air oven at 110–120° to constant weight. From Allen and Johnston's [1910] results the following corrections were made for the solubility of barium sulphate:—

2·2 cc. dilute HCl add 0·5 mgm. BaSO₄.

4·5 cc. dilute HCl add 0·6 mgm. BaSO₄.

Phosphorus was estimated in the filtrate from BaSO₄ by Gregerson's [1907] modification of Neumann's [1900] method.

Caseinogen.

Ash $\left\{ \begin{smallmatrix} 0\cdot5764 \\ 0\cdot5750 \end{smallmatrix} \right\} = 0\cdot57\%$ except where otherwise stated.

C & H 0·2118 g. containing 10·30% H₂O; 0·3690 g. CO₂; 0·1403 g. H₂O.

0·2214 g. ,, 6·54% H₂O; 0·4010 g. CO₂; 0·1492 g. H₂O.

0·2415 g. ,, 6·54% H₂O; 0·4377 g. CO₂; 0·1549 g. H₂O.

N (Dumas) 0·2227 g. containing 6·54% H₂O; 27·2 cc. nitrogen over 50% KOH at 15·5° C. and 760 mm.

N (Kjeldahl) Ash = $\left\{ \begin{smallmatrix} 0\cdot32 \\ 0\cdot20 \end{smallmatrix} \right\} = 0\cdot26\%$; H₂O = 11·30%.

0·2510 g. required 24·63 cc. N/10 H₂SO₄.

0·2494 g. ,, 24·72 cc. ,,

0·2507 g. ,, 24·66 cc. ,,

0·2493 g. ,, 24·43 cc. ,,

S 0·5008 g. containing 7·69% H₂O; 0·0332 g. BaSO₄.

0·5055 g. ,, 7·69% H₂O; 0·0354 g. BaSO₄.

P 0·4936 g. containing 7·69% H₂O required 29·61 cc. N/10 NaOH.

0·5055 g. ,, 7·69% H₂O ,, 30·71 cc. ,,

Ash-free dry caseinogen:

	Mean
C = 53·25, 53·15, 53·20	53·20%
H = 7·03, 7·32, 6·93	7·09
N (Dumas) = 15·63	15·63
N (Kjeldahl) = 15·54, 15·76, 15·61, 15·54	15·61
S = 0·992, 1·048	1·015
P = 0·727, 0·734	0·731

Casein (Prep. I).

Ash = 0·31% of dry casein.

C & H 0·2408 g. containing 11·75% water; 0·4133 g. CO₂; 0·1576 g. H₂O.

0·2040 g. ,, 11·70% water; 0·3493 g. CO₂; 0·1390 g. H₂O.

N (Dumas) 0·2792 g. containing 11·75% water; 33·6 cc. nitrogen over water at 12° C. and 742 mm.

0·3342 g. containing 11·70% water; 40·0 cc. nitrogen over water at 15·3° C. and 749 mm.

S 0·5063 g. containing 11·75% water; 0·0323 g. BaSO₄.

0·4939 g. ,, 10·09% water; 0·0295 g. BaSO₄.

0·4250 g. dry casein; 0·0312 g. BaSO₄.

P 0·5063 g. containing 11·75% water required 33·33 cc. N/10 NaOH.

0·4939 g. ,, 10·09% ,, ,, 32·30 cc. N/10 NaOH.

Dry ash-free casein (Prep. I):				Mean
C=53·21, 53·06	...	...	...	53·14 %
H=6·82, 7·17...	...	...	...	7·00
N (Dumas)=15·85, 15·68	...	...	...	15·77
S=0·999, (0·919), 1·008	...	...	...	1·004
P=0·829, 0·809	...	...	...	0·819

Casein (Prep. II).

Ash=0·45 % of dry casein.

C & H 0·2268 g. containing 11·95 % water; 0·3867 g. CO₂; 0·1539 g. H₂O.
 0·2127 g. ,, 11·94 % water; 0·3616 g. CO₂; 0·1413 g. H₂O.

N (Dumas) 0·2487 g. containing 11·95 % water; 30·1 cc. nitrogen over water at 17·2° C.
 and 754 mm.

0·2886 g. containing 11·94 % water; 33·7 cc. nitrogen over 50 % potash at
 15·7° C. and 756 mm.

S 0·5318 g. containing 11·94 % water; 0·0340 g. BaSO₄.

0·5154 g. ,, 11·94 % water; 0·0337 g. BaSO₄.

P 0·5318 g. containing 11·94 % water required 34·05 cc. N/10 NaOH.

0·5154 g. ,, 11·94 % ,, ,, 32·08 cc. ,,

Dry ash-free casein (Prep. II):				Mean
C=53·02, 52·88	...	...	...	52·95 %
H=7·14, 6·95...	...	...	...	7·05
N (Dumas)=15·88, 15·81	...	...	...	15·85
S=1·002, 1·024	...	...	...	1·013
P=0·810, 0·787	...	...	...	0·799

Kjeldahl nitrogen estimations of casein were carried out with a third specimen prepared by the same method.

Ash=1·03 % in dry casein.

Water = $\frac{11·41}{11·46}$ = 11·44 %.

0·2478 g. required 24·13 cc. N/10 H₂SO₄.

0·2517 g. ,, 24·61 cc. ,,

N=15·59, 15·65. Mean=15·62 %.

The mean compositions of caseinogen and casein are thus :—

	Caseinogen %	Casein %
C	53·20	53·05
H	7·09	7·03
N (Dumas)	15·63	15·81
N (Kjeldahl)	15·61	15·62
S	1·015	1·009
P	0·731	0·809

Hausmann Numbers.

The caseinogen used was Kahlbaum's "Casein nach Hammarsten." The ash and water were estimated and allowed for as above.

Three samples of casein were used.

(1) Prepared by the action of rennet on a solution of caseinogen in disodium hydrogen phosphate. The casein was precipitated by calcium chloride and purified by Hammarsten's method.

(2) and (3) Prepared by the action of rennet on a solution of caseinogen in the minimum amount of NaOH. The rennet was destroyed by heating momentarily to 90° C. with steam and the casein precipitated with glacial acetic acid. It was purified by Hammarsten's method. At each purification after the first a portion was washed with alcohol and ether and not further purified. In this way four fractions were obtained.

From 110 g. air-dry (about 100 g. dry) caseinogen were obtained:—

	Prep. (2)	Prep. (3)
	g.	g.
Fraction <i>a</i>	12	13
<i>b</i>	11	16
<i>c</i>	13	12
<i>d</i>	14	11
	<hr/> 50	<hr/> 52

The fractions (2) *a*, (2) *d*, (3) *b* and (3) *d* were used for Hausmann numbers.

The Hausmann numbers were estimated by the method given by Röhmann [1908]. Usually the diamino nitrogen was estimated in the phosphotungstic acid precipitate and the monamino nitrogen in the filtrate from this, in some cases only one of these two was estimated and the other obtained by difference. Figures thus obtained by difference are given below in brackets. The results are given in percentages of the total nitrogen obtained by addition of the four separate nitrogen percentages.

Caseinogen.

1st Series.

	(1)	(2)	(3)	(4)	Mean
Ammoniacal N	10·52	10·64	10·06	9·73	10·24
Melanin N	1·79	0·58	2·07	1·57	1·50
Diamino N	(23·07)	(26·19)	20·00	20·92	22·55
Monamino N	64·62	62·59	67·87	67·78	65·72
					<hr/> 100·01

2nd Series.

	(5)	(6)	(7)	(8)	(9)	Mean
Ammoniacal N	10·03	10·32	10·35	10·26	10·17	10·23
Melanin N	1·34	1·55	1·57	1·59	1·71	1·55
Diamino N	23·28	22·66	23·77	22·98	23·56	23·25
Monamino N	65·35	65·48	64·31	65·23	64·57	64·99
						<hr/> 100·02

The second series is the more reliable,

Casein (Prep. (1)).

	(1)	(2)	(3)	(4)	Mean
Ammoniacal N	9.92	10.38	10.26	10.51	10.27
Melanin N	1.50	1.57	1.63	1.37	1.52
Diamino N	(23.89)	(25.59)	24.05	(23.96)	24.37
Monamino N	64.69	62.46	64.07	64.16	63.85
					<hr/> 100.01

Casein (Prep. 2 a).

	(1)	(2)	(3)	Mean
Ammoniacal N	10.78	10.38	9.87	10.34
Melanin N	1.46	1.43	1.49	1.46
Diamino N	23.94	25.14	22.18	23.75
Monamino N	63.82	63.05	66.48	64.45
				<hr/> 100.00

Casein (Prep. 2 d).

	(1)	(2)	(3)	(4)	Mean
Ammoniacal N	9.91	10.31	10.17	10.22	10.13
Melanin N	1.66	—	2.28	2.14	2.01
Diamino N	—	—	25.30	(23.40)	24.35
Monamino N	—	62.99	62.24	64.24	63.24
					<hr/> 99.73

Casein (Prep. 3 b).

	(1)	(2)	(3)	(4)	Mean
Ammoniacal N	10.42	10.29	10.55	10.69	10.49
Melanin N	1.59	2.00	1.51	1.64	1.69
Diamino N	(28.06)	(20.67)	24.66	(24.97)	24.60
Monamino N	59.92	67.04	(63.28)	62.70	63.28
					<hr/> 100.02

Casein (Prep. 3 d).

	(1)	(2)	Mean
Ammoniacal N	10.65	10.00	10.33
Melanin N	1.65	1.70	1.68
Diamino N	22.47	22.11	22.29
Monamino N	65.23	66.19	65.71
			<hr/> 100.01

The mean of all the results gives the following values for caseinogen and casein :—

	Caseinogen	Casein
Ammoniacal N	10.23	10.31
Melanin N	1.53	1.66
Diamino N	22.94	24.03
Monamino N	65.31	63.90
	<hr/> 100.01	<hr/> 99.90

CONCLUSIONS.

The difference between caseinogen and casein is thus scarcely appreciable. If for caseinogen the second series alone is taken, the difference is still less than that given above. No difference could be established between the different fractions of casein.

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