CCXX. ANALYSIS OF PROTEINS
IX. THE CONTENT IN AMINO-ACIDS OF THE
CASEINOGEN AND LACTALBUMIN
OF WOMAN'S MILK

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THOUGH analyses of the amino-acids present in caseinogen and lactalbumin of cow's milk have been frequently made, analyses of the corresponding proteins of woman's milk are lacking. The reason is probably the difficulty of procuring sufficient woman's milk for the preparation of an adequate quantity of each protein for analysis. Through the kind help of the Directors of the Mothercraft Training Society it has been possible to collect sufficient woman's milk to make from 10 to 20 g. of each of the proteins. These proteins have been analysed for those amino-acids which can now be quantitatively estimated. At the same time the proteins of cow's milk have been prepared and analysed concurrently so as to obtain comparative figures. This research has arisen from the question whether the nutritive value of the proteins of cow's milk, generally diluted with an equal volume of water, is equal to that of undiluted woman's milk. With the receipt of many samples of woman's milk the opportunity was taken of recording the distribution of the nitrogen as caseinogen, as lactalbumin and as non-protein nitrogen, as well as the calcium and total and inorganic phosphorus. The several samples of caseinogen and lactalbumin have also been analysed for their nitrogen, sulphur and phosphorus contents. A summary is given of the various amino-acids in the proteins and in woman's and cow's milks.

EXPERIMENTAL

*Determination of nitrogen of caseinogen, lactalbumin, the non-protein
nitrogen and the calcium and phosphorus of woman's milk*

Quantities of 100–200 ml. of woman's milk were received several times a week. Only occasional analyses of the first series of samples were made, but every sample of the second series was analysed for the nitrogen of the two proteins, non-protein nitrogen, calcium and phosphorus. This distribution serves well as an indication of genuine woman's milk.

1 ml. of milk was used for determination of total N. 5 ml. were diluted with water, acidified with 1 ml. of 0.5 *N* acetic acid, the volume made to 50 ml. and the caseinogen and fat filtered off. Of the filtrate 5 ml. (=0.5 ml. milk) were taken for determination of N (=lactalbumin + non-protein-N), and 40 ml. were treated with 40 ml. of 10% trichloroacetic acid and the lactalbumin filtered off. 60 ml. of this filtrate (=3 ml. milk) were taken for determination of non-protein-N. All nitrogen estimations were made by the semi-micro-Kjeldahl method.

1 ml. of milk was used for determination of total phosphorus by the semi-micro-method of Plimmer [1933], and 10 ml. of the trichloroacetic acid filtrate were used for determination of inorganic phosphorus by the same method.

1 ml. of milk was oxidized with 2 or 3 drops of H_2SO_4 and HNO_3 , the residue diluted and precipitated with ammonium oxalate and ammonia. The calcium oxalate after filtering and washing with ammonia was dissolved in dilute HCl and titrated with $N/20$ KMnO_4 .

In the 31 samples thus analysed the highest, lowest and mean figures were:

g. per 100 ml. woman's milk			
Total N	0.245	and 0.155	Mean 0.191
Caseinogen-N	0.085	0.020	0.046
Lactalbumin-N	0.140	0.062	0.099
Non-protein-N	0.068	0.037	0.047
Calcium	0.060	0.020	0.040
Phosphorus, total	0.024	0.011	0.016
Phosphorus, inorganic			0.013

These figures for partition of nitrogen correspond with those of Scott [1934] who found 0.213 and 0.177 for total N and 0.063 and 0.033 for non-protein-N. There is a considerable variation in the different amounts.

The ratios of the different forms of nitrogen are:

Caseinogen-N:lactalbumin-N	= 1 : 2.15
Caseinogen-N:non-protein-N	= 1 : 1
Lactalbumin-N:non-protein-N	= 2 : 1

Six samples of cow's milk analysed in the same way showed:

g. per 100 ml. cow's milk			
Total N	0.495	and 0.485	Mean 0.490
Caseinogen-N	0.365	0.320	0.347
Lactalbumin-N	0.127	0.085	0.102
Non-protein-N	0.050	0.033	0.042
Calcium	0.150	0.133	0.140
Phosphorus, total	0.106	0.097	0.101
Phosphorus, inorganic	0.085	0.079	0.082
Caseinogen-N:lactalbumin-N	= 3.27 : 1		
Caseinogen-N:non-protein-N	= 8.3 : 1		
Lactalbumin-N:non-protein-N	= 2.4 : 1.		

There is little variation in commercial samples of cow's milk.

Preparation of caseinogen and lactalbumin

The samples of woman's milk, in portions of 100–500 ml. were centrifuged to remove the fat. A small amount of the caseinogen separated with the fat. The aqueous portion after straining through glass-wool was diluted with 2–3 volumes of warm tap water and acidified with 1/10 of its volume of 0.5 *N* acetic acid. The precipitate of caseinogen was filtered off on two or three filter-papers of 15 cm. diameter and in the course of 24 hr. had completely drained from the solution. It was suspended in water and dissolved in 10–20 ml. of 0.5 *N* sodium carbonate and filtered clear through the same papers. The clear solution was acidified with a slight excess of 0.5 *N* acetic acid and the caseinogen again filtered off. Solution and reprecipitation were repeated twice. The caseinogen was then soaked in 75% alcohol, 90% alcohol, absolute alcohol twice, filtered off, washed with ether and dried *in vacuo* over sulphuric acid and sodium hydroxide.

The filtrate containing the lactalbumin, in the case of the first 18 samples was partially neutralized with 15–20 ml. 0.5 *N* sodium carbonate and boiled for 5 min. The solution then had pH 5.5–6. In the case of the second 17 portions the filtrate was not partially neutralized. The coagulum of lactalbumin was washed with boiling water, soaked in 75% alcohol, 90% alcohol, absolute alcohol twice, filtered off, washed with ether and dried *in vacuo* over H₂SO₄ and NaOH.

The yields of caseinogen were from 0.2 to 0.4%, and of lactalbumin from 0.3 to 0.5%. Altogether 13 g. of caseinogen and 18.6 g. of lactalbumin were obtained from the first 18 portions totalling 5095 ml. and 8.7 g. of caseinogen and 9.4 g. of lactalbumin from the second portions totalling 3135 ml. The lower yield of lactalbumin is accounted for by the non-neutralization of the solution before coagulation.

Analyses of the samples of caseinogen and lactalbumin

Each of the samples of the proteins was dried at 100° *in vacuo* over P₂O₅. The nitrogen, phosphorus, and sulphur contents were determined by semi-micro-Kjeldahl, semi-micro-phosphorus [Plimmer, 1933] and semi-micro-sulphur [Pirie, 1932] methods. The analytical figures showed distinct variations. The highest and lowest data only are shown:

<i>Woman's milk</i>							
	Caseinogen %		All mixed		Lactalbumin %		All mixed
			First 18 lots				
N	13.96 to 14.98		14.70	N	14.24 to 15.26		14.94
P	0.42 0.73		0.45	P	0.01 0.31		0.09
S	*		0.79	S	*		1.71
			Second 17 lots				
N	14.20 to 14.83		14.37	N	14.10 to 14.77		14.57
P	0.41 0.53		0.45	P	0.07 0.23		0.16
S	0.64 1.02		0.78	S	1.37 1.81		1.65

* These analyses were made by the Benedict method and are not reliable.

The variation in the N, P and S contents of the samples would be in agreement with the results of the experiments of Linderstrøm-Lang [1929] on caseinogen, that it consists of hetero-molecular substances, or a more or less homogeneous mixture of several colloidal molecules following a co-precipitation system.

A similar variation in the N, P and S contents was shown by a series of the proteins prepared from cow's milk:

<i>Cow's milk (six samples)</i>							
	Caseinogen %		All mixed*		Lactalbumin %		All mixed
N	14.51 to 14.99		15.24	N	14.17 to 14.50		14.23
P	0.76 0.87		0.84	P	0.07 0.30		0.19
S	—		0.65	S	1.32 1.62		1.52

* Dried again over P₂O₅.

Hydrolysis of the proteins

With the limitation of the quantities of the proteins to 10–15 g. only those methods requiring from 1 to 5 g. of material for analysis could be employed. Comparison of the contents of the amino-acids was made (1) by the Van Slyke method of nitrogen distribution, (2) by the Block method for the diamino-acids,

(3) by the Folin-Looney-Ciocalteu method for tryptophan and tyrosine, (4) by the Baernstein method for cystine and methionine, (5) by the Folin-Marenzi method for cystine.

(1) *Nitrogen distribution of the amino-acids.* The analyses of the proteins were carried out by the procedure adopted by Plimmer & Rosedale [1925]. It has been found that in the amino-nitrogen determination better results are secured with 2.8 *N* HCl (2 ml. conc. HCl to 5 ml. H₂O), and that lysine then reacts quantitatively on standing for half an hour followed by 5 min. shaking at 11° [Plimmer & Lowndes, 1938]. The results, expressed in the usual way in percentages of the total nitrogen, were:

Distribution of nitrogen of amino-acids

	Caseinogen		Lactalbumin	
	Cow's	Woman's	Cow's	Woman's
Amide-N	11.70	11.82	8.73	9.04
Humin-N	0.68	0.79	0.81	1.69
Arginine-N	7.77	7.11	7.23	10.62
Histidine-N	2.61	1.96	3.85	1.20
Lysine-N	11.77	7.92	10.91	13.05
Monoamino-N	60.94	59.97	66.02	63.50
Non-amino-N	6.41	9.24	2.65	2.01
Total	101.88	98.81	100.20	101.11

There is a distinct difference in the amounts of humin-N in the lactalbumins. Also arginine-N is greater in woman's lactalbumin than that of the cow. The data for histidine-N and lysine-N must be regarded with caution. They show greater differences after calculation in terms of g. amino-acid in 100 g. protein. The quantities of these amino-acids in the proteins are obtainable from the nitrogen contents of the solutions and of the amino-acids and proteins respectively:

Nitrogen contents of the solutions

	Caseinogen		Lactalbumin	
	Cow's	Woman's	Cow's	Woman's
Total N	0.3025 g.	0.2673 g.	0.2905 g.	0.2805 g.
Arginine-N	0.0235	0.0185	0.0262	0.0295
Histidine-N	0.0079	0.0052	0.0112	0.0034
Lysine-N	0.0356	0.0212	0.0317	0.0366

Amino-acid content of the proteins

	Caseinogen				Lactalbumin			
	Cow's		Woman's		Cow's		Woman's	
	g.	%	g.	%	g.	%	g.	%
Protein	1.9849	—	1.8184	—	2.0414	—	1.9493	—
Arginine	0.0730	3.68	0.0575	3.16	0.0814	3.99	0.0917	4.70
Histidine	0.0291	1.47	0.0192	1.05	0.0413	2.02	0.0126	0.65
Lysine	0.1857	9.36	0.1146	6.30	0.1654	8.11	0.1909	9.79

The values for arginine compare favourably with those by the Block method (see below). The values for histidine are lower; that in the case of woman's lactalbumin must be erroneous. There is no correspondence in the values for lysine. It is generally considered that other amino-acids are precipitated by phosphotungstic acid and are included in the figure for lysine by Van Slyke's method. A further examination of the diamino-solutions has been made by us,

and in the mono-amino-fraction we have found that tyrosine can be estimated by the colorimetric method [1938].

(2) *Estimation of the diamino-acids by Block's method.* The four proteins were analysed by the shortened Vickery-Leavenworth method [1928] described by Block [1934]. The experiments were performed as far as possible concurrently for each kind of protein exactly as stated by Block. The completion of the numerous operations of the process took longer than 8 hr., the working time mentioned by Block. It was observed that the lysine solution became distinctly yellow on the addition of conc. H_2SO_4 before precipitation by phosphotungstic acid owing to the action of nitrous acid on tyrosine and that the lysine phosphotungstate was yellow, though the colour was mostly removed during washing. It is believed that the values for lysine may be low in consequence of the presence of nitrous acid (from AgNO_3). The amounts of the diamino-acids isolated as flavianates or picrate were always less than expected from the amounts of nitrogen in the fractions. The following figures of the analyses are from the nitrogen contents:

	Caseinogen		Lactalbumin	
	Cow's %	Woman's %	Cow's %	Woman's %
Arginine	3.73	3.58	4.01	5.03
Histidine	1.67	1.52	1.82	1.66
Lysine	6.13	5.46	6.27	6.57

The only differences are 1% more arginine in woman's lactalbumin and 0.6% more lysine in cow's caseinogen.

(3) *Estimation of tryptophan and tyrosine.* The colorimetric estimation of these two amino-acids was described by Folin & Looney [1922]. The procedure was improved by Folin & Ciocalteu [1927].

Folin & Looney hydrolysed the protein with barium hydroxide, Folin & Ciocalteu with sodium hydroxide. In our experience hydrolysis of the protein with NaOH causes a large destruction of tryptophan. Hydrolysis was therefore effected with $\text{Ba}(\text{OH})_2$. The hydrolysed solutions of the proteins after treatment with sulphuric acid and filtration were then treated as stated by Folin & Ciocalteu. Both ways of estimating tryptophan were used, and tyrosine was estimated by the blue colour reaction with the phenol reagent and by the Millon reaction. The data were:

	Caseinogen		Lactalbumin	
	%	%	%	%
Tryptophan:				
By NaCN	1.36	0.99	1.90	2.42
HgS	—	1.10	—	2.52
Mean	1.36	1.05	1.90	2.47
Tyrosine:				
By Millon	5.80	5.41	3.54	4.51
Phenol reagent	5.79	5.49	3.74	4.45
Mean	5.80	5.45	3.64	4.48

There is no great difference between the two caseinogens, but both tryptophan and tyrosine are greater in woman's lactalbumin. We have found that the tryptophan in these solutions can equally well be estimated by bromination [1938].

(4) *Estimation of methionine and cystine.* These amino-acids have been determined in the four proteins by the latest procedure of Baernstein [1936]. The only alteration that has been found necessary is in the removal of the hydriodic acid. Instead of distilling off through the upright condenser, it was

boiled off through a short adapter piece to the second condenser. The quantities of these amino-acids in the proteins were found to be:

	Caseinogen		Lactalbumin	
	Cow's %	Woman's %	Cow's %	Woman's %
Cystine	0.36	0.60	3.31	4.14
Methionine	2.89	2.70	2.29	1.40

There are differences especially in the lactalbumins.

(5) *Estimation of cystine colorimetrically.* The amount of cystine in the four proteins has also been determined by the colorimetric method of Folin & Marenzi [1929] with the special uric acid reagent. We have found the colour matching more troublesome than with tyrosine and tryptophan. The colours seemed to develop unequally and fade rather rapidly. The values were lower than by the Baernstein method.

	Caseinogen		Lactalbumin	
	Cow's %	Woman's %	Cow's %	Woman's %
Cystine	0.29	0.51	2.55	2.87

Summary of the contents of amino-acids in proteins of milk

The various data for the proteins of cow's and woman's milk are summarized in Table I.

Table I

	Caseinogen		Lactalbumin	
	Cow's %	Woman's %	Cow's %	Woman's %
Nitrogen	15.24	14.37	14.23	14.57
Sulphur	0.65	0.78	1.52	1.65
Phosphorus	0.84	0.45	0.19	0.16
Arginine	3.73	3.58	4.01	5.03
Histidine	1.67	1.52	1.82	1.66
Lysine	6.13	5.46	6.27	6.57
Tryptophan	1.36	1.05	1.90	2.47
Tyrosine	5.80	5.45	3.64	4.48
Cystine	0.36	0.60	3.31	4.14
Methionine	2.89	2.70	2.29	1.40

Comparison of the amounts of the constituents of cow's and woman's milk

By taking the mean values of 2.28 g. caseinogen and 0.71 g. lactalbumin in cow's milk and 0.32 g. of caseinogen and 0.68 g. lactalbumin in woman's milk per 100 ml. the contents of the several amino-acids can be calculated. The full data for all constituents are given for convenience in Table II.

Except for cystine, which is the same in amount in cow's and woman's milks, cow's milk contains 2-3 times the amounts of the other amino-acids.

Cow's milk diluted with an equal volume of water will contain half the amount of cystine present in woman's milk. If diluted with two volumes of water then tryptophan and possibly arginine will be below the amounts in woman's milk.

Cystine is stated to be replaceable by methionine in nutrition. Cow's milk diluted with an equal volume of water will have an equal amount of the sum of these sulphur-containing amino-acids. Cystine cannot replace methionine [Rose, 1936] which is an essential amino-acid.

Table II. *Composition of milk in g. per 100 ml.*

	Cow's		Total	Woman's		Total
Calcium			0.140			0.040
Phosphorus			0.101			0.016
Nitrogen			0.491			0.192
Non-protein-N			0.042			0.047
	Caseinogen	Lactalbumin		Caseinogen	Lactalbumin	
Protein-N	0.347	0.102	0.449	0.046	0.099	0.145
(factor for protein)	(6.562	7.027	—	6.959	6.863)	—
Protein	2.280	0.710	2.990	0.320	0.680	1.000
Protein-S	0.015	0.011	0.026	0.002	0.011	0.013
Arginine	0.085	0.028	0.113	0.012	0.034	0.046
Histidine	0.038	0.013	0.051	0.005	0.011	0.016
Lysine	0.140	0.044	0.184	0.017	0.045	0.062
Tryptophan	0.031	0.013	0.044	0.003	0.017	0.020
Tyrosine	0.132	0.026	0.158	0.017	0.030	0.047
Cystine	0.008	0.024	0.032	0.002	0.030	0.032
Methionine	0.066	0.016	0.082	0.009	0.009	0.018

Supposing that cystine is not replaceable by methionine in the nutrition of the human infant, then 16 mg. per 100 ml. (or $\frac{1}{3}$ grain per 5 oz.) should be added to diluted cow's milk (1 : 1).

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