XXXV. A COMPARATIVE STUDY OF THE PROTEINS OF THE COLOSTRUM AND MILK OF THE COW AND THEIR RELATIONS TO SERUM PROTEINS.

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Very diverse views have been propounded from time to time as to the nature of the protein constituents of cow's milk. On the one hand Duclaux and others have asserted that only one protein, caseinogen, is present, existing in coagulable and non-coagulable forms. On the other hand the literature of milk proteins includes claims to the isolation of some nine or ten distinct proteins. There can be little doubt that the majority of these latter preparations were of very doubtful purity, and from a close survey of the literature it is clear that the presence of only two distinct proteins has been demonstrated with reasonable certainty, viz. caseinogen and lactalbumin. In normal cow's milk of average quality these are present to the extent of about 3.0 % and 0.4 % respectively. A third protein, with the general characteristics of a globulin, was isolated by Sebelien [1885] and independently by Emmerling [1888], the amount present in milk being stated to be not more than about 0.005 %. More recently Halliburton [1890] has denied the existence of this lactoglobulin, the material isolated by Sebelien being regarded by him as lactalbumin.

There can be no such doubt as to the existence of a globulin in the colostrum—the fluid secreted in the earliest stages after parturition. The secretion at this stage is characterised by an extraordinary richness in protein material, this forming quite commonly 18 to 20 % of the total secretion. The proportions of caseinogen (4–5 %) and of albumin (0.7–1.5 %) are appreciably higher than in the normal milk of later stages of lactation, but

the great bulk (6–12 %) of the colostrum protein consists of a globulin. With the development of mammary activity this preponderance of globulin rapidly vanishes, and usually within 48 hours of calving the proportion of globulin in the secretion has become similar to, or less than that of albumin, the total protein being correspondingly reduced.

These changes with advance of lactation in the proportions of the three proteins present are well illustrated by the following averages obtained by us with seven cows of the herd at Manor Farm, Garforth (Experimental Farm of the University of Leeds and the Yorkshire Council for Agricultural Education). The cows were milked twice daily after calving.

	Nitrogen present				
	Total %	as caseinogen %	as albumin %	as globulin %	as non-protein %
1st milking	2.40	0.75	0.14	1.32	0.19
2nd "	2.01	0.68	0.17	1.02	0.14
3rd "	1.44	0.59	0.14	0.59	0.12
4th "	0.97	0.51	0.11	0.31	0.04
5th "	0.76	0.46	0.07	0.20	0.03
6th "	0.75	0.46	0.06	0.20	0.03
7th "	0.69	0.42	0.06	0.18	0.03
8th "	0.65	0.46	0.05	0.12	0.02

A more prolonged series of analyses made by Sutherst [1902] with a single cow gave very similar results.

It has been commonly assumed that the protein preparations obtained from colostrum are identical with the similarly named preparations obtainable by the same methods from milk, but little evidence has yet been adduced in justification of this assumption. Even in the case of caseinogen the existing evidence amounts to little more than the close agreement in physical properties and ultimate chemical composition.

In the work described in the present communication we have endeavoured to obtain really conclusive evidence on this point by a study of the chief products of hydrolysis of preparations of caseinogen, globulin and albumin isolated with the greatest care from the colostrum and later from the normal milk of the same cows. Two cows (2A1 and 10A1) of the dairy herd at the Manor Farm, Garforth, were used for the purpose, the colostrum being taken in each case immediately after calving, whilst three months later a quantity of the milk of each cow was worked up in precisely similar fashion.

The methods adopted for the isolation and purification of the different

proteins are described fully later. For the characterisation of the proteins the method of analysis elaborated by van Slyke [1911] was used. This method has now become widely known and it will suffice here to indicate that it consists of a quantitative study of the distribution of the nitrogen of the protein between the different nitrogenous ingredients of the mixture obtained by acid hydrolysis of the protein. The determinations made lead finally to a classification of the nitrogen distribution under the following heads:

Ammonia-N, melanin- (or humin-) N, cystine-N, arginine-N, histidine-N, lysine-N, and nitrogen in forms not precipitable by phosphotungstic acid (subdivided into mono-amino-N and non-amino-N).

The method requires only 3-4 g. of protein, and with due allowance for experimental errors, all the nitrogen can be accounted for. The limits of accuracy which must be kept in mind in discussing the results may be gauged from the following data given by van Slyke [1911] which represent as large differences between duplicates as are normally to be expected.

			%			%
Ammonia-N	...	...	0.37	Lysine-N	...	1.23
Melanin-N	...	...	0.39	Total N of filtrate	...	1.50
Cystine-N	...	...	0.11	Amino-N of filtrate	...	1.10
Arginine-N	...	...	1.27	Non-amino-N of filtrate	...	1.20
Histidine-N	...	...	0.93			

It should be remarked that van Slyke's analyses, from which the foregoing table of errors is deduced, were done on duplicate portions of the same hydrolysis solution. In the experiments described in this paper, however, each protein was hydrolysed separately, and thus any errors incurred during the preparation of the proteins or during their hydrolysis would be added to the experimental errors described above.

In order to ensure the strictest possible comparability of the results throughout, the whole of the experimental work has been carried out by one of us (H. R.) and every effort has been made to secure the maximum of accuracy of which the method is capable.

The determination of the histidine-N, lysine-N and non-amino-N by difference rather than by direct estimation would seem to make these figures liable to considerable errors, but experience, as indicated above, shows that the errors are, in actual fact, relatively small [cf. van Slyke, 1911].

In addition to furnishing evidence as to the identity or otherwise of the corresponding proteins of colostrum and milk, our results, taken in conjunction with those obtained by the same methods by Hartley [1914] with the globulins and albumin of ox-serum, enable conclusions to be drawn as

to the identity or otherwise of the globulins and albumin of colostrum and milk with the analogous proteins of ox-serum.

Further, since we have obtained lactoglobulin in pseudoglobulin and eu-globulin forms we have been able to compare the chemical structure of the protein in these two forms.

CASEINOGEN.

Evidence of the identity of caseinogen prepared from colostrum with that prepared from milk was first adduced by Sebelien [1885] and later by Winterstein and Strickler [1906]. In each case the evidence amounted to little more than the close agreement in ultimate chemical composition, and the similarity of response to familiar qualitative protein tests. Their conclusions have been substantiated, however, by the more recent work of Bauer and Engel [1911] and others in which the precipitin and other biological tests have been used.

Our own results are summarised in Table I, which gives the results of analyses of two preparations each from colostrum and milk.

TABLE I.

Summary of the Corrected Analyses of Different Preparations of Caseinogen in Percentages of the Total Nitrogen.

	From colostrum		From milk		Average		Average of all four samples %
	(1) Cow 2AI %	(2) Cow 10AI %	(3) Cow 2AI %	(4) Cow 10AI %	Colostrum (1 and 2) %	Milk (3 and 4) %	
Ammonia-N	10.42	10.07	10.15	10.35	10.25	10.25	10.25
Melanin-N	1.29	1.35	1.27	1.12	1.32	1.20	1.26
Total N of bases	25.94	26.76	27.52	26.28	26.35	26.90	26.62
Cystine-N	1.34	1.40	1.23	1.24	1.37	1.24	1.30
Arginine-N	8.95	9.87	9.73	8.71	9.41	9.22	9.31
Histidine-N	6.45	6.12	7.17	6.47	6.29	6.82	6.55
Lysine-N	9.20	9.37	9.39	9.86	9.29	9.62	9.46
Total N of filtrate	63.30	62.25	61.00	62.70	62.78	61.85	62.31
Amino-N of filtrate	56.90	55.34	54.06	55.46	56.12	54.76	55.44
Non-amino-N of filtrate	6.40	6.91	6.94	7.24	6.66	7.09	6.87
Sum	100.95	100.43	99.94	100.45	100.69	100.19	100.44

The differences between the results for the four samples are in no case greater than would normally be expected between duplicate analyses of any one preparation of caseinogen, so that one may reasonably conclude that, so

far as the protein part of the molecule is concerned, the caseinogen of colostrum is identical with that of milk.

GLOBULIN.

Hammarsten seems to have been the first to suggest the presence of a globulin in milk, and a substance with globulin-like characteristics was indeed isolated from milk by Sebelien [1885]. He expressed a doubt, however, whether this substance could be regarded as a true globulin.

Halliburton [1890] on repeating Sebelien's work obtained a small quantity of a protein preparation, which, however, he maintained was not a globulin but merely lactalbumin. As a result of further investigation he arrived at the conclusion that milk does not contain a globulin.

There can be no doubt that this conclusion is erroneous. Not only do biological tests point clearly to the presence of a globulin in milk, but we have succeeded in obtaining from milk small quantities of material which, as the results in Table II indicate, is in every way identical with the undoubted globulin prepared from colostrum.

In the case of the preparation from colostrum the total globulin was resolved into pseudoglobulin and euglobulin by dialysis, but owing to the small quantities involved this was not feasible in the case of the preparation from milk.

TABLE II. *Summary of the
of Globulin in Per-*

				From colostrum			
				Cow 2A1		Cow 10A1	
				(5) Eu globulin %	(6) Pseudo- globulin %	(7) Eu- globulin %	(8) Pseudo- globulin %
Ammonia-N	...	...		7.28	7.19	6.92	7.33
Melanin-N	...	...		2.00	2.03	2.40	2.20
Total N of bases	...	24.95		24.57		24.52	23.90
Cystine-N	...	...		2.00	1.83	2.04	1.81
Arginine-N	...	...		10.93	10.70	10.69	10.41
Histidine-N	...	...		3.87	4.16	3.68	3.25
Lysine-N	...	...		8.15	7.88	8.11	8.43
Total N of filtrate	...	65.31		65.82		63.97	64.57
Amino-N of filtrate	...			64.46	64.49	63.08	63.31
Non-amino-N of filtrate	...			0.85	1.33	0.89	1.26
Sum	...	...		99.54	99.61	97.81	98.00

From Table II it is clear that the total globulin of milk is identical in composition with that of colostrum. Further, it is obvious that, so far as the protein part of their molecules is concerned, eu-lactoglobulin and pseudo-lactoglobulin are identical. This is in complete accord with the similar observations of Hartley [1914] on euglobulin and pseudoglobulin from ox-serum, and lends further support to the hypothesis of Chick [1914] that euglobulins are protein-lipoid complexes resulting from the interaction and mutual precipitation of the colloidal solution of pseudoglobulin with a lipid emulsion. That differences do exist between the pseudoglobulin and euglobulin prepared from ox-serum is clear from the work of Hardy [1905] and Haslam [1913], but Hartley's results show that this difference is limited to the non-protein ingredients of the preparations, as do our results for the parallel, if not identical, case of the lactoglobulin preparations.

ALBUMIN.

The results of analysis of three samples of albumin from colostrum and two samples from milk are summarised in Table III.

They show clearly that the lactalbumin of colostrum is identical with that of milk, and thus substantiate the indications of biological tests.

Corrected Analyses of Different Preparations centages of the Total Nitrogen.

From milk			Average					Average of all seven samples %
Cow 2A1		Cow 10A1	From colostrum			From milk		
(9) Total globulin %	(10) Total globulin %	(11) Total globulin %	Eu- globulin (5 and 7) %	Pseudo- globulin (6 and 8) %	Total globulin (5, 6, 7 and 8) %	Total globulin (9, 10 and 11) %		
	8.03	8.04	8.22	7.10	7.26	7.18	8.10	7.57
	2.23	1.95	2.30	2.20	2.12	2.16	2.16	2.16
25.80	26.82	25.99	24.74	24.24	24.49	26.20	25.23	
	2.01	2.00	1.59	2.02	1.82	1.92	1.87	1.90
	10.59	11.30	10.92	10.81	10.56	10.68	10.94	10.79
	3.92	4.13	4.68	3.78	3.70	3.74	4.24	3.96
	9.28	9.39	8.80	8.13	8.16	8.15	9.16	8.58
62.89	62.54	63.55	64.64	65.20	64.92	63.00	64.10	
	61.39	61.52	62.52	63.77	63.90	63.83	61.81	62.97
	1.50	1.02	1.03	0.87	1.30	1.09	1.19	1.13
	98.95	99.35	100.06	98.68	98.81	98.74	99.46	99.06

Comparison of the Three Proteins.

In view of the divergent views which have been expressed from time to time as to the inter-relation of the proteins of milk it is of interest to compare the results of analysis, for which purpose Table IV gives a convenient summary.

TABLE IV.

	Caseinogen %	Lactoglobulin %	Lactalbumin %
Ammonia-N	10.25	7.57	7.93
Melanin-N	1.26	2.16	1.82
Total N of bases	26.62	25.23	26.72
Cystine-N	1.30	1.90	2.18
Arginine-N	9.31	10.79	7.56
Histidine-N	6.55	3.96	4.44
Lysine-N	9.46	8.58	12.54
Total N of filtrate	62.31	64.10	62.49
Amino-N of filtrate	55.44	62.97	59.84
Non-amino-N of filtrate	6.87	1.13	2.65

These results are conclusive as to the distinct chemical individuality of each of the three proteins. Thus caseinogen is sharply characterised by its relatively high proportion of ammonia-N, histidine-N and "non-amino-N of filtrate" (*i.e.* of proline-N + oxyproline-N + tryptophane-N) and relatively low proportion of melanin-N, cystine-N and α -mono-amino-acids ("amino-N of filtrate"). The arginine and lysine figures for caseinogen also mark it off as a separate protein.

Similarly the lactoglobulin is sharply differentiated from the lactalbumin by its lower content of lysine and its higher content of arginine. This large difference in lysine-content is in agreement with the observations of Hartley on the analogous proteins of ox-serum and horse-serum. The higher cystine-content of lactalbumin is in agreement with the sulphur-content of the two proteins. Thus, in lactalbumin from milk Sebelien found 1.73 % S, whilst in lactoglobulin from colostrum Tiemann [1898] found only 1.24 % S.

The foregoing conclusions as to the distinct chemical individuality of the three milk proteins are in complete accord with conclusions as to the biological differentiation of the three proteins which have been arrived at by the precipitin method [Hamburger, 1901], the method of complement deviation [Bauer and Engel, 1911] and anaphylactic methods [Besredka, 1909; Heuner, 1911].

TABLE V.
*Showing the Similarity in Composition of the Globulins of Cow's Colostrum,
 Cow's Milk and Ox-Serum.*

	Total globulin		Euglobulin		Pseudoglobulin		Average of all samples	
	From milk (average of three samples) (9, 10, 11) %	From ox-serum (one sample) (Hartley) %	From colostrum (average of two samples) (5, 7) %	From ox-serum (average of two samples) (Hartley) %	From colostrum (average of two samples) (6, 8) %	From ox-serum (one sample) (Hartley) %	From milk and colostrum (seven samples) (5-11) %	From ox-serum (four samples) (Hartley) %
Ammonia-N	8.10	7.7	7.10	8.65	7.26	7.5	7.57	8.13
Melanin-N	2.16	2.0	2.20	2.25	2.12	1.9	2.16	2.10
Total N of bases	26.20	28.2	24.74	27.65	24.24	27.1	25.23	27.65
Cystine-N	1.87	2.0	2.02	1.7	1.82	1.9	1.90	1.82
Arginine-N	10.94	10.9	10.81	11.65	10.56	10.8	10.79	11.25
Histidine-N	4.24	6.3	3.78	5.15	3.70	4.8	3.96	5.35
Lysine-N	9.16	9.0	8.13	9.15	8.16	9.6	8.58	9.23
Total N of filtrate	63.00	62.0	64.64	60.05	65.20	63.3	64.10	61.35
Amino-N of filtrate	61.81	59.8	63.77	57.95	63.90	61.7	62.97	59.35
Non-amino-N of filtrate	1.19	2.2	0.87	2.1	1.30	1.6	1.13	2.00
Sum	99.46	99.9	98.68	98.60	98.81	99.8	99.06	99.23

Comparison with the Proteins of Ox-Serum.

The question of the relationship of the proteins of milk to those of the blood is one of obvious interest and has already received considerable attention. The earlier investigators, relying upon ultimate chemical analysis and methods of precipitation, concluded that the albumins of milk and of blood were identical. Later workers, using the biological methods elaborated by workers on immunity, have confirmed this conclusion and extended it further to the globulins [Bauer and Engel, 1911].

Our results obtained with lactoglobulin and lactalbumin, when taken in conjunction with those obtained by Hartley [1914] with ox-serum globulin and ox-serum albumin, whilst confirming the identity of the globulins, do not harmonise with the foregoing conclusions, so far as the albumins are concerned. For convenience of comparison we have assembled the data necessary for the comparison in Tables V and VI.

(a) Comparison of Globulins.

Table V contains a summary of the average results obtained by Hartley and by us in the analysis of the respective globulins.

Comparison of these results shows a striking resemblance throughout between the different preparations. The greatest variation is seen in the histidine values, but, as pointed out earlier, these are subject to relatively greater errors than any other of the values. If due allowance be made for unavoidable experimental error, and for the varying personal error of the different observers, there can be little doubt that these results, so far as they go, indicate that the respective globulins, whether total globulin, pseudo-globulin, or euglobulin, of colostrum and milk are identical with those of ox-serum.

(b) Comparison of Albumins.

The average results obtained by us for lactalbumin are summarised in Table VI, together with Hartley's analysis of serum albumin. This table also includes in the last column the average of three analyses by van Slyke [1915] of a sample of lactalbumin obtained from milk by precipitation of the caseinogen by acetic acid and coagulation of the lactalbumin by heating. This preparation would obviously be contaminated with the small amount of lactoglobulin present in the milk, but the analysis serves as a useful check upon our results obtained with purer preparations.

TABLE VI.

*Showing the Differences in Composition of the Albumins of
Cow's Milk and Ox-Serum.*

	Lactalbumin			Serum- albumin One sample (Hartley) %	Lactalbumin Average of three samples (van Slyke) %
	From colostrum (average of three samples, 12-14) %	From milk (average of two samples, 15, 16) %	Average of all five samples (12-16) %		
Ammonia-N	8.07	7.72	7.93	5.8	8.57
Melanin-N	1.93	1.65	1.82	1.1	2.32
Total N of bases ...	26.61	26.90	26.72	36.9	25.31
Cystine-N	2.07	2.34	2.18	3.5	1.30
Arginine-N	7.75	7.27	7.56	10.4	7.20
Histidine-N	4.60	4.20	4.44	6.7	4.57
Lysine-N	12.19	13.08	12.54	16.3	12.24
Total N of filtrate ...	62.19	62.93	62.49	56.5	63.50
Amino-N of filtrate ...	59.13	60.91	59.84	54.2	61.53
Non-amino-N of filtrate	3.06	2.03	2.65	2.3	1.97
Sum	98.80	99.20	98.96	100.3	99.70

Comparison of the results reveals marked differences between our lactalbumin and Hartley's serum albumin. This is strikingly manifest in every item of the analyses, but notably in the basic nitrogenous constituents. The reliability of our results for lactalbumin may be gauged from the separate analyses set out in Table III, and further from the reasonably close concordance with van Slyke's results. On the other hand, we have for serum albumin only the one analysis by Hartley, although his results are substantially confirmed by those obtained by him with albumin from horse-serum.

As already mentioned most of the older chemical work and the more recent biological work has pointed to the identity of lactalbumin with serum albumin, but observations that do not harmonise with this conclusion have not been lacking.

Thus Sebelien [1885] found that albumin whether prepared from colostrum or milk had a specific rotatory power of $[\alpha]_D = -36^\circ$ to -37° , whilst serum albumin isolated by a similar method from ox-blood gave $[\alpha]_D = -62.6^\circ$. Further, Halliburton [1890] showed that lactalbumin differed from serum albumin in its behaviour on heat-coagulation, and in its precipitability by certain neutral salts.

There is obvious need for further investigation here, but it is clear that biological tests alone will not suffice for the purpose. With the material at our disposal we propose to investigate this point more closely.

EXPERIMENTAL DETAILS.

Preparations of the Proteins.

Summary. *Caseinogen* was prepared by precipitation from the diluted colostrum or milk with saturated potash alum solution (Schlossmann); *Total Globulin* by saturation of the neutralised filtrate from the caseinogen with anhydrous magnesium sulphate; the two globulins were separated by dialysis; *Albumin* was obtained from the filtrate from the total globulin by saturation with anhydrous sodium sulphate at 40° C. (Pinkus). Ammonium sulphate was not used as a precipitant for the globulin and albumin in order to avoid the possibility of adsorption of extraneous nitrogen by the proteins.

Caseinogen. Freshly drawn colostrum was diluted with five times its volume of *distilled* water¹ and heated to 40° in order to produce a more satisfactory settling of the caseinogen. Saturated potash alum solution was then added in sufficient amount to produce complete precipitation of the caseinogen. In the case of milk, dilution with only three times its volume of water was necessary and less alum solution was also needed. After settling for half an hour, the clear supernatant liquid was syphoned off, the residuum of caseinogen and fat strained on muslin, washed three times by stirring vigorously with water, and the filtered washings added to the main bulk of liquid, for the preparation of globulin and albumin.

Before proceeding with the purification of the caseinogen, it was necessary to free it from the large amount of fat carried down with it on precipitation. This was done at first by extracting the semi-solid mass with alcohol and then with ether. It was found preferable, however, to extract the fat with ether from the solution of caseinogen in alkali. For this purpose the caseinogen, suspended in distilled water, which was kept well stirred, was dissolved in the minimum amount of 1% soda and the solution extracted with ether. The ethereal layer carried with it most of the insoluble matter, and filtration of the solution of caseinogen in alkali was thereby rendered much easier. The filtered solution was diluted with distilled water and the caseinogen reprecipitated with 1% acetic acid, added with constant stirring. After settling, the supernatant liquid was syphoned off and the caseinogen washed by sedimentation. This process of dissolving in alkali and reprecipitating was repeated five times. The final solution in alkali was filtered clear through

¹ It is essential, in order to produce a perfectly white sample of caseinogen, that all dilutions should be done with distilled water. The use of tap water, especially if of peaty origin, causes the production of a brownish coloured product.

filter paper pulp, diluted, and precipitated with the minimum amount of 1% hydrochloric acid. After washing and draining, the caseinogen was dried by grinding with alcohol, and finally with ether. The final product (air-dried) was a perfectly white, dusty powder.

Globulin. The filtrate from the caseinogen preparation was neutralised with dilute soda, and saturated with anhydrous magnesium sulphate, thus obviating the large increase in volume which occurs if the crystalline salt is used. The precipitated globulin was filtered off on large Buchner funnels and the filtrate used for the isolation of albumin. The crude globulin was dissolved in distilled water to which a little magnesium sulphate had been added, the solution filtered through pulp, saturated with magnesium sulphate, and the globulin filtered off. This reprecipitation was repeated four times at the same volume each time, and the filtrate in each case was added to the albumin preparation. The final solution of globulin was then dialysed in large sausage-skin dialysers for six days in running water, and then in relays of distilled water until free from sulphates. The globulin was thus separated into two fractions; euglobulin, insoluble in distilled water, and pseudoglobulin, soluble in distilled water.

Euglobulin. This was filtered from the soluble pseudoglobulin, dissolved in the minimum amount of 0.6% sodium chloride solution, filtered, and reprecipitated by diluting the solution with ten times its volume of distilled water. The precipitate was allowed to settle, washed with distilled water, and finally dried with alcohol and ether, thus giving an almost white powder.

Pseudoglobulin. The filtered solution of pseudoglobulin in water was precipitated with alcohol, filtered, and the protein dried with alcohol and ether, giving a perfectly white powder.

Albumin. This protein was isolated and purified by Pinkus' method [1901]. The combined filtrates from the globulin preparation were heated to 40°, saturated with anhydrous sodium sulphate (free from chlorides) and allowed to stand at this temperature for a few hours. The precipitated albumin was then filtered off through a warm jacketed filter, care being taken to prevent the salt from crystallising. The crude albumin was dissolved in cold water, filtered, and the solution saturated with magnesium sulphate. The slight precipitate, probably globulin, was filtered off, and the filtrate saturated with anhydrous sodium sulphate at 40° as before. This process was repeated four times, saturation with magnesium sulphate being from the same volume each time. The last saturation with magnesium sulphate gave no precipitate. The final precipitate of lactalbumin was dissolved in water

and the filtered solution precipitated with alcohol, the protein being finally dried with alcohol and ether, and gave a white, light powder, readily soluble in water.

In the preparation of the proteins from colostrum the first three milkings after calving from two cows (2A1 and 10A1) were taken, and the proteins isolated from each of these separately. Had any appreciable differences between the respective proteins of colostrum and milk been found, it was proposed to analyse separately each sample of protein obtained. Since, however, no such differences could be detected, only the preparations from the first sample of colostrum after calving were analysed.

The proteins from milk were prepared from samples taken from the same cows three months after calving, when it could be safely assumed that the milk had become quite normal. The method of isolation was the same as for colostrum. It has been remarked, however, that in the purification of the albumin from colostrum, when the solution of the albumin was saturated with magnesium sulphate, it gave in the first two or three reprecipitations a slight precipitate with magnesium sulphate, which was supposed to be a globulin. In working up the colostrum these small amounts of globulin were rejected, an ample yield being otherwise obtained. In the case of milk, however, the combined precipitates obtained by saturation with magnesium sulphate in the purification of the albumin, were added to the main bulk of the globulin precipitate, and the whole purified by reprecipitation with magnesium sulphate six times. That it was really a part of the globulin which remained dissolved in the albumin solution is clear from the analyses, and the recovery of these portions probably accounts for the fact that a much bigger yield of globulin was obtained from milk than has hitherto been described. Twenty litres of milk from each cow were worked up separately, but only a portion of the caseinogen was purified. The following amounts of lactoglobulin and lactalbumin were isolated. From 20 litres of milk in each case

Cow 2A1 gave 7.28 g. lactoglobulin and 43.0 g. lactalbumin.

Cow 10A1 gave 4.54 g. lactoglobulin and 35.4 g. lactalbumin.

Owing to the difficulty of obtaining more than small quantities of the globulin in milk, and since preliminary analyses had pointed to the fact that euglobulin and pseudoglobulin from colostrum are indistinguishable by the method of analysis used, the samples of globulin obtained from milk were not fractionated into eu- and pseudoglobulin, so that in this case data for total globulin only are recorded in the tables.

METHODS OF ANALYSIS.

Van Slyke's method of protein analysis was used throughout these experiments. For details the original paper must be consulted.

In order that the analyses might be strictly comparable the conditions of analysis of each protein were made as nearly the same as possible. Whenever the amount available permitted it, 3.5 g. of the protein, air dried, were used for an analysis, the lowest amount used (analysis 10, Table VII) being 2.5 g. In all cases the protein was hydrolysed by heating with thirty-five times its weight of 20 % hydrochloric acid for 48 hours at 100°, using flasks with air-condensers ground into the necks. The resultant mixture was evaporated *in vacuo* to remove as much HCl as possible and was then made up to 100 cc., and the following estimations were made:

- (i) *Total N* estimated (Kjeldahl) on two separate portions of 10 cc. each.
- (ii) 75 cc. were taken for the subsequent analysis and gave
 - (a) *Ammonia-N* in the 75 cc.
 - (b) *Melanin-N* in the 75 cc.

After removal of the ammonia and melanin the bases were precipitated with phosphotungstic acid, and after removal of the phosphotungstic acid with baryta, the solution of the bases was made up to 100 cc., and the following estimations made on this solution:

- (c) *Cystine-N* in 20 cc. of the solution of the bases.
- (d) *Arginine-N* in 50 cc. of the solution of the bases.
- (e) *Residual-N* (*i.e.* half the arginine-N and total N of cystine, histidine, and lysine) in the same 50 cc. of solution as (d), so that (d) + (e) gave the total N of the bases.
- (f) *Amino-N of the bases* in 2 cc. of the solution: done in triplicate.

The filtrate from the phosphotungstic acid precipitate of the bases was made up to 200 cc. and the following estimations were made on this solution:

- (g) *Total N of the filtrate* in 50 cc.: done in duplicate.
- (h) *Amino-N of the filtrate*; 15 cc. were diluted to 25 cc. and the amino-N estimated in 2 cc.: done in triplicate.
- (i) *Non-amino-N of the filtrate*, was calculated by taking the differences of (g) and (h).

Table VII contains a record of the whole of the analytical observations performed on each protein, as described in the above scheme, whilst Table VIII gives a summary of the whole of the results obtained.

TABLE VII. *Details of Analyses.*

TABLE VII. <i>Details of Analyses.</i>																									
Nature of preparation analysed		Total N		Ammonia		Melanin		Cystine		Arginine		Total N of bases			Amino-N of bases			Amino-N of filtrate				Total N of filtrate		Total N recovered g. per 75 cc.	
		cc. thio. per 10 cc.	g. N per 75 cc.	cc. thio. per 75 cc.	g. N per 75 cc.	cc. thio. per 75 cc.	g. N per 75 cc.	BaSO ₄ g. per 20 cc.	g. N per 75 cc.	cc. thio. per 50 cc.	g. N per 75 cc.	cc. thio. per 50 cc.	cc. thio. + arginine titration	g. N per 75 cc.	cc. gas per 2 cc.	t°	p mm.	g. N per 75 cc.	cc. gas per 2 cc.	t°	p mm.	g. N per 75 cc.	cc. thio. per 50 cc.		g. N per 75 cc.
1.	Caseinogen from colostrum. Cow 2AI ...	{34.63 34.48}	0.3545	27.02	0.03695	3.35	0.004581	0.0071	0.00213	5.12	0.02853	24.26	29.38	0.08186	{1.952 1.948 1.940}	17.0 16.4 16.0	{770.3 770.6 770.7}	0.04774	{2.375 2.363 2.388}	19.0 18.2 18.8	{765.7 765.1 764.4}	0.2069	{42.04 42.11}	0.2345	0.3579
2.	Caseinogen from colostrum. Cow 10AI ...	{33.61 33.84}	0.3458	25.45	0.03480	3.41	0.004663	0.0075	0.00225	5.55	0.03093	24.04	29.59	0.08244	{1.965 1.955 1.940}	16.1 15.6 17.0	{763.5 764.0 770.6}	0.04766	{2.265 2.255 2.252}	18.6 18.1 18.0	{767.3 767.3 767.3}	0.1966	{40.50 40.40}	0.2254	0.3473
3.	Caseinogen from milk. Cow 2AI ...	{36.61 36.69}	0.3777	27.89	0.03832	3.51	0.004822	0.0068	0.00204	5.97	0.03356	27.41	33.38	0.09380	{2.158 2.160 2.170}	15.7 16.0 16.2	{757.6 757.6 757.0}	0.05312	{2.347 2.380 2.355}	13.1 14.2 14.1	{761.0 761.0 761.0}	0.2094	{43.09 43.06}	0.2405	0.3774
4.	Caseinogen from milk. Cow 10AI ...	{34.88 34.85}	0.3577	27.07	0.03701	2.92	0.003993	0.0061	0.00183	5.02	0.02796	25.10	30.12	0.08391	{2.020 2.028 2.023}	12.7 13.0 15.7	{761.4 761.4 763.4}	0.05004	{2.332 2.336 2.335}	18.8 19.2 19.0	{768.2 768.0 768.0}	0.2035	{42.03 42.04}	0.2343	0.3592
5.	Euglobulin from colostrum. Cow 2AI ...	{37.39 37.27}	0.3922	20.39	0.02856	5.61	0.007859	0.0175	0.00525	7.06	0.03968	24.17	31.23	0.08776	{2.085 2.070 2.075}	15.4 15.3 14.6	{749.5 750.0 755.0}	0.05041	{2.900 2.905 2.880}	15.0 15.0 13.5	{753.3 753.2 755.0}	0.2580	{47.41 47.39}	0.2663	0.3905
6.	Pseudoglobulin from colostrum. Cow 2AI	{37.43 37.53}	0.3939	20.20	0.02830	5.70	0.007986	0.0154	0.00462	6.93	0.03895	23.91	30.84	0.08667	{2.015 2.020 2.045}	14.7 15.0 14.9	{754.8 754.8 746.6}	0.04907	{2.910 2.905 2.915}	14.7 15.0 14.6	{752.6 754.7 754.7}	0.2592	{47.96 47.91}	0.2693	0.3923
7.	Euglobulin from colostrum. Cow 10AI ...	{38.25 38.25}	0.4020	19.84	0.02780	6.89	0.009651	0.0186	0.00558	7.08	0.03979	24.39	31.47	0.08844	{2.135 2.135 2.115}	15.5 15.9 16.0	{746.3 746.3 747.0}	0.05129	{2.900 2.902 2.880}	15.0 15.0 15.0	{756.2 756.2 761.2}	0.2588	{47.58 47.58}	0.2673	0.3932
8.	Pseudoglobulin from colostrum. Cow 10AI	{38.50 38.71}	0.4056	21.23	0.02974	6.37	0.008923	0.0157	0.00471	6.94	0.03900	23.94	30.88	0.08677	{2.125 2.110 2.100}	15.0 16.0 16.1	{750.6 751.8 752.0}	0.05127	{2.900 2.900 2.890}	15.0 13.2 13.0	{760.6 762.0 762.0}	0.2620	{48.45 48.40}	0.2720	0.3974
9.	Total globulin from milk. Cow 2AI ...	{36.46 36.43}	0.3807	22.45	0.03058	6.08	0.008470	0.0168	0.00504	6.66	0.03712	24.96	31.62	0.08811	{2.150 2.140 2.140}	18.2 17.8 17.6	{767.2 767.2 767.2}	0.05286	{2.713 2.697 2.718}	15.0 14.5 14.8	{751.7 751.4 750.8}	0.2389	{44.69 44.83}	0.2495	0.3767
10.	Total globulin from milk. Cow 2AI ...	{25.35 25.59}	0.2661	15.33	0.02139	3.72	0.005182	0.0091	0.00273	4.82	0.02686	17.18	22.00	0.06130	{1.575 1.585 1.580}	18.0 18.1 17.9	{765.8 765.3 765.7}	0.03637	{1.965 1.975 1.965}	13.8 14.0 13.0	{750.0 750.0 755.8}	0.1689	{31.67 31.66}	0.1765	0.2644
11.	Total globulin from milk. Cow 10AI ...	{30.72 30.72}	0.3210	18.91	0.02638	5.29	0.007370	0.0083	0.00249	5.72	0.03187	20.60	26.32	0.07333	{1.770 1.775 1.778}	18.1 17.9 17.7	{764.3 764.7 765.0}	0.04196	{2.355 2.355 2.360}	15.4 14.1 14.7	{754.4 754.4 754.0}	0.2059	{38.42 38.44}	0.2141	0.3212
12.	Albumin from colostrum. Cow 2AI ...	{28.79 28.69}	0.3003	17.46	0.02378	4.24	0.005907	0.0103	0.00309	3.48	0.01939	21.23	24.71	0.06886	{1.967 1.955 1.970}	16.9 17.0 16.0	{768.8 768.8 768.2}	0.04802	{2.142 2.128 2.126}	19.2 19.0 19.0	{763.4 763.1 763.1}	0.1826	{35.75 35.83}	0.1995	0.2980
13.	Albumin from colostrum. Cow 2AI (Duplicate of 12.)	{28.61 28.59}	0.2988	16.68	0.02328	4.04	0.005628	0.0144	0.00432	3.50	0.01950	21.64	25.14	0.07005	{2.004 1.997 1.985}	18.0 17.6 16.7	{768.8 768.8 770.0}	0.04877	{2.095 2.105 2.105}	17.6 17.9 17.9	{760.7 760.2 760.2}	0.1802	{34.72 34.72}	0.1936	0.2926
14.	Albumin from colostrum. Cow 10AI ...	{33.76 33.76}	0.3528	22.01	0.02997	4.95	0.006900	0.0148	0.00444	4.57	0.02547	25.59	30.16	0.08405	{2.245 2.258 2.265}	16.7 18.2 17.6	{768.6 767.8 766.3}	0.05624	{2.478 2.476 2.482}	17.5 16.9 17.4	{758.0 757.8 757.3}	0.2161	{41.17 41.11}	0.2293	0.3502
15.	Albumin from milk. Cow 2AI ...	{34.97 34.93}	0.3584	20.31	0.02777	3.90	0.005333	0.0211	0.00633	3.99	0.02224	27.02	31.01	0.08640	{2.480 2.505 2.490}	17.7 17.0 17.8	{758.4 757.6 759.4}	0.06222	{2.565 2.578 2.568}	16.8 16.7 17.5	{751.2 751.2 751.2}	0.2228	{42.26 42.26}	0.2355	0.3550
16.	Albumin from milk. Cow 10AI ...	{34.88 34.84}	0.3538	19.98	0.02722	4.57	0.006366	0.0171	0.00513	4.15	0.02313	26.35	30.50	0.08498	{2.432 2.435 2.420}	18.9 18.7 17.1	{758.8 758.8 759.3}	0.06022	{2.540 2.535 2.530}	16.0 15.8 15.6	{751.5 752.6 753.2}	0.2214	{41.82 41.79}	0.2329	0.3515

Observations on the Method of Analysis.

(i) In all estimations of nitrogen by the Kjeldahl method the excess of acid used for neutralising the ammonia was determined by adding to the liquid to be titrated a little solid potassium iodide and then a few cc. of a saturated solution of normal potassium iodate. The iodine liberated by the free acid present was then estimated by titration with standard (N/10) sodium thiosulphate, using starch paste as indicator. A sharper end-point was thereby obtained than with the ordinary acidimetric method of titration. The strength of the thiosulphate solution was determined at short intervals by checking it against the standard acid used, and by titration with weighed amounts of pure iodine.

(ii) The filtration of the gelatinous melanin precipitate produced by the lime is very slow if carried out on an ordinary filter paper. It was found to be performed more expeditiously and the precipitate needed less washing to free it from chlorides by filtering by suction. In this, as in the filtration and washing of the phosphotungstates of the bases, the filtering tube as described by Irvine [1915] was used. A double gravimetric filter paper was fitted into the Buchner funnel, and a very slightly reduced pressure maintained all the time in the tube. The precipitate, if not allowed to become dry, when it cracks, is thus easily and quickly washed free from chlorides.

(iii) In the estimation of amino-nitrogen, van Slyke's micro-apparatus [1914] was used throughout. By this means all the estimations could be done in triplicate, and thus with greater accuracy than with the large apparatus. Increased accuracy was further obtained by enclosing the gas measuring burette in a glass jacket through which cold water of known temperature was slowly circulated, so that the volume of gas could be conveniently read off without any disturbance of its temperature. By this means, and with the aid of a lens in taking readings—the burette being of 5 cc. capacity with divisions of 0.01 cc.—it was found possible to read the volume of the gas to 0.002 cc. and to obtain triplicates agreeing within 0.01 cc. The liquid to be analysed was measured from a calibrated 2 cc. pipette and washed into the apparatus with a few drops of distilled water.

(iv) *Corrections for Reagents.* The following were the corrections determined for the impurities in the reagents. All the readings in Table VII have been corrected except the amino-nitrogen estimations.

Total N. 0.16 cc. thio. *Ammonia-N* nil. *Melanin-N.* 0.24 cc. thio.

Cystine-N. 0.0023 g. BaSO₄. *Arginine-N.* 0.16 cc. thio. .

Residual N of bases 0.18 cc. thio. *Amino-N of bases* 0.188 mgm. N.

Total N of filtrate 0.18 cc. thio. *Amino-N of filtrate* 0.129 mgm. N.

(v) *Corrections for the solubility of the phosphotungstates of the bases.*

The phosphotungstates of the bases are slightly soluble in the liquid from which they are precipitated. In order to correct for this error the bases were always precipitated from the same volume of solution (200 cc.) and the corrections as determined by van Slyke [1911, p. 32] applied.

The analyses given in Tables I–VI have all been corrected in this way, while Table VIII contains a summary of the analyses, expressed as percentages of the total nitrogen, before and after correction.

SUMMARY.

The chief proteins of cow's colostrum and cow's milk were isolated, and analysed by the method of van Slyke, with the following results.

1. Caseinogen, total lactoglobulin, and lactalbumin are sharply differentiated, distinct proteins, and have respectively the same composition whether prepared from colostrum or normal milk.

2. The presence in milk of a globulin in small quantities (0.03 % isolated) was proved.

3. Eu-lactoglobulin and pseudo-lactoglobulin are identical so far as the protein part of their molecules is concerned.

4. Lactoglobulin from either colostrum or milk is very closely allied to, and is probably identical with, serum globulin from ox-blood.

5. Lactalbumin from either colostrum or milk is very different in composition from serum albumin from ox-blood.

A considerable portion of the work described in this communication has been carried out in the Animal Nutrition Research Laboratory of the School of Agriculture, Cambridge, and we would take this opportunity of acknowledging our indebtedness to Professors Wood and Hopkins for the facilities kindly accorded for the purpose.

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