ELECTROPHORETIC STUDIES OF MILK

I. INVESTIGATIONS ON COLOSTRUM OF DAIRY COWS

by

G. V. HEYNDRICKX AND A. DE VLEESCHAUWER

Chair of Technology, State Agricultural College, Ghent (Belgium)

INTRODUCTION

In view of the manifold importance of the milk proteins and the limited knowledge we have of them, a scientific analytical examination appeared very desirable. In a search of the literature it is surprising to find that the methods of fractionation of the proteins in milk which have been developed up to the present have a chemical basis, whereas modern protein research has a physico-chemical basis. One of the best developed physico-chemical procedures—that of electrophoresis—has already had many applications in biochemistry but has not in practice been applied to milk, apart from a couple of qualitative investigations of skim milk or of proteins separated from milk.

METHODS

Since a protein solution intended for electrophoresis must be completely transparent, an important part of our work was to devise and develop a method of clearing the milk with the minimum of alteration in the qualitative and quantitative composition of the proteins. After trying a number of tests, the following process was finally adopted. The milk was dialysed* against MICHAELIS veronal-sodium buffer[1] of p_H 8 and ionic strength 0.1. After three days dialysis at low temperature (2° C) the milk was centrifuged at 10,000 to 15,000 r.p.m. in order to remove the fat, this giving complete clearing. This high-speed centrifuging may be done only after dialysing for some days, as otherwise the calcium casein compound, which is probably converted into the more stable sodium casein by the dialysis, would come out as a sediment. After centrifuging, the milk was again dialysed for a couple of days with repeated centrifuging in order to obtain finally a perfectly clear and completely dialysed serum. Separate tests showed that this clearing process occurred with practically no loss of protein.

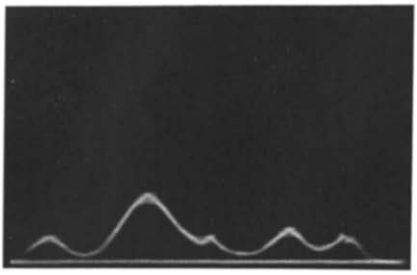
The so-prepared serum was diluted with the dialysis buffer and electrophoresis was carried out at 2° C with a current strength of 25 mA. For this purpose we used the Strübin apparatus** with the Philpot Svensson optical system, which is characterised by specially great accuracy and is described in detail in the numerous publications of WIEDEMANN[2]. The resolution of the diagrams obtained into ideal GAUSS curves was done according to the semi-automatic method of WIEDEMANN[3]. In calculating the percentual composition of the different fractions, the correction formulae of LONGSWORTH AND MCINNES[4] and WIEDEMANN[5] for the delta and epsilon effect were used. The mobilities were calculated from the classical formula:

$$u = \frac{q.s.\ K}{I.t}$$

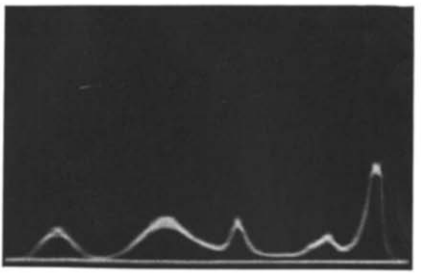
* The sausage casings used were from the Visking Corporation, 6733 West 65th. Street, Chicago (U.S.A.).

** Messrs. Strübin, Gerbergasse, 25, Basle (Switzerland).

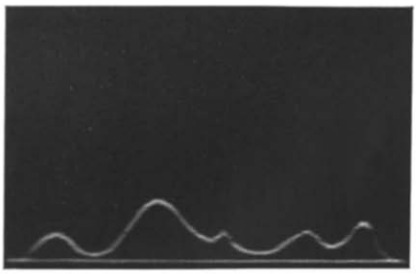
References p. 493.



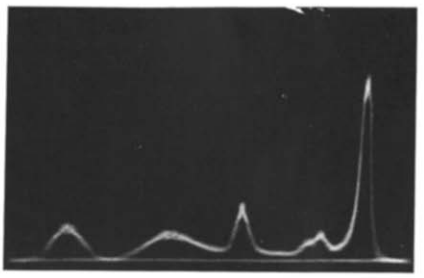
0 hours



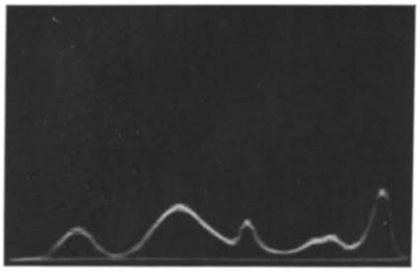
20 hours



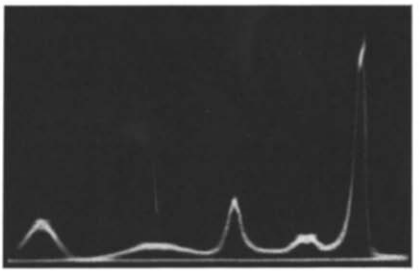
6 hours



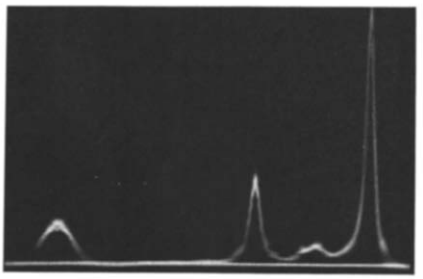
30 hours



12 hours



43 hours



8 days

Fig. 1. Electrophoresis diagrams of colostrum of dairy cows, at different intervals post partum
Cow B – Rising boundaries

In addition to the electrophoresis tests, a Kjeldahlometric fractionation according to the method of ROWLAND[6] was carried out, the total protein being determined by precipitation with trichloracetic acid at a final concentration of 12%, the casein by precipitation at p_H 4.6, the albumin by heating, the globulin by saturation of the casein-free filtrate with magnesium sulphate, and the proteose by difference.

EXPERIMENTAL RESULTS AND DISCUSSION

The milk of two cows was examined periodically during the colostrum period and the results are collected in Tables I and II and Fig. 1.

From the results of the Kjeldahlometric fractionation given in Table I, it appears in the first place that the samples taken immediately after calving had a very high total nitrogen content. In the sample from cow A the total nitrogen amounted to 1624.3 mg N per 100 g milk, decreasing very rapidly through the colostrum period to 1390.3 mg after 4 hours, 1221.9 mg after 8 hours, 987.3 mg after 18 hours, 785.5 mg after 30 hours and 453.1 mg in the normal state. With the samples from cow B the values went from 3245.5 mg after 0 hours, 2466.6 mg after 6 hours, 1676.8 mg after 12 hours, 1319.9 mg after 20 hours, 985.2 mg after 30 hours, 809.5 mg after 43 hours to 597.9 mg in the normal state. Similarly the content of protein nitrogen ranged from 1578.8–1343.8–1166.6–934.4–744.4 to 422.9 mg N per 100 g of milk for the samples from cow A and from 3151.8–2387.6–1604.9–1252.5–923.6–752.1 to 555.4 mg N for the samples from cow B.

In the sample from cow A, taken immediately after calving, the content of casein nitrogen attained a value of 664.2 mg, which is however only 42.1% of the total protein nitrogen. This casein nitrogen, as a percentage of the total protein nitrogen, rose gradually with time after calving and attained successively the values of 46.5 after 4 hours, 54.9 after 8 hours, 66.0 after 18 hours, 73.6 after 30 hours and 82.8% in the normal lactation state. The samples from cow B showed the same increasing values: the original percentage, lower than with cow A, *i.e.* 29.7%, becoming in succession 32.2–40.3–47.8–60.2–71.0 and finally 81.0%.

With regard to the globulin content, it appears from Table I that in the samples from cow A the percentage, calculated on total protein, was 41.7 on calving, and that this figure dropped through the values 38.2–33.1–21.7 and 13.4 to 4.5% in the normal state. The samples from cow B showed the same character but more pronounced: the globulin content dropped from 61.2 to 6.4%. While the percentage of casein to total protein rose during the first hours after calving in the proportion of about 1 to 2 or 1 to 2.7, the globulin percentage dropped from practically 9 to 1 or 10 to 1 according to whether we are considering the samples from cow A or from cow B.

In both samples the contents of albumin and proteose, as a function of total protein, showed only slight variations during the colostrum period.

The results of the Kjeldahlometric fractionation show that colostrum milk, as against normal milk, is characterised by—among other factors—an appreciably lower proportion of casein together with a still more conspicuous high proportion of globulin; as the time after calving increases, the casein rises and the globulin drops, both to the normal values.

On careful examination of the photographs of the electrophoresis diagrams, collected together on Fig. 1, the changes in composition of the milk proteins during the colostrum period appear very clearly. Since, on the photographs, the visual course of

References p. 493.

TABLE I

RESULTS OF THE KJELDAHLOMETRIC FRACTIONATION ON COLOSTRUM OF DAIRY COWS

Experiment no.	Time post partum	Total N-mg/100 g milk	Non-protein N-mg/100 g milk	Protein N-mg/100 g milk	Casein-N		Globulin-N		Albumin-N		Proteose-N	
					mg/100 g milk	in percent of total protein N	mg/100 g milk	in percent of total protein N	mg/100 g milk	in percent of total protein N	mg/100 g milk	in percent of total protein N
					Cow A							
66	0 hours	1624.3	45.5	1578.8	664.2	42.1	658.4	41.7	202.5	12.8	53.7	3.4
67	4 hours	1390.3	46.5	1343.8	625.6	46.5	512.8	38.2	145.0	10.8	60.4	4.5
68	8 hours	1221.9	55.3	1166.6	640.4	54.9	386.7	33.1	91.0	7.8	48.5	4.2
69	18 hours	987.3	52.9	934.4	616.0	66.0	202.8	21.7	77.0	8.2	38.6	4.1
70	30 hours	785.5	41.1	744.4	547.7	73.6	100.0	13.4	59.8	8.0	36.9	5.0
72	28 days	453.1	30.2	422.9	350.3	82.8	19.2	4.5	30.2	7.2	23.2	5.5
					Cow B							
166	0 hours	3245.5	93.7	3151.8	936.7	29.7	1926.6	61.2	202.9	6.4	85.6	2.7
172	6 hours	2466.6	79.0	2387.6	769.3	32.2	1383.1	57.9	180.3	7.6	54.9	2.3
168	12 hours	1676.8	71.9	1604.9	647.5	40.3	792.7	49.4	129.8	8.1	34.9	2.2
174	20 hours	1319.9	67.4	1252.5	599.1	47.8	527.4	42.1	95.7	7.7	30.3	2.4
170	30 hours	985.2	61.6	923.6	555.8	60.2	262.4	28.4	79.5	8.6	25.9	2.8
171	43 hours	809.5	57.4	752.1	534.0	71.0	131.5	17.5	66.4	8.8	20.2	2.7
176	8 days	597.9	42.5	555.4	450.0	81.0	35.5	6.4	50.3	9.1	19.6	3.5

TABLE II

RESULTS OF THE ELECTROPHORETIC EXAMINATION ON COLOSTRUM OF DAIRY COWS

Time post partum	A	B	C	D	E	F	G	H
1. Relative percentual concentrations for the different fractions								
Cow A								
0 hours	1.3	21.8	6.4	6.4	4.4	3.8	9.0	46.9
4 hours	1.8	25.5	8.3	6.6	4.7	5.4	9.5	38.2
8 hours	1.1	30.8	7.5	6.2	5.2	6.3	10.1	32.8
18 hours	1.3	43.0	9.4	7.4	4.9	4.9	10.9	18.2
30 hours	1.8	50.4	8.0	7.7	4.7	4.7	11.9	10.8
30 days	2.0	51.8	11.9	5.8	6.1	4.4	14.7	3.3
Cow B								
0 hours	1.0	11.9	7.0	8.4	3.4	2.6	6.5	59.2
6 hours	0.9	14.1	7.1	8.4	4.7	3.1	8.3	53.4
12 hours	1.0	16.4	7.5	9.4	4.5	2.6	11.2	47.4
20 hours	0.8	26.2	5.7	9.0	3.5	2.7	12.3	39.8
30 hours	0.8	39.1	5.8	7.9	3.9	2.9	13.8	25.8
43 hours	0.8	45.3	5.2	7.2	4.4	5.0	17.1	15.0
8 days	0.9	48.6	8.3	7.0	9.0	3.6	18.4	4.2
2. Mobilities ($\times 10^{-5}$) for the different fractions								
Cow B								
0 hours	13.2	7.3	6.7	6.0	5.2	4.4	3.6	1.8
6 hours	13.4	7.4	6.9	6.2	5.3	4.5	3.8	2.1
12 hours	13.5	7.4	6.8	6.0	5.3	4.4	3.7	2.0
20 hours	13.1	7.4	6.8	6.1	5.3	4.4	3.6	1.9
30 hours	13.4	7.6	6.8	6.3	5.5	4.5	3.7	1.9
43 hours	13.3	7.5	6.8	6.3	5.6	4.6	3.5	1.6
8 days	13.4	7.8	7.1	6.5	6.0	4.9	3.6	2.0

the protein composition during the colostrum period is similar with both animals both on the rising and descending boundaries, only the rising boundaries of cow B are reproduced. Moreover the most quickly moving, but very small, A peak is not visible on Fig. 1 as when exposed it had disappeared from the field of view; for the calculation this peak was photographed separately.

The results obtained from the electrophoresis diagrams, given in Table II, show that with colostrum, as with normal milk, there were eight fractions which we call, in decreasing order of mobility, A, B, C, D, E, F, G and H. The B component, the dominant fraction with normal milk, represents merely 21.8% in the sample from cow A taken immediately after calving. This percentage increased during the post partum period through the values 25.5 after 4 hours, 30.8 after 8 hours, 43.0 after 18 hours, 50.4 after 30 hours to 51.8% in the normal state. The samples from cow B showed a similar rise

References p. 493.

of the B component during the post calving period, actually from 11.9 after 0 hours, 14.1 after 6 hours, 16.4 after 12 hours, 26.2 after 20 hours, 39.1 after 30 hours, 45.3 after 43 hours to 48.6% in the normal state. The mobility of the B component appeared to be on an average $7.5 \cdot 10^{-5}$.

The G component also shows a striking rise during the post calving period. This component, of which the mobility varies slightly around $3.6 \cdot 10^{-5}$, rises, with the samples from cow A, from 9.0 to 14.7% and with cow B from 6.5 to 18.4%. As we had already been able to show from the results of the electrophoresis of normal milk*, the B and G components have a casein character, which is also confirmed by the results obtained with colostrum milk.

With regard to the H fraction, it appears from Table II that, in the sample taken from cow A at calving, this amounted to 46.9%; this value dropped very quickly to 38.2 after 4 hours, 32.8 after 8 hours, 18.2 after 18 hours, 10.8 after 30 hours and 3.3% in the normal state. The results obtained with the samples from cow B are similar: here the value of the H component dropped from 59.2 after 0 hours to 53.4 after 6 hours, 47.4 after 12 hours, 39.8 after 20 hours, 25.8 after 30 hours, 15.0 after 43 hours and 4.2% in the normal state. The H component is also the slowest moving of the eight fractions; its average mobility appeared to be $1.9 \cdot 10^{-5}$. In comparison with the chemical fractionation, this H component agrees very well with the globulin percentage as determined by salting-out. The results of the electrophoretic examination of normal milk showed merely the casein character of the B and G components, while nothing definite could be said of the other fractions on account of their secondary character. The results of this investigation have now allowed us to establish the globulin character of the H fraction, both in colostrum and in normal milk.

With respect to the A, C, D, E and F components, it appears from Table II that these do not undergo any significant change during the colostrum period.

SUMMARY

Electrophoresis tests were carried out on samples of colostrum of dairy cows. The milk was first cleared by dialysis against MICHAELIS veronal-sodium buffer ($p_H = 8$; $\mu = 0.1$) and high-speed centrifuging. The results of chemical fractionation by the method of ROWLAND show an increase in the casein, in the samples from cow A, from 42.1 to 82.8%; with cow B from 29.7 to 81.0%. Together with this increase in casein content there is a drop in globulin content during the colostrum period from 41.7 to 4.5% with the samples of cow A and from 61.2 to 6.4% with cow B. In the electrophoresis ($p_H = 8$; $\mu = 0.1$; $t = 2°$ C; I = 25 mA) the same number of fractions were obtained as with normal milk, but in different proportions.

From the results obtained it appears that the B component, of mobility $7.5 \cdot 10^{-5}$, rises during the colostrum period from 21.8 to 51.8% and from 11.9 to 48.6% respectively for the samples from cow A and B. The G component also, of mobility $3.6 \cdot 10^{-5}$, rises during the colostrum period, *i.e.* from 9.0 to 14.7% in the samples from cow A, and from 6.5 to 18.4% with cow B. As we were able to establish previously from the results obtained in the electrophoresis of normal milk, the B and G components show a casein character, which is also confirmed by the results obtained with colostrum milk. Moreover, during the colostrum period there is a considerable drop in the H component, the slowest moving fraction with a mobility of $1.9 \cdot 10^{-5}$. With the samples from cow A this drop is from 46.9 to 3.3% and with cow B from 59.2 to 4.2%. On account of the remarkable agreement between the globulin percentage and the magnitude of the H component, we ascribe a globulin character to this fraction. The other components A, C, D, E and F show only slight variations during the colostrum period.

* To be published.

References p. 493.

RÉSUMÉ

Nous avons soumis à l'électrophorèse des prises de colostrum de vaches laitières. Le lait était d'abord clarifiée par dialyse contre du tampon veronal-sodium de MICHAELIS ($p_H = 8$; $\mu = 0.1$) et par centrifugation à grand nombre de tours. Les résultats du fractionnement chimique par la méthode de ROWLAND montrent une augmentation de la teneur en caséine de 42.1 à 82.8% dans les échantillons de colostrum de la vache A et de 29.7 à 81.0% pour la vache B. En même temps que cette augmentation de la teneur en caséine on observe pendant la période de colostrum une baisse de la teneur en globuline de 41.7 à 4.5% pour la vache A, et de 61.2 à 6.4% pour la vache B. A l'électrophorèse ($p_H = 8$; $\mu = 0.1$; $t = 2°$ C; I = 25 mA) nous avons obtenu le même nombre de fractions que pour le lait normal, mais en proportions différentes.

Il ressort des résultats obtenus que la composante B, de mobilité $7.5 \cdot 10^{-5}$, augmente pendant la période de colostrum de 21.8 à 51.8% et de 11.9 à 48.6% pour les vaches A et B respectivement. La composante G, de mobilité $3.6 \cdot 10^{-5}$, elle aussi, augmente pendant la période de colostrum de 9.0 à 14.7% (vache A) et de 6.5 à 18.4% (vache B). Nous avons pu établir par des expériences antérieures que les fractions B et G ont le même caractère que la caséine; ce fait a été confirmé par les expériences effectuées avec le colostrum. De plus, pendant la période de colostrum, la fraction H, c.-à-d. la fraction la plus lente de mobilité $1.9 \cdot 10^{-5}$, baisse en quantité: de 46.9 à 3.3% (vache A) et de 59.2 à 4.2% (vache B). En vue de la concordance remarquable entre le pourcentage de globuline et la grandeur de la fraction H nous attribuons à cette fraction le caractère de globuline. Les autres composantes A, C, D, E et F ne montrent que des variations faibles pendant la période de colostrum.

ZUSAMMENFASSUNG

Elektrophoretische Versuche wurden mit Colostrumproben von Milchkühen ausgeführt. Die Milch wurde erst durch Dialyse gegen MICHAELIS'schen Veronalnatrium-Puffer ($p_H = 8$; $\mu = 0.1$) und Zentrifugieren bei hoher Tourenzahl geklärt. Die Ergebnisse der chemischen Fraktionierung nach der ROWLAND'schen Methode zeigen eine Zunahme des Kaseingehaltes und zwar in den Proben von Kuh A von 42.1 auf 82.8%, und von Kuh B von 29.7 auf 81.0%. Gleichzeitig mit dieser Zunahme des Kaseingehaltes beobachtet man während der Colostrumperiode eine Abnahme des Globulingehaltes von 41.7 auf 4.5% für Kuh A und von 61.2 auf 6.4% für Kuh B. Bei der Elektrophorese ($p_H = 8$; $\mu = 0.1$; $t = 2°$ C; I = 25 mA) wurde die gleiche Anzahl Fraktionen wie bei gewöhnlicher Milch, aber in verschiedenen Mengen-verhältnissen, erhalten.

Aus den Versuchsergebnissen geht hervor, dass die B-Komponente, Mobilität $7.5 \cdot 10^{-5}$, während der Colostrumperiode, respektive für Proben von Kuh A und B, von 21.8 auf 51.8% und von 11.9 auf 48.6% zunimmt. Auch die G-Komponente, Mobilität $3.6 \cdot 10^{-5}$, nimmt während der Colostrumperiode an Menge zu, und zwar von 9.0 auf 14.7 für Kuh A und von 6.5 auf 18.4 für Kuh B. Aus den Ergebnissen der Elektrophorese von normaler Milch haben wir bereits früher feststellen können, dass die B- und G-Komponente Kaseincharakter haben; dies wird durch die, mit Colostrummilch erhaltenen, Ergebnisse bestätigt. Ausserdem nimmt während der Colostrumperiode die H-Komponente, das ist die langsamste Fraktion mit einer Mobilität von $1.9 \cdot 10^{-5}$, bedeutend ab. Für die Proben von Kuh A beträgt dieser Abfall von 46.9 auf 3.3% und für diejenigen von Kuh B von 59.2 auf 4.2%. Auf Grund der bemerkenswerten Übereinstimmung zwischen dem Globulingehalt (in %) und der Grösse der H-Komponente wird dieser Fraktion Globulincharakter zugeschrieben. Die anderen Komponenten A, C, D, E und F zeigen nur geringe Variationen während der Colostrumperiode.

REFERENCES

[1] L. MICHAELIS, *Biochem. Z.*, 234 (1931) 139.
[2] E. WIEDEMANN, *Helv. Chim. Acta*, 30 (1947) 639, 648; 31 (1948) 40, 2037.
E. WIEDEMANN, *Chimia*, 2 (1948) 25.
E. WIEDEMANN, *Experientia*, 3 (1947) 341.
E. WIEDEMANN, *Revue Hématologie*, 3 (1948) 251.
[3] E. WIEDEMANN, *Helv. Chim. Acta*, 30 (1947) 892.
[4] L. G. LONGSWORTH AND D. A. MCINNES, *J. Am. Chem. Soc.*, 62 (1940) 705.
[5] E. WIEDEMANN, *Helv. Chim. Acta*, 30 (1947) 168.
[6] S. J. ROWLAND, *J. Dairy Research*, 9 (1938) 42.

Received November 6th, 1950