CXXXIX. ULTRACENTRIFUGAL AND ELECTROPHORETIC STUDIES ON THE MILK PROTEINS.

II. THE LACTOGLOBULIN OF PALMER.

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It is generally assumed that milk serum contains two different proteins: lactalbumin and lactoglobulin. However, Palmer [1934] recently isolated a crystalline, globulin-like protein from the albumin fraction of cow's milk. Recent ultracentrifugal studies by Philippi¹ have shown the presence of at least three different proteins in milk serum: a protein of rather low molecular weight, isolated later on by Kekwick,¹ the lactoglobulin studied by Palmer [1934] and a protein with a molecular weight higher than that of the latter.

Recent investigations by the author gave similar results [Pedersen, 1936]. This paper gives the result of an ultracentrifugal and electrophoretic study of the protein isolated by Palmer.

The lactoglobulin used throughout this investigation was kindly put at the disposal of Prof. The. Svedberg by Prof. Sørensen. It was prepared by Dr Palmer at the Carlsberg Laboratory from cow's milk according to a modification of the method worked out by Dr Palmer himself. This preparation had been recrystallised four times. The material was received in Upsala as a wet crystal paste containing some toluene as preservative and was stored in this condition in the refrigerator (3–5°). A part of the lactoglobulin was later dried in a desiccator over P₂O₅ and kept in the desiccator. These two materials behaved identically in the centrifuge and in electrophoresis. At the end of this investigation a few experiments were carried out using another material prepared by R. A. Kekwick in Upsala according to the method of Palmer. The sedimentation values found for this material were identical with the earlier ones.

*The specific refraction increment and specific volume of lactoglobulin.*² The determinations of the specific refraction increment were carried out on solutions prepared by dissolving the lactoglobulin in 0.5 *M* NaCl solution and dialysing it in cellophane bags against a 0.5 *M* NaCl solution in water. The determinations were carried out by means of a differential prism apparatus arranged for photographic registration of the undeviated and refracted beams. The concentrations were determined by drying a weighed amount of protein solution and a weighed amount of dialysate to constant weight (105–110°).

In Table I are given the results of two independent determinations. A micro-Kjeldahl determination (Gunning-Pregl method) gave for total nitrogen of the first solution 15.14 % and for the second 15.26 % in good agreement with Palmer's value which was 15.3 %.

The specific volume of lactoglobulin was found to be 0.7514 at 20° which is in good agreement with the values found for other proteins.

¹ Results to be published soon.

² The author is much indebted to Mr Kjell Andersson for having carried out these determinations.

Table I. *The specific refraction increment (α) of lactoglobulin in 0.5M NaCl solution.*

Protein concentration		λ in $m\mu$			
g./100 g. sol.	g./100 ml.	366	436	546	579
1.231	1.2566	196.2	189.3	181.3	181.2
1.853	1.8934	196.4	189.2	182.3	180.9
Mean for α :		196.3	189.2	181.8	181.0

The isoelectric point and the electrophoretic mobility of the lactoglobulin. These experiments were carried out by means of a special moving boundary method worked out by Tiselius [1930] and Pedersen [1933]. In this method the protein solution is introduced into a quartz U-tube below a solution of the same buffer

Table II. *Electrophoretic mobility of lactoglobulin.*

Exp. No.	p_H	Buffer	Migration	$u \cdot 10^5$
17	3.22	Acetate	Cathodic	19.6
3	3.66	"	"	17.3
7	4.18	"	"	12.1
6	4.48	"	"	8.5
2	4.65	"	"	6.8
8	4.95	"	"	2.2
4	5.26	"	Anodic	1.1
5	5.49	"	"	2.9
9	5.55	"	"	4.6
13	5.76	Phosphate	"	5.9
10	6.18	"	"	8.5
12	6.46	"	"	9.8
19	6.66	Phosphate-borate	"	11.8
11	7.00	Phosphate	"	14.0
14	7.47	"	"	15.6
20	8.27	Phosphate-borate	"	18.0
15	8.92	"	"	19.7
16	10.07	Phosphate-carbonate	"	21.5
18	10.38	"	"	21.7

Fig. 1. Electrophoretic mobility of lactoglobulin.

composition as that in which the protein is dissolved. The migration of the boundary is followed by taking photographs from time to time (in the short wave ultraviolet, λ below $270m\mu$). These photographs are registered later by

the microphotometer in the same way as the centrifuge plates and the movement of the boundary is determined from these records.

All the experiments were carried out at 20.0°. The protein concentration in these experiments varied between 0.15 and 0.20 g. per 100 ml. The acetate buffers had all a constant sodium acetate and a varied acetic acid concentration, *viz.* 0.02 *M* sodium acetate + *x M* acetic acid. The other buffers were made up to constant ionic strength $\mu = 0.02$. The mobility *u* is measured in cm.²/sec.⁻¹/volt⁻¹.

In Table II and Fig. 1 are given the values found for the mobility of lactoglobulin in the different buffers. From these figures it is seen that the isoelectric point of lactoglobulin is at p_H 5.20 in acetate buffers of the composition 0.02 *M* sodium acetate + *x M* acetic acid. The slope of the mobility curve at the isoelectric point $\left(\frac{du}{dp_H}\right) = 11.9 \cdot 10^{-5}$. The curves show no sign of inhomogeneity.

The (*u*, p_H) curve is of a very characteristic shape with abrupt changes in the slopes at the following p_H values: 3.8, 5.6 and 7.7; otherwise the points lie on straight lines. The change in the slope at p_H 7.7 corresponds to the jump in the p_H stability curve (see later). The change in slope at p_H 5.6 may perhaps be explained by a change from the simple uni-univalent acetate system to the more complicated phosphate system; on the other hand there is a change in the sedimentation constant slightly above p_H 5. The change at p_H 3.8 does not seem to correspond to a change in the sedimentation constant.

Sedimentation velocity experiments. The sedimentation constant was determined between p_H 1 and 11. The buffer solutions generally were made up in such a manner that the concentration of NaCl was 5–10 times the concentration of the proper buffer substance in order to reduce both primary and secondary charge effects.¹ The protein solutions were made up immediately before starting the experiment except in some cases indicated in Table IIIb. Experiments 21, 34 and 35 were carried out in order to see whether it was possible to detect a secondary charge effect at these p_H values, where the protein must be very strongly charged; the experiments however did not give a very definite answer, although the values for s_{20} are of the expected orders, *viz.* $2.79 \cdot 10^{-13}$ for KCl, $2.92 \cdot 10^{-13}$ for NaCl and $2.98 \cdot 10^{-13}$ for LiCl. From the values given in Table IIIa and Fig. 2, it is seen that the sedimentation constant of lactoglobulin is divided into three different groups. The first group between p_H 1 and 5 shows a mean sedimentation constant of $2.95 \cdot 10^{-13}$ (mean of 19 values). The next group between p_H 5.2 and 7 has a mean sedimentation constant of $3.12 \cdot 10^{-13}$ (mean of 13 values). The third group, p_H 7.6–9.8, gives $s_{20} = 2.76 \cdot 10^{-13}$ (13 values). Between these three groups intermediate values are found. On the alkaline side of the third group there seems to be a rise in the sedimentation constant which increases, if the solution is allowed to stand for some time at room temperature (compare too equilibrium Exp. 13). At still higher p_H values (above 11) there is a strong fall in the sedimentation constant at the same time as the light absorption is increased to about three times its normal value indicating profound changes in the lactoglobulin molecule.

The influence of the lactoglobulin concentration on the sedimentation constant (compare Exps. 40–46) is extremely small and hardly beyond the limits

¹ The primary charge effect is caused by the potential gradient set up by the unequal "sedimentation tendency" of the protein and its "gegenionen". The secondary charge effect is caused by the influence of the potential gradient set up by the sedimentation of the unequally dense salt ions on the sedimenting charged colloidal particles. Whereas the primary charge effect is reduced by the mere addition of electrolytes, the secondary effect is reduced by the addition of electrolytes with equally dense cations and anions.

Table IIIA. *Sedimentation velocity of lactoglobulin.*

Centrifugal force 240,000–290,000 times gravity except in Exp. 7, where it was 570,000 times gravity. Thickness of column of solution: 0.2–2.2 cm. Source of light: mercury lamp. Light filter: chlorine and bromine (except in the experiments marked with *L*, where the refractometric method of Lamm was used). Plates: imperial process. Time of exposure: 20–45 sec. Metol quinol developer 1 min.

Exp. No.	Lactoglobulin concentration %	Solvent system	Total salt molarity	p_H of solution	$s_{20} \cdot 10^{13}$
23	0.31	NaCl, HCl	0.59	1.04	2.88
21	0.3	"	0.55	1.28	2.92
35	0.3	LiCl, HCl	0.55	1.24	2.98
34	0.3	KCl, HCl	0.55	1.33	2.79
25	0.31	NaCl, HCl	0.51	1.98	2.94
22	0.35	NaCl, citrate, HCl	0.55	2.53	2.97
12	0.30	NaCl, sodium acetate, acetic acid	0.55	3.32	3.01
48	0.31	" "	0.24	3.81	2.86
14	0.30	" "	0.15	4.00	3.14 ?
50	0.32	" "	0.53	4.28	2.88
5	0.3	" "	0.16	4.6	3.00
47	0.31	" "	0.24	4.68	2.99
27	0.3	" "	0.58	4.80	3.02
45	0.05	" "	0.275	5.00	2.99
43	0.06	" "	0.275	5.00	2.92
44	0.12	" "	0.275	5.00	2.90
42 L	0.32	" "	0.275	5.00	3.03
41 L	0.64	" "	0.275	5.00	2.99
40 L	1.28	" "	0.275	5.00	2.97
46 L	4.47	" "	0.275	5.00	(2.85)*
38	0.3	NaCl, KH_2PO_4 , Na_2HPO_4	0.59	5.12	3.11
39	1.04	" "	0.59	5.12	2.99
3	0.3	NaCl	0.2	About 5.2	3.12
1	0.15	"	0.2	About 5.2	3.17
49	0.31	NaCl, KH_2PO_4 , Na_2HPO_4	0.29	5.33	2.94
8	0.31	KH_2PO_4 , Na_2HPO_4	0.18	5.36	3.14
9	0.30	" "	0.16	5.81	3.19
16	0.30	NaCl, KH_2PO_4 , Na_2HPO_4	0.12	5.87	3.08
15 L	1.46	" "	0.12	5.87	3.21
7	0.3	" "	0.2	6.33	3.13
52	0.33	" "	0.58	6.41	3.14
53	0.34	" "	0.60	6.41	3.10
51	0.32	" "	0.58	6.5	3.03
2	0.15	" "	0.23	6.57	3.12
54	0.33	" "	0.25	6.77	3.16
4	0.3	" "	0.20	7.09	3.07
37	0.3	" "	0.54	7.36	3.02
10	0.3	KH_2PO_4 , $Na_2B_4O_7$	0.27	7.69	2.88
32	0.33	NaCl, KH_2PO_4 , $Na_2B_4O_7$	0.64	7.81	2.84
19	0.3	" "	0.64	7.81	2.66
31	0.33	" "	0.64	7.84	2.75
11	0.3	KH_2PO_4 , $Na_2B_4O_7$	0.25	8.25	2.65
55	0.33	NaCl, KH_2PO_4 , $Na_2B_4O_7$	0.53	8.38	2.66
56	0.33	" "	0.55	8.38	2.76
13	0.30	KH_2PO_4 , $Na_2B_4O_7$	0.28	8.4	2.78
17 L	1.22	NaCl, KH_2PO_4 , $Na_2B_4O_7$	0.11	8.5	2.75
28	0.3	NaCl, $Na_2B_4O_7$, Na_2CO_3	0.54	9.06	2.73
36	0.3	" "	0.58	9.33	2.78
6	0.3	" "	0.19	9.74	2.85
18	0.3	" "	0.58	9.75	2.85
29	0.3	" "	0.58	10.05	3.05
20	0.1	NaCl, Na_2HPO_4 , NaOH	0.53	11.16	2.42†

* Not used in averaging, owing to the high protein concentration.

† Strong change in light absorption.

values from Table III A.

+ values from Table III B.

Fig. 2. p_H -stability diagram of lactoglobulin.Table III B. *Sedimentation velocity of lactoglobulin.*

Conditions as in Table III A with the exception that in this case the lactoglobulin solution was made some time before the experiment started. (The solutions contained toluene as a preservative.)

Exp. No.	Protein conc. %	Solvent system	Total salt molarity	p_H of solution	$s_{20} \cdot 10^{13}$	Time interval between making up solution and experiment
24	0.31	NaCl, HCl	0.59	1.04	2.89	2 days at 20°
30	0.3	NaCl, citrate, HCl	0.55	2.15	3.07	?
12 a	0.3	NaCl, sodium acetate, acetic acid	0.55	3.32	2.76	2 days at 20°
33	0.3	NaCl, KH_2PO_4 , $Na_2B_4O_7$	0.64	7.84	2.92	4 days at 20°
26	1.03	NaCl, $Na_2B_4O_7$, Na_2CO_3	0.58	9.75	3.15	14 days at 20°

of experimental error except perhaps in the case of the 4.5% solution. In this respect lactoglobulin differs from other proteins that have so far been studied over a wider concentration range.

In the experiments marked with L the initial concentration of the sedimenting protein was calculated from the sedimentation diagrams as described by McFarlane [1935]. For these calculations the exposures were used only where the whole sedimentation peak was free from the meniscus and where it had not yet reached the bottom of the cell. In Table IV the values calculated from the single exposures are given in chronological order. It is seen that the mean values differ very little from the analytical concentration except in Exp. 46, where possibly the value given for analytical concentration is too high.

Table IV. *Concentration of lactoglobulin calculated from sedimentation diagram.*

Exp. No.	Concentration calculated from the single exposures	Mean conc. g./100 ml.	Anal conc. g./100 ml.
15	1.50, 1.50, 1.46, 1.45, 1.59, 1.46, 1.48, 1.51	1.49	1.46
17	1.24, 1.22, 1.23, 1.22, 1.20, 1.19	1.22	1.22
40	1.23, 1.24, 1.25, 1.21	1.23	1.28
41	0.615, 0.667, 0.637	0.640	0.64
42	0.313, 0.324, 0.349, 0.329, 0.337, 0.326, 0.326, 0.319	0.328	0.32
46	4.24, 4.17, 4.39, 4.27, 4.05, 4.11	4.21	4.47*

* No exact analysis on this solution, the value 4.47 is the maximum possible value.

The sedimentation diagrams often showed a small irregularity between the meniscus and the lactoglobulin curve indicating the presence of a small amount of a substance with a lower sedimentation constant than that of lactoglobulin: the concentration of this substance was too small to give even an approximate value for its sedimentation, but in all cases it was lower than that for lactoglobulin. This substance is probably either some degradation product of lactoglobulin or more likely the low-molecular protein of Kekwick mentioned before.

Diffusion constant. The author is indebted to Mr Polson for carrying out some diffusion measurements on lactoglobulin. The experiments were carried out by means of the refractometric method described by Lamm and Polson [1936]. The values given in Table V for D_{20} are the values reduced to a water basis and infinite protein dilution.

Table V. *Diffusion of lactoglobulin.*

(Determinations by A. G. Polson.)						
Exp. No.	Protein conc. %	Solvent	p_H	$D_{20} \cdot 10^7$	Mean $s_{20} \cdot 10^{13}$ at same p_H	M_D
1	1.0	0.2 <i>M</i> NaCl	5.0	7.27	2.95	39,700
		0.038 <i>M</i> sodium acetate				
		0.012 <i>M</i> acetic acid				
2	1.0	0.001 <i>M</i> Na ₂ HPO ₄	5.4	7.19	3.12	41,600
		0.019 <i>M</i> KH ₂ PO ₄				
		0.2 <i>M</i> NaCl				
3	1.0	0.0375 <i>M</i> KH ₂ PO ₄	6.1	7.32	3.12	42,400
		0.0125 <i>M</i> Na ₂ HPO ₄				
		0.2 <i>M</i> NaCl				
4	1.0	0.02 <i>M</i> Na ₂ CO ₃	9.3	6.88	2.76	39,200
		0.02 <i>M</i> Na ₂ B ₄ O ₇				
		0.2 <i>M</i> NaCl				
					Mean value	40,700

According to Svedberg [1925; 1927] it is possible to calculate the mol. wt. of a solute from its sedimentation and diffusion constants, if one assumes that the frictional coefficient is the same for a sedimenting as for a diffusing particle. Then we have

$$M_D = \frac{RT \cdot s}{D(1 - v\rho)}$$

In the deduction of this formula no assumptions have been made about the shape of the particle; it is therefore of general use.

In column 7 of Table V are given the values calculated from this formula.

Sedimentation equilibrium experiments. The ultracentrifuge technique by means of sedimentation equilibrium measurements allows a direct determination of the mol. wt. [Svedberg, 1925; 1926]. 17 equilibrium experiments were carried out with different concentrations of lactoglobulin and different buffers. The speed was varied between 8700 and 12500 r.p.m. The temperature was in all cases 20°. Equilibrium was generally obtained after 5–7 days.

The measurements of the concentration gradient in the centrifuge cell were made by means of the refractometric method of Lamm [1928; 1929; 1933] and the calculations of the mol. wt. were carried out in the two different ways described earlier [Pedersen, 1936]. Only in the case of a monodisperse system and where the gas laws are valid can it be expected that the two methods should give the same result, *viz.* the correct mol. wt. All the average mol. wts. were calculated according to the method of Lansing and Kraemer [1935] as described in the preceding paper [Pedersen, 1936].

In Tables VI and VII are given the more detailed data for equilibrium in Exp. 5 at p_H 6.36 where the lactoglobulin is monodisperse, and Exp. 13 at p_H 9.75 where it is polydisperse.

Table VI. *Lactoglobulin equilibrium, Exp. 5.*

Concentration of lactoglobulin at start: 0.1625 g./100 ml. Buffer solution: 0.5 *M* NaCl + 0.0625 *M* KH_2PO_4 + 0.0625 *M* Na_2HPO_4 . p_H = 6.36. Thickness of cell: 1.201 cm. Bottom of cell: 5.17 cm. from axis of rotation. Meniscus of solution: 4.83 cm. from axis of rotation. Optical scale distance: 8.77 cm. G = 1.015. Light filter: Corning nickel oxide (λ = 366 $m\mu$). α_{366} = $196.3 \cdot 10^{-5}$. Temperature: 20.0°. Rev. per sec.: 182.8. V = 0.7514. ρ = 1.034. $(1 - V\rho)$ = 0.2231. Exposures used taken 119, 125, 144, 149 and 167 hours from the start. Cell not sector-shaped but with

$$f(x) = -2.016x + 11.483 \quad (\text{Eq. 8 and 9 Pedersen [1936]}).$$

x	z in μ	$\frac{dc}{dx}$	c	M_{wx}	M_{zx}
4.87	57.5	—	—	—	—
4.90	62	0.295	0.130	38,300	39,600
4.95	70.5	0.336	0.146	38,400	32,300
5.00	78.5	0.374	0.164	37,800	32,600
5.05	87.5	0.417	0.184	37,200	37,000
5.10	99	0.471	0.206	37,200	39,500
5.15	113	0.538	0.231	37,500	—
				$M_w = 37,600$	Mean 36,200*

* Too strong fluctuation in the M_{zx} to allow an accurate determination of M_z .

Table VII. *Lactoglobulin equilibrium Exp. 13.*

Concentration of lactoglobulin at start: 0.341 g./100 ml. Buffer solution: 0.5 *M* NaCl + 0.0208 *M* $\text{Na}_2\text{B}_4\text{O}_7$ + 0.0625 *M* Na_2CO_3 . p_H = 9.75. Thickness of cell: 0.999 cm. Bottom of cell: 5.18 cm. from axis of rotation. Meniscus of solution: 4.75 cm. from axis of rotation. Optical distance: 8.79 cm. G = 1.011. Light filter: Corning nickel oxide (λ = 366 $m\mu$). α_{366} = $196.3 \cdot 10^{-5}$. Temperature: 20.0°. Rev. per sec.: 180.0. V = 0.7514. ρ = 1.032. $(1 - V\rho)$ = 0.2247. Exposures used in calculations taken 187, 209, 215, 230, 239 and 256 hours from start. Cell not sector-shaped but with parallel walls.

x	z in μ	$\frac{dc}{dx}$	c	M_{wx}	M_{zw}
4.80	77	0.442	0.209	37,400	62,600
4.85	93	0.534	0.234	39,900	61,000
4.90	112	0.643	0.263	42,300	65,900
4.95	137	0.786	0.299	45,000	70,000
5.00	170	0.975	0.343	48,200	75,800
5.05	215	1.234	0.397	52,200	83,800
5.10	279	1.601	0.468	56,800	87,500
5.15	367	2.106	0.560	61,800	—
				$M_w = 50,200$	$M_z = 77,800$

The drift in the values in Exp. 5 is within limits of experimental errors [Pedersen, 1936] whereas the drift in Exp. 13 certainly is real and means that the molecules at this p_H are aggregating. It is characteristic that the equilibrium in Exp. 13 took a much longer time to be established than usual, probably because the aggregation process is taking place slowly which is in agreement with the two velocity Exps. 18 and 26. The first of these was done immediately after mixing a portion of the same solution as the one used in equilibrium Exp. 13; the sedimentation constant was $2.85 \cdot 10^{-13}$ which is a little higher than the mean value for that p_H . Exp. 26 was done on the stock solution used to prepare the equilibrium solution; this had been standing at 20° for a fortnight, and the sedimentation constant was $3.15 \cdot 10^{-13}$, which is much higher than the mean value for that p_H , likewise indicating aggregation.

Table VIII gives a summary of all the equilibrium experiments. It can be seen that the most probable mol. wt. found from sedimentation equilibrium is about 38,000. There is some discrepancy between this value and the values

Table VIII. *Sedimentation equilibrium experiments on lactoglobulin.*

Exp. No.	Initial protein conc. %	Buffer solution	Total buffer conc. M	p_H	Rev. per sec.	M_w	M_z
1	0.21	NaCl, KH_2PO_4 , Na_2HPO_4	0.24	5.4	180	37,700	38,500
2	0.42	"	0.24	5.4	180	37,300	38,600
3	0.21	"	0.24	5.4	204	35,300	35,700
4	0.42	"	0.24	5.4	204	36,400	36,600
5	0.16	"	0.63	6.4	183	37,600	(36,200)*
6	0.16	"	0.63	6.4	208	36,000	(36,300)*
7	0.30	"	0.12	5.9	145	37,900	39,800
8	1.47	"	0.12	5.9	145	40,700	42,500
9	0.30	"	0.12	5.9	180	40,800	37,800
10	1.47	"	0.12	5.9	180	41,300	40,500
11†	1.47	"	0.12	5.9	145	37,500	—†
12‡	1.47	"	0.12	5.9	180	36,700	—†
13	0.34	NaCl, $\text{Na}_2\text{B}_4\text{O}_7$, Na_2CO_3	0.58	9.8	180	50,200	77,800
14	0.41	NaCl, KH_2PO_4 , $\text{Na}_2\text{B}_4\text{O}_7$	0.64	7.8	180	35,500	40,900
15	0.84	"	0.64	7.8	180	36,400	(40,500)*
16	0.41	"	0.64	7.8	204	37,100	39,900
17	0.84	"	0.64	7.8	204	36,300	41,000
Average for No. 1-12						37,900	38,800
,, 14-17						36,300	40,600

* Ordinary mean values due to strong fluctuation in M_{zx} values.

† The new refractometric method of Lamm [1934] used.

‡ Too strong fluctuations in M_{zx} to allow any calculations of average values.

calculated from the diffusion and sedimentation constants, although some of the single values for M_w and M_z agree with the M_D values. If we consider all the values for M_w , M_z and M_D we get as probable mol. wt. for lactoglobulin 39,000.

DISCUSSION.

This investigation shows that the lactoglobulin prepared according to the method of Palmer [1934] from the lactalbumin fraction of cow's milk is a monodisperse protein with a mol. wt. of 39,000 and an isoelectric point of 5.19 (in acetate buffers). From the result of the sedimentation equilibrium experiments and from the mol. wts. calculated from sedimentation and diffusion constants we must assume that the mol. wt. of lactoglobulin is independent of the hydrogen ion concentration of the solution from the most acid values studied up to p_H 9. At still higher p_H values the lactoglobulin undergoes a slow aggregation as found from sedimentation equilibrium and velocity experiments. Above p_H 11 the light absorption increases strongly and the sedimentation constant decreases, indicating profound changes in the molecule (probably hydrolysis).

If we assume that the mol. wt. is constant between p_H 1 and 9 and take the small variations in M_D as being due to experimental errors, then we have to explain the change in the sedimentation constant at p_H around 5 and 7.5. For an unchanged mol. wt. change of the sedimentation constant must be due to change of the molecular frictional constant. A change in the molecular frictional constant can be due to two causes: either the shape or the kinetic volume of the particle has been altered, *e.g.* due to change in hydration. From sedimentation

and diffusion data alone it is impossible to distinguish between these two modes of change. The electrophoretic mobility (u , p_H) curve shows a characteristic jump at about the same p_H values as the sedimentation constant. A mere change in the shape of the particles would probably not explain the change in slope in the (u , p_H) curve, since it has often been found (for instance in Abramson's work with protein-covered quartz particles) that the size and shape of the particle influence the electrophoretic mobility very little. The jump in the (u , p_H) curve is therefore probably due to some change in the ionisation of the molecule.

The fact that the sedimentation constant hardly changes with concentration shows that the dissociation tendency of lactoglobulin is negligible.

From the constancy of the mol. wt. one cannot infer that the lactoglobulin molecule remains unchanged in such a manner that it should still possess its original properties, when it is brought back to a p_H around the isoelectric point after having been exposed to acid or alkali. Two electrophoresis experiments have shown that changes in the lactoglobulin molecule take place under these conditions. In one of these experiments some lactoglobulin was dissolved in dilute HCl to give a p_H of about 1.8 and was left in this solution for 1 day; after that time the solution was first dialysed against distilled water and later against phosphate buffer at p_H 6.15. The dialysate was changed several times. The electrophoretic mobility was determined and gave $u = 9.05 \cdot 10^{-5}$, whereas the normal value is $8.3 \cdot 10^{-5}$, so that it may be assumed that the lactoglobulin has probably undergone some change. Another sample of lactoglobulin was first treated at p_H 10 for 1 day and then dialysed against water and phosphate buffer (p_H 6.15). While the HCl-treated lactoglobulin gave uniform curves but a somewhat different mobility from the normal, this lactoglobulin gave quite heterogeneous curves showing that more particles were present with different mobilities. This indicates that profound changes in the molecule have occurred at p_H 10.

SUMMARY.

1. An ultracentrifugal study has been carried out on the lactoglobulin prepared according to Palmer.
2. The protein was found to be homogeneous and to have a mol. wt. of about 39,000.
3. The mol. wt. was found to be constant between p_H 1 and 9 but the sedimentation constant varied indicating a change in the molecular frictional constant at about p_H 5 and 7.5.
4. An electrophoretic study of lactoglobulin has shown that its isoelectric point is at p_H 5.19 in acetate buffers.

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