STUDIES ON THE FORMATION AND IONIZATION OF THE COMPOUNDS OF CASEIN WITH ALKALI.

IV. The Transport Numbers of the Compounds of Casein with the Alkali Earth Elements.*†

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In a previous communication (1) it was shown that data obtained from transference experiments with alkali solutions of casein could be best interpreted on the assumption that the carriers of the current are alkali metal cations and casein anions. In the present studies on the transport numbers of solutions of casein with the alkali earth elements, the data appear to indicate that *part* of the metallic element is bound by casein in such a way as to produce a complex ion, which, in migrating, carries with it the metallic element in a direction opposite to the path of the cation. The idea of complex ions is not wholly new. Northrop and Kunitz (2) have suggested the formation of complex ions between certain cations and gelatin to account for the deviations which they obtained from the calculated values for the distribution of the ions according to the Donnan membrane equilibrium theory. These authors, however, were unable to show any stoichiometric relations governing the formation of the complex ions. The theory proposed by Robertson (3) postulates that only complex ions of protein with the metallic element exist in solution. These are formed by the union of the acid or alkali with the $-COHN-$ groups of the protein molecule. The experimental results of the present investigation afford considerable evidence in favor of the view that definite stoichiometric relationships govern the formation of complex ions of casein

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and the alkali earth elements. The data, moreover, are in harmony with our previously expressed view that part of the current is carried by the metallic cation. There is no indication that the $-$ COHN $-$ groups play a rôle in the union of casein with the alkali earth elements.

The transference cell, and the technique which was used in preparing the casein, as well as the analytical methods, are the same as those which have been previously described (1). All of the experiments were carried out in an air bath which was maintained at a temperature constant within $\pm$ 0.5°C. The alkaline earth hydroxides were Kahlbaum's best brand. On account of the insolubility of magnesium hydroxide it was necessary for the preparation of the casein solutions to weigh out definite quantities of magnesium hydroxide and to add these to the casein and water. The mixture was shaken to effect solution. A small amount of residue was removed by filtration through a clean flannel cloth.

The average values of the transport numbers are given in Table I. As in the previous work the transport number of the casein was calculated by dividing the increase in concentration of the anode portion by the amount of casein which was deposited on the anode. In the studies with the alkali caseinates it was shown that Q, the electrochemical equivalent, varies with the amount of alkali in solution. This relationship was expressed by the formula,

$$Q \times B = K,$$

where B represents the cc. of 0.1 N alkali which is combined with 1 gm. of casein and K is a constant. Since the publication of these data the same relationship has been found to hold for solutions of casein in lithium hydroxide. There are no *a priori* grounds for expecting that different values for K would be obtained with caseinates of the alkaline earths. However, the data from the latter experiments give values for K which are 15 to 25 per cent lower than the average value obtained in the experiments with the alkali caseinates. The significance of this is not at present clear.

The values for the transport numbers of casein in solutions of the alkali earth elements are abnormally high as compared with the values which were obtained for casein in solutions of the alkali metals. The abnormal values are probably due to the fact that part of the

TABLE I.

Transport Numbers. (Average Values at 25°C.)*

Time.	Casein.	B approximately.	pH	Q	K	T_{casein}	T_{cation}
				(*a*) *Casein* + $Mg(OH)_2$.			
hrs.	*per cent*	*cc.*					
3.5	1.85–2.25	6.75	7.0	1.20	8.1	0.84	0.26
3	2.27	7.5	7.7	0.90	6.8	0.82	0.31
2.5–3.5	1.85–2.55	9.5	9.3	0.74	7.1	0.74	0.40
3	2.28	10.45	9.8	0.66	6.9	0.71	0.27
3.5	1.7–2.2	10.9	10.0	0.56	6.2†	0.79	0.20
				Average =	7.2		
				(*b*) *Casein* + $Ca(OH)_2$.			
3.5–4.0	1.94–2.57	6.6	7.2	1.15	7.6	1.00	(−0.12)
3.25	1.77	7.7	7.7	0.99	7.6	1.05	(−0.18)
3.5–4.0	1.6–2.1	9.5	9.4	0.85	8.1	0.78	—
3.25	1.84	11.0	10.3	0.63	7.0	0.64	—
				Average =	7.5		
				(*c*) *Casein* + $Sr(OH)_2$.			
3.5–4	2.13	6.25	6.8	1.31	8.2	0.73	0.24
3.0–4.25	2.3	8.0	7.6	1.02	8.2	0.75	0.25
3.25	2.2	9.0	8.7	0.88	7.9	0.70	0.23
2.5	2.25	10.5	10.0	0.71	7.4	0.63	0.43
				Average =	7.9		
				(*d*) *Casein* + $Ba(OH)_2$.			
3.75	1.85	7.4	7.3	0.94	7.0	1.24	(−0.07)
4	1.85	8.0	8.0	0.80	6.4	1.06	(−0.09)
4	1.9	9.0	9.5	0.80	7.2	0.84	0.07
3.25	1.81	10.5	9.9	0.70	7.3	0.77	0.31
2.3	1.82	13.55	10.7	0.42	5.7†	0.54	0.42
				Average =	7.0		

B = cc. 0.1 N alkali per gm. of casein.

Q = electrochemical equivalent per millifaraday.

$K = Q \times B$.

* Lack of space prevents publication of the data in detail.

† Not used in obtaining average.

alkali earth element is bound by the casein ion in a non-ionic form, producing a complex which carries a certain amount of the element in a direction opposite to that of the cation.

This explanation is not at all novel. It has been used to explain abnormal transport numbers since the pioneer researches of Hittorf (4). The formation of complexes can be shown by an equation:

$$M_a \text{ casein} = x \overset{++}{M} + M_{(a-x)} \text{ casein}^{-2x}$$

where M stands for the alkali earth element. This equation may be used to develop a formula for the transport number of the cation in terms of the fraction of the cation in the form of free ions and the mobilities of the ions in solution. It is possible that more than one kind of complex ion exists in solution, each type having a different mobility. In the following calculation, however, this makes no difference since we can consider a mean value for the mobility. If i be the fraction of the alkali earth element which exists as cations, n the equivalent concentration of the solution, u the mobility of the cations and v the mobility of the complex ions, the number of cations which are carried toward the cathode will be: niu. At the same time, a certain number of the alkali elements $n\ (1 - i)v$ will be carried to the anode; so that the total change in concentration of the alkali element will be $niu - n(1 - i)v$. The total current which passes through the solution will be $ni\ (u + v)$, and the transport number of the cation is:

$$T_{\text{cation}} = \frac{iu - (1-i)\ v}{i\ (u + v)},$$

the equivalent concentration n cancelling out. This equation may be used to calculate the value for i, provided the numerical values for u and v are known. The values for u, the cation mobility, may be obtained from tables such as Landolt and Börnstein (5), but no numerical data are available for v, the mobility of the complex ion or ions. However, some value for v which possesses a certain probability of being near to the correct value may be assumed and used for the purpose of calculation. The results which are obtained in this way will have the value of being comparative. From conductivity measurements the value for the mobility of the casein ion in alkali caseinate

solutions at 25°C. was found to be 35 mhos (6). Since in alkaline earth solutions of casein part of the metallic element is apparently held as a casein complex and hence is not in the form of cation, it appears probable that the mobility of the complex ions is somewhat less than that of the casein ion. In the following calculations 30 mhos was decided upon as the value for v. There is additional justification for using a single value for v, since previous transference experiments with alkali caseinate solutions have shown that within the limits studied the mobility of the casein ion is independent of the concentration of alkali in the solution. The following numerical values were used for the mobilities of the cations: Barium, 65 mhos; strontium, 60 mhos; calcium, 60 mhos; magnesium, 55 mhos. The results of the calculations are given in Table II. It should be noted that the results of the calculations have a higher degree of certainty than might be expected in view of the necessity of assigning an arbitrary value for v. An error of $\pm$ 5 units in v will introduce into the calculations an error which in the average is less than 3 per cent. This is less than the error inherent in the measurement of the transport numbers.

The results of these calculations throw some light upon the nature of the binding of the alkali earth cation by casein. In a series of studies on adsorption by charcoal, Odén and Andersson (7) found that the adsorption from alkali nitrates and alkali earth nitrate solutions did not, with different cations, take place in equivalent amounts but instead followed the order Mg < Ca < Sr < Ba with the alkali earth elements and Li < Na < K < NH_4 < Cs for the alkali elements. Previous experiments (1, 6) with the alkali caseinates supported the view that the union between casein and alkali is chemical in nature rather than a phenomenon of adsorption. An examination of the transport numbers of casein which are given in Table I seems to indicate that in solutions of the alkali earth caseinates the phenomenon is one of adsorption, since with the exception of strontium caseinate there is fairly regular increase in the initial values for the transport number of casein in each of the experiments. However, as shown in the last column of Table II, the amount of alkali earth element expressed in cc. of 0.1 N solution which is held as complex by 1 gm. of casein, in each of the experiments tends to reach a maximum value as

the amount of alkaline earth hydroxide per gm. of casein is increased. This maximum value is constant and independent of the alkaline earth element with which the casein is combined. This fact apparently indicates that casein combines with the alkali earth elements in stoi-

TABLE II.

Free Cations and Bound Cations in Solutions of Alkaline Earth Caseinates.

B approximately.	T_{casein}	i*	Bound cations.†
	(*a*) *Casein* + $Mg(OH)_2$.		
cc.		*per cent*	*cc.*
6.75	0.84	42	3.9
7.5	0.82	43	4.3
9.6	0.74	48	5.1
10.45	0.71	50	5.2
10.9	0.79	45	6.0
	(*b*) *Casein* + $Ca(OH)_2$.		
6.6	1.00	33	4.4
7.7	1.05	32	5.2
9.5	0.78	43	5.4
11.0	0.64	52	5.3
	(*c*) *Casein* + $Sr(OH)_2$.		
6.25	0.73	46	3.4
8.0	0.75	45	4.4
9.0	0.70	48	4.7
10.5	0.63	53	5.0
	(*d*) *Casein* + $Ba(OH)_2$.		
7.4	1.24	26	5.5
8.0	1.06	30	5.6
9.0	0.84	38	5.6
10.5	0.77	41	6.2
13.55	0.54	58	5.7

* i = proportion of total alkaline earth element as cation.

† 0.1 N alkali per gm. of casein.

chiometric proportions, and that with the maximum amounts of alkali which were used the complex ion of casein with alkaline earth element possesses a definite composition.

The idea that casein combines with the hydroxides of the alkaline

earths in a somewhat different manner than it does with the alkali hydroxides is supported by certain conductivity measurements which were carried out by Robertson (8). In Table III are given the equivalent conductivities of calcium, barium, and strontium caseinates which have been obtained by recalculating Robertson's data; for purposes of comparison the more recent data (6) relating to solutions of sodium and potassium caseinate are also given. While the two sets of data are not strictly comparable, the table, nevertheless, shows a striking difference between the equivalent conductivities of the alkali and alkaline earth caseinates. The latter values range from one-fifth

TABLE III.

Comparison of Conductivities of Alkaline Earth Caseinates with Conductivities of Alkali Caseinates. 30°C.

Concentration of alkali.	Calcium caseinate. λ	Barium caseinate. λ	Strontium caseinate. λ	Sodium caseinate.* λ	Potassium caseinate.† λ
0.032 N	9.3	10.2	—	—	70.7
0.024 N	10.6	—	18.8	50.0	74.8
0.016 N	12.0	12.8	20.1	55.0	78.6
0.012 N	13.5	—	21.2	57.7	—
0.008 N	16.3	17.0	24.1	62.0	84.5
0.004 N	22.5	22.7	28.8	68.5	90.8

Approximately 8 cc. 0.1 N alkali used to dissolve each gm. of casein.

* The figures for the sodium caseinate are for a solution containing approximately 5 cc. of 0.1 N alkali per gm. of casein.

† Data not previously published. pH of solution, 7.75.

to one-third of the values for similar solutions of the alkali caseinates. The conductance of solutions of strontium caseinate is somewhat greater than what might be expected from its relationship to calcium and barium. At 30°C. the mobilities for the cations are: $\frac{1}{2}$ Ba, 71.5 mhos; $\frac{1}{2}$ Sr, 66.1 mhos; $\frac{1}{2}$ Ca, 66.1 mhos; Na, 61.5 mhos; and K, 88.4 mhos. On the basis of these figures and on the assumption that the mobility of the casein ion is the same in all of the solutions, the conductivity of barium caseinate should be greater than that of strontium caseinate and both of these should be greater than the conductivity of sodium caseinate solutions. Robertson's data, however, do not support such conclusions but distinctly show that the conductance of

solutions of the alkaline earth caseinates are markedly less than the alkali caseinates.

While the experimental data in this paper relate only to solutions of casein, it is possible that complex ions such as were found to exist in solutions of the alkaline earth caseinates may be formed in an analogous manner with other proteins. The significance of such a possibility in life processes need not be mentioned at this time. Support for such a conclusion is gained from the findings of Robertson (9) and of Adolf (10) that the conductivity of the serum globulinates of the alkaline earths is markedly lower than similar solutions of the alkalies.

SUMMARY.

1. Data from the results of transference experiments on solutions of the alkaline earth caseinates are given.

2. The data support the idea that part of the alkaline earth element is held by the casein in the form of complex ions.

3. Grounds are given for believing that the complex anions have a definite composition.

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