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APPARENT SPECIFIC VOLUME OF THE CALCIUM CASEINATE–CALCIUM PHOSPHATE COMPLEX IN MILK

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SUMMARY

Skimmilks were progressively depleted of casein colloids by centrifuging, supernatant liquids and deposited colloids being thus obtained. The deposits were washed by redispersing in distilled water and recentrifuging. Densities were determined on the supernatant liquids and on water suspensions of the washed colloids. The analysis of the data on supernatant liquids is novel, in that the apparent specific volume is first calculated for the casein complex hydrate and the specific volume of the unhydrated complex derived from this, a procedure required because the solvent is not water but milk serum. Within close limits, the values obtained are the same as those calculated directly from the densities of suspensions of washed deposits, and also agree with values calculated from the separate specific volumes of the components of the complex. The apparent specific volumes found vary slightly with composition, and are to a first approximation linearly related to the calcium content of the complex. For a colloid fraction of milk of average calcium content the apparent specific volume found is 0.697, and the extremes are 0.694 and 0.700, all with probable errors of about 0.4%. Use of these values in calculating the densities of the hydrated and solvated casein colloid particles as they exist in milk is discussed. The data are for Jersey and Holstein milks.

Data presented in previous papers (9, 10) show that the casein colloid in milk, in the broad intermediate range of sizes, contains a phosphoprotein which has the same phosphorus: nitrogen ratio in all these sizes. Similar data have since been obtained for the very smallest particles (7), and give the same ratio. There is evidence that the extremely large particles may differ in phosphoprotein composition (9), but to what extent this apparent difference may be due to inclusion of foreign matter such as leucocytes is unknown. In some skimmilks the large particles may represent as much as 15% of the total casein; in other milks they may be entirely absent.

It has also been shown that the casein colloid particles as they exist in milk, i.e., in their native state, sediment in the ultracentrifuge with a limited number of definite sedimentation velocities (6, 7). This observation, combined with the constancy of phosphoprotein composition, strongly suggests that all of these particles are aggregates of a single-unit particle.

In raw milk native casein, the phosphoprotein above discussed is intimately associated with calcium and inorganic phosphorus, in such stoichiometric proportions as to indicate a calcium caseinate–calcium phosphate complex with some substitution of magnesium for calcium (1, 9, 13). The dispersed casein colloid particles also contain hydrate water (4) and considerable milk serum which is present as interstitial or solvate liquid (5).

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This paper has to do first with the apparent specific volume—the reciprocal of the apparent density—of the complex; second, with the specific volumes and densities of the actual hydrated and solvated particles.

Although the phosphoprotein contained in the particles is apparently of definite composition, and the proportion of calcium as calcium caseinate may also be considered to be definite, the proportion of calcium phosphate is variable over the particle-size range and between milks. The limits of this variability have been established (9, 10). It is necessary, similarly, to establish a median value of the apparent specific volume, and to set probable limits of deviation from this mean value. Although the deviations turn out to be relatively small, they must be considered in careful calculations of particle sizes from sedimentation velocity data, and in other calculations.

Apparently, only one other measurement of the specific volume of the casein complex, formerly called calcium caseinate, has been reported. This is by Nichols *et al.* (12) and is in error. A recalculated value, given in the fourth section of this paper, is in good agreement with the present results.

EXPERIMENTS AND CALCULATIONS

Values Based on Density, Total Nitrogen, and Casein Nitrogen Determinations, on Skimmilk Samples Progressively Depleted of Casein Colloids by Centrifuging. Figure 1 is a typical plot showing the relationship between density and total nitrogen in liquid samples as the casein-containing colloids are pro-

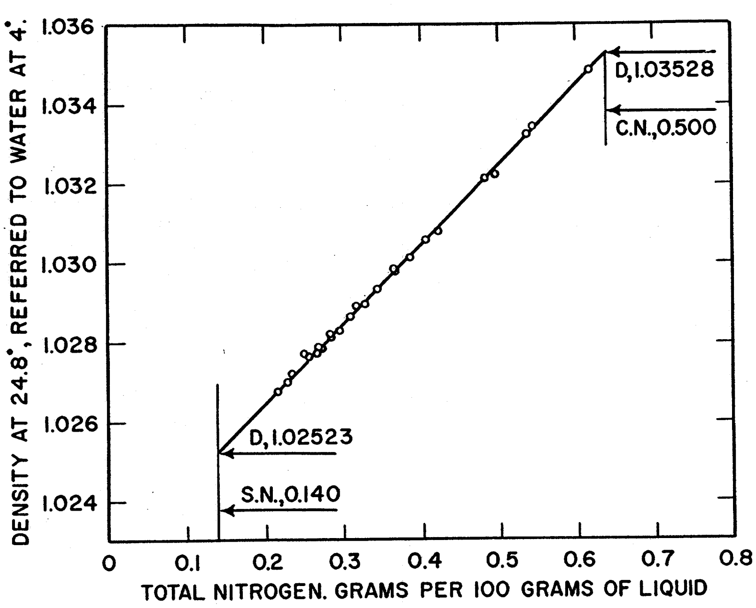

FIG. 1. Relationship of density: total nitrogen for skimmilk samples centrifuged for different lengths of time. (S.N. = serum nitrogen; C.N. = casein nitrogen. Densities measured at 24.8° and referred to water at 4°.)

gressively removed by centrifuging. Each point gives the density and nitrogen content of the liquid phase after a definite time of centrifuging. The serum nitrogen value shown was obtained by extrapolating a casein nitrogen: total nitrogen plot to zero casein nitrogen. The slope of the casein nitrogen plot, i.e., the ratio of casein nitrogen removed to total nitrogen removed is 1.00, which is the reason 0.50% casein nitrogen falls at 0.640% total nitrogen in this case.

The least-squares straight line is drawn through the set of points in Figure 1. Six such plots were obtained, for six milks, and in only one case was there any indication of curvature. This curvature, if real, is very slight. Some downward curvature should exist because of differences in composition over the size range, but it would be difficult to detect. The approximate linearity of such plots justifies only calculation of single average values for the specific volume for all of the particles in the size ranges represented.

The apparent specific volumes were calculated by the equation of Svedberg and Chirnoaga (15).

$$V = \frac{\omega - (l - h)}{\rho h}, \tag{1}$$

where ω is the weight of serum or solvent that can be contained in the pycnometer, l is the weight of solution contained by the pycnometer, h is the weight of solute in the solution, and ρ is the density of the serum or solvent.

In calculations based on data such as those of Figure 1, it is convenient to choose a liquid sample containing, say, 0.5% casein nitrogen, and to consider this to be the solution. Assuming an average complex: nitrogen factor of 6.89 (10) and a hydration of 0.52 g. of hydrate water per gram of dry complex (4, 10), such a solution contains (0.5) (6.89) (1.52) or 5.236 g. of hydrated complex. The hydrated complex is considered to be the solute. The solvate liquid associated with the hydrated complex is by definition the same as the serum or solvent and is considered to be a part of the solvent. Referring to Figure 1, as an example, and assuming a 100-ml. pycnometer, since the density of the solution at 0.5% casein nitrogen (0.640% total nitrogen in this case) is 1.03528, the value to be used for h in Equation (1) is (5.236) (103.528)/(100) or 5.241, and $l = 103.528$. The hypothetical 0.5% casein nitrogen skimmilk contains 0.140 g. of serum nitrogen per 100 g., as does the casein-free serum, but this serum nitrogen of the skimmilk is all in the serum phase. The true concentration of serum nitrogen in the uncentrifuged serum or solvent is, therefore $\{100/(100 - 5.236)\}$ $\{0.140\}$, or 0.148 g. per 100 g. As an approximation, the density of such a serum may be taken from the plot at 0.148% total nitrogen. This makes $\rho = 1.02541$ and $\omega = 102.541$. The serum density calculated by adding to 100 g. and the corresponding volume of centrifuged casein-free serum that weight and volume of serum protein centrifugally removed with 5.236 g. of hydrated complex is 1.02540, or practically the same as found by the simple approximation. The effect of the small amount of lipid material removed is negligible. Putting the above numerical values for ω, l, h, and ρ in Equation (1) gives $V_{hydrate} = 0.798$.

If the partial specific volumes of the dry complex and hydrate water are additive, then

$$V_{\text{complex}} = (l + y)\ V_{\text{hydrate}} - {}^{y}V_{H_2O}, \qquad (2)$$

where y is the hydration in grams per gram. Making $V_{\text{hydrate}} = 0.798$ as found above, $y = 0.52$, and as a first approximation $V_{H_2O} = 1.003$, the reciprocal of the density of water in bulk at 25°, this equation gives $V_{\text{complex}} = 0.692$ for this particular milk.

The assumption that the density of hydrate water is the same as the density of water in bulk is arbitrary and may not be justified. It is the usual assumption made in such calculations. More refined interpretation of such data must wait on better understanding of the nature of hydrate water.

Table 1 gives the values for the apparent specific volume, calculated as described above, for the six skimmilks for which density data were obtained. In Table 1 the corresponding calucium : casein nitrogen and complex : casein nitrogen ratios also are given, together with the serum nitrogen and the density values used in the calculations. Of these experiments, those for October, 1946, and for August, 1955, have been previously reported in part (*cf.* References 9 and 10). Figure 1 is a plot of the data for December, 1946. Fewer points were obtained in the last three experiments, but with greater accuracy, over wider particle-size ranges, and the plots show even less deviation from linearity.

Values Based on Density and Total Solids Determinations on Skimmilks and Their Casein-Free Serums. If only total solids and density are known for a skimmilk and its casein-free serum, an apparent specific volume can be calculated which is to a close approximation the apparent specific volume of the complex. In the October, 1946, and the two August, 1955, experiments (Table 1), total solids as well as densities and nitrogens were determined. The data are given in the preceding paper (10). Taking the August, 1955, experiment on Jersey milk as an example, the casein-free serum total solids is 6.29%, and the total solids at 0.5% casein nitrogen is 9.40%. If the amount of total solids removed from 100 g. of this skimmilk is x and if a hydration of 0.52 g. per gram of dry complex be assumed, then $9.40 - 6.29\{(100 - 1.52\ x/100\} = x$, and $x = 3.44$. Since the density of this skimmilk is 1.03300, $h = (3.44)\ (1.52)\ (1.03300)$, or 5.40. The serum density is 1.02330. Putting these values in Equation (1) gives $V_{\text{hydrate}} = 0.802$, and $V_{\text{complex}} = 0.697$. In this case, correction of the serum total solids for the serum protein and fat or lipids removed by centrifuging, and correction of the serum density for the serum protein removed, is possible. The effect of the small amount of fat on the density can be ignored. Recalculating on this basis, the apparent specific volume found is 0.696. The agreement with the value given in Table 1, 0.696, is exact as it should be, since the complex : nitrogen factors used in the calculations for Table 1 were based on total solids results and involved the same corrections for serum protein and fat.

Reference has been made to a specific volume reported by Nichols *et al.* (12). These authors give total solids and densities for a skimmilk and for the casein-free serum obtained from it by ultrafiltration. Using their data, and calculating as above, the apparent specific volume obtained is 0.703. Nichols *et al.* corrected

TABLE 1

Apparent specific volume of the casein complex, calculated from data on progressively depleted samples of skimmilks, and the corresponding calcium: nitrogen and complex: nitrogen ratios; together with the serum nitrogen and the density values used in the calculations

Date	S.N.[a] gram per 100 g.	Serum density (24.8°/4°)	Density at 0.50% C.N. (24.8°/4°)	Ca/C.N.[a]	Complex/C.N.	A.S.V.[a]	Milk
October, 1946	0.105	1.02615	1.03605	0.182	6.83	0.692	Jersey
November, 1946	0.146	1.02532	1.03505	....	(6.89)	0.699	Herd
December, 1946	0.140	1.02523	1.03528	....	(6.89)	0.692	Jersey
September, 1954	0.167	1.02487	1.03450	....	(6.89)	0.701	Jersey
August, 1955	0.119	1.02330	1.03300	0.182	6.77	0.696	Jersey[b]
August, 1955	0.140	1.02270	1.03240	0.184	6.90	0.703	Holstein[b]
Averages	0.146	1.02460	1.03438	0.183	6.86	0.6972[c]	

[a] S.N. = serum nitrogen; C.N. = casein nitrogen; A.S.V. = apparent specific volume.
[b] About 20% of the colloid was removed from these two skimmilks by preliminary centrifugation. The results, therefore, apply to intermediate- and small-particle sizes only.
[c] Standard deviation, 0.0039.

their serum total solids for the albumin-globulin held up on the filter, but they did not make a corresponding correction for the serum density. They give a specific volume of 0.665. Aside from the failure to correct the serum density, however, they also ignored hydration, and they obtained a value for total solids removed by simple subtraction of their corrected serum total solids from the skimmilk total solids.

Values Based on Density Measurements on Water Suspensions of Centrifugally Deposited Water-Washed Casein Colloids. The experiments previously reported (9, 10), have shown that the casein complex contained in the colloids centrifugally deposited from milk is not sensibly altered in composition by redispersing the deposit in distilled water and recentrifuging. The colloids are, however, thus washed free of lactose, and presumably washed free of serum salts. They are also, to a large extent, freed of ether-extractable substances which are always present in the unwashed deposits. They are not, however, freed of serum proteins.

Several twice-washed water suspensions of the casein colloids were prepared as indicated, analyzed for total nitrogen, casein nitrogen, calcium, and total solids, and the densities determined. In the calculations based on these experiments, the quantity h in Equation (1) was taken as referring to the total solids. The apparent specific volume of the total solids was then corrected for the contained serum protein by use of the relationship

$$V_{\text{complex}} = \frac{(T.S.)(V_{T.S.}) - (S.P.)(0.751)}{(T.S. - S.P.)}, \qquad (3)$$

where $T.S.$ means total solids, $S.P.$ means serum protein (serum nitrogen × 6.38), and $V_{T.S.}$ is the specific volume of the total solids as directly determined. The factor 0.751 is the specific volume given by Svedberg and Pedersen (16) for albumin and globulin.[4] In these calculations, since the solvent is water, hydration is disregarded, as is common practice. The results obtained in this way, with corresponding calcium: nitrogen and complex: nitrogen ratios, are given in Table 2. In the last three experiments, the complex: nitrogen ratios were not determined, but calculated from the calcium: nitrogen ratios (*cf.* Reference 10).

Calculations of the Specific Volume of the Casein Complex Based on the Specific Volumes of Its Components, for Minimum, Average, and Maximum Calcium: Nitrogen Ratios. The method of calculating specific volumes of proteins by adding the weights and the volumse of constituent groups and dividing the sum of the volumes by the sum of the weights was described by Cohn and Edsall (2). It has been used by McMeekin, Groves, and Hipp (11) for casein. The method is here employed for the casein complex.

The casein complex will be considered to be composed by adding the amounts of calcium caseinate, tricalcium phosphate, and trimagnesium phosphate equiva-

[4] A value 0.734 has recently been reported (3) for bovine serum albumin, which is commonly presumed to be identical with milk serum albumin. The best value for lactalbumin remains 0.751. In the absence of a new value for isolated serum albumin, 0.751 is used here. Substitution of 0.734 would affect the final result by less than 0.15%.

TABLE 2

Apparent specific volumes of the casein complex based on analyses and density determinations on suspensions of centrifugally deposited, water-washed colloids; and corresponding calcium: nitrogen and complex: nitrogen ratios[a]

Ca/C.N.[b]	Complex/C.N.	A.S.V.[b]	Milk	Particle-size fraction
0.198	6.87	0.696	Jersey	Composite
0.200	6.80	0.697	Jersey	Composite
0.209	6.85	0.698	Jersey,[c] 24 hr. at 4° C.	Composite
0.215	6.91	0.700	Jersey, 48 hr. at 4° C.	Composite
0.216	6.90	0.700	Jersey, 1 wk. at 4° C.	Composite
0.202	6.83	0.697	Jersey[d]	Small particles
0.204	6.96	0.692	Jersey	Intermediate
0.181	6.82	0.701	Holstein[e]	Small
0.196	6.86	0.698	Holstein	Intermediate
0.204	6.89	0.698	Holstein	Large
0.203 Average	6.87 Average	0.6977[f] Average		

[a] There is a discrepancy between the Ca/C.N. and Complex/C.N. values for the 1-wk. Jersey milk as between this table and the third part of Table 2 of the preceding paper on the nitrogen factor (10). The values given here are correct. The error in the previous paper has no effect on the final result.
[b] C.N. = casein nitrogen; A.S.V. = apparent specific volume.
[c] This and the following two samples were made from the same milk.
[d] This and the following sample were made from the same milk.
[e] This and the following two samples were made from the same milk.
[f] Standard deviation, 0.0024.

lent to 1 g. of nitrogen, using minimum, average, and maximum analyses previously reported (9). The three compositions, in grams and milliliters of the assumed components are given in Table 3.

The weight of calcium caseinate used in Table 3, 6.519 g., is equal to 6.45 + 0.0727 − 0.0037, the casein: nitrogen ratio, plus the grams of calcium equivalent to the phosphorus in casein, less the grams of hydrogen displaced by the calcium

TABLE 3

Minimum, average, and maximum calculated specific volumes of the casein complex in milk, based on analyses of liquid samples progressively depleted of the casein colloid by centrifuging

Components	Minimum Ca content found by analysis Ca/N = 0.181		Average Ca content found by analysis Ca/N = 0.206		Maximum Ca content found by analysis Ca/N = 0.232	
	(g.)	*(ml.)*	*(g.)*	*(ml.)*	*(g.)*	*(ml.)*
Calcium caseinate per gram nitrogen $\bar{V} = 0.715$	6.519	4.661	6.519	4.661	6.519	4.661
$Ca_3(PO_4)_2$ per gram nitrogen[a] $\bar{V} = 0.38$	0.280	0.106	0.344	0.131	0.410	0.156
$Mg_3(PO_4)_2$ per gram nitrogen[b] $\bar{V} = 0.38$	0.016	0.006	0.019	0.007	0.023	0.009
Totals	6.815	4.773	6.882	4.799	6.952	4.826
Specific volume *(ml/g)*	0.700_4		0.697_3		0.694_2	

[a] $Ca_3(PO_4)_2$ equivalent to the total calcium diminished by calcium equivalent to organic phosphorus.
[b] $Mg_3(PO_4)_2$ equivalent to 1/15th the total mols of calcium (*cf.* Reference 1).

(*cf.* Reference 10). The weight of tricalcium phosphate is that equivalent to the total calcium in the complex, less the 0.0727 g. of calcium assumed to be present as calcium caseinate. The weight of trimagnesium phosphate assigned is based on a mol ratio of magnesium to calcium of 1 to 15 (1).

The volumes, in Table 3, are the products of the weights of the components and the specific volumes. The specific volumes used are based on supplementary measurements and calculations.

The specific volume of calcium caseinate was determined by density measurements on filtered and centrifugally clarified solutions of freshly precipitated and washed casein in lime water. The average of ten values is 0.714, and the standard deviation, 0.004. Equation (1) was used. The specific volume was also estimated by starting with 6.45 g. of casein of specific volume 0.731, the value given by McMeekin, Groves, and Hipp (11), adding the equivalent weight and volume of calcium, and subtracting the weight and volume of hydrogen displaced. Calculating the specific volumes of calcium and hydrogen from the ionic radii 0.98 and 1.36 Angstroms, due to Zachariason (14), the specific volume thus obtained for calcium caseinate is 0.720. However, it is permissible to increase the ionic radius of the hydrogen by at least about 0.5 Angstroms, on the presumption that in the dissolved state the ester phosphate hydrogens of calcium caseinate are exposed and, when this is done, the specific volume found is 0.714. If the ionic radius of calcium is increased by a like amount the specific volume becomes 0.716. A similar calculation can be based on the specific volumes of ortho-phosphoric acid and calcium dihydration phosphate, 0.448 and 0.258, obtained from density data. Using these figures, the decrease in specific volume resulting when two hydrogens are replaced by calcium, in phosphoric acid, is found to be 0.706 cc. per gram of calcium. If the same decrease in volume occurs when two ester phosphate hydrogens of casein are replaced by calcium, then a simple calculation gives 0.715 for the specific volume of calcium caseinate.

The specific volume of tricalcium phosphate was determined by density measurements on suspensions prepared by mixing solutions of $Ca(OH)_2$ and $CaHPO_4$. The value 0.38 was obtained. By adding up the ionic volumes, the result is 0.36, and if the calcium is presumed to be in part exposed, a value of about 0.42 is obtained. The experimental value is used in Table 3. The specific volume of trimagnesium phosphate was not determined, but the value 0.38 was used. The amount of magnesium phosphate involved is so small that even a large error in the specific volume would have little effect on the final result.

The three specific volumes for the complex given in Table 3 are in good agreement with the experimental values. The purpose of these calculations is not, however, to show agreement between theory and experiment, but primarily to set limits of variability of the specific volume with composition. This variability is about 0.45% of the mean value.

In accordance with the method of calculation of the theoretical values, it is seen that the relationship between the apparent specific volume and the calcium: nitrogen ratio is not linear. In the useful range, however, between the extreme calcium : nitrogen ratios, 0.181 and 0.232, found by experiment (9), it is approximately linear, and can be described by the equation

$$V_{\text{complex}} = 0.700 - (0.121)(\text{Ca/C.N.} - 0.181). \qquad (4)$$

DISCUSSION

Assumptions Involved in the Calculations Based on Progressively Depleted Skimmilk Samples. These calculations are complicated by two factors: the centrifugal casein-free serum does not have the same composition as the serum or solvent phase of the skimmilk, and the disperse phase is not the anhydrous complex but a hydrate. Centrifugal casein-free serum has been partially depleted of serum protein and, possibly, small amounts of lactose and salts also, by centrifugation. It is for this reason that the true serum nitrogen concentration must be calculated from the analysis of the skimmilk, assuming a value for hydration; and an approximate density for this serum then taken from the density : total nitrogen plot, or calculated. Taking the December, 1946, experiment, Figure 1, for example, if the serum density were assumed to be that of the centrifugal casein-free serum, and the calculations carried out otherwise as indicated, assuming a hydration of 0.52 g. per gram, the apparent specific volume obtained would be 0.687 rather than 0.693, as given. If hydration were ignored also, i.e., assumed to be zero, then the apparent specific volume 0.701 would be obtained.

Hydration can be disregarded in determinations of apparent specific volumes for pure proteins dissolved in water, if the density of hydrate water is assumed to be the same as that of water in bulk. It is commonly disregarded also for solutions of proteins in dilute buffers. It can not be disregarded in the present case, because the density of the solvent differs a great deal from that of water. Again taking the December, 1946, experiment as an example, correcting serum densities as indicated but assuming hydrations of zero, 0.52 g. per gram, and 1.0 g. per gram, the apparent specific volumes found are 0.706, 0.693, and 0.679, respectively.

The assumption that the density : total nitrogen plots can be extrapolated to zero casein nitrogen may or may not be justified. In one of the experiments reported in Table 1, however, for August, 1955 (Jersey milk), 95% of the casein nitrogen was removed with no deviation in linearity of the density plot, and in other experiments in which densities were not determined, as much as 99% of the casein nitrogen has been centrifugally removed. Whether the extrapolation is justified or not, however, makes no difference in the calculations. The serum may be assumed to contain 5, 10, or 20% casein nitrogen, and when the calculations are carried out as indicated, the same apparent specific volume for the remaining complex is obtained. This could be predicted, since the specific volume : total nitrogen plots, like the density plots, are straight lines within the accuracy of the data.

Collected results. The 13 sets of apparent specific volume–calcium : casein nitrogen ratio values of Tables 1 and 2 were analyzed by the least-squares method, giving equal weight to all the data. The values obtained for the constants a and b

in the equation $V = a + b(Ca/N - 0.181)$ are 0.6974 and − 0.007. Using only the data for washed colloids, Table 2, the constants 0.6986 and − 0.058 are obtained. In the second case, the apparent specific volumes given by the equation at the minimum, average, and maximum calcium: nitrogen ratios, 0.181, 0.206, and 0.232 are 0.6986, 0.6971, and 0.6956, respectively. The data are too meager to establish a slope with any precision, but since the experimental average specific volume agrees so well with the theoretical value, 0.6973, it seems permissible to assume the slope to be the theoretical slope, −0.121, given in Equation (4). When this is done, and the sum of the squares of the deviations from a line having this slope is made a minimum, the value obtained for a, using all the data, is 0.6992; the apparent specific volumes at the minimum, average, and maximum calcium: casein nitrogen ratios are 0.6992, 0.6961, and 0.6930, respectively, and the standard error of estimate is 0.0033. If only the data for washed colloids are used, a becomes 0.6998, the three apparent specific volumes are 0.6998, 0.6968, and 0.6936, and the standard error of estimate is 0.0024.

These statistical results indicate that the constant a in Equation (4) should probably be about 0.700, as given; that the apparent specific volume for a complex of average composition (calcium: nitrogen, 0.206) is about 0.697; and that the extreme deviations to be expected in this value are about equal to the probable experimental error, 0.003.

Specific volume of the actual casein complex particle in milk. The apparent specific volume calculated here is the specific volume of the calcium caseinate–calcium phosphate complex. As it exists in milk or in any aqueous medium, this complex is hydrated and solvated. The specific volume of the actual dispersed particle or micelle is, therefore, determined by the contributions of the complex, the hydrate water, and the solvate liquid to the total weight and volume. These contributions can be calculated. For purposes of illustration, a complex of average composition will be assumed for which $V = 0.697$ in accordance with Equation (4). It will be further assumed that the hydration is 0.52 g. per gram; that the density of hydrate water is 0.9970 at 25° C.; that the ratio of the volume of the total particle to the weight of contained dry complex is about 3.1 to 1 (5); that the solvate liquid is identical with casein-free milk serum and has a density of 1.0264; and that no serum proteins or other solids are present, except those in the solvate liquid. Using these values, for the total particle:

$$V_{\text{particle}} = \frac{3.1}{1.000 + 0.52 + (3.1 - 0.697 - 0.522)\,(1.0264)} = 0.898 \qquad (5)$$

The corresponding density is 1.114. This is the density that would be used in calculations of particle sizes from sedimentation velocities by use of Stokes' law, for example. These hydrated, solvated colloids can be separated by centrifugation, and obtained as wet jelly-like deposits (*cf.* Reference 9), with some inclusion of serum proteins in excess of those in the solvate liquid, and with variable entrainment of excess milk serum, depending on the centrifugal force used. Densities of such wet deposits determined by displacement of xylene approach the value 1.114 as the centrifugal force is increased and the amount of entrained serum approaches zero. Such experiments will be reported in detail later.

CONCLUSIONS

1. Skimmilks were progressively depleted of casein colloids by centrifuging, supernatant liquids and deposited fractions being thus obtained. The deposits were twice washed by redispersing in water and recentrifuging. Densities were determined on the supernatant liquids and on water suspensions of the washed colloids.

2. Apparent specific volumes were calculated by the equation of Svedberg and Chirnoaga. In the calculations based on densities of supernatant liquids the mathematical treatment is novel in that the apparent specific volume of the casein complex hydrate considered as the solute is first calculated, and the apparent specific volume of the unhydrated complex is then calculated from this value. This procedure is necessary because the solvent is not water in this case but milk serum of density appreciably different from that of water. In the calculations based on densities of water suspensions of washed colloids this refinement is not necessary.

3. For six sets of data on supernatant liquids the average apparent specific volume found for all fractions for the casein complex was 0.697 ± 0.0038. For ten sets based on water suspensions of washed colloids the average value found was 0.698 ± 0.0018. The corresponding average calcium : nitrogen ratios were 0.183 and 0.203.

4. The apparent specific volume of the complex calculated from the specific volumes of its components, as indicated by analyses previously reported, is a function of the calcium : nitrogen ratio in the complex, and is given by the equation

$$\text{A.S.V.} = 0.700 - 0.121\,[(\text{Ca/N}) - 0.181]$$

This equation is in statistical agreement with the experimental results. For a large number of samples the minimum, average, and maximum calcium : nitrogen ratios found are 0.181, 0.206, and 0.232. These values used in the above equation give theoretical, and actual, apparent specific volumes of 0.700, 0.697, and 0.694, respectively, with a probable error of $\pm$ 0.003 or about 0.4%. These values apply to the total complex and to the complex in any particular size fraction, for Jersey or Holstein milk.

5. It is emphasized that the apparent specific volumes calculated are for the casein complex and not for the total colloidal particle as it exists in milk. The specific volume of the actual dispersed particle can be calculated from the specific volume of the complex by taking hydration and solvation into account. An example of such a calculation is given.

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