VIII. THE DENSITY AND SOLUTION VOLUME OF SOME PROTEINS.

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In a previous communication dealing with the viscosity and other properties of caseinogen solutions [1912], we pointed out that there was a shrinkage in volume and corresponding increase in density when that protein formed colloidal solution. In order to investigate the point the method employed was to determine the density of the dry, powdered protein by weighing in benzene or some other indifferent fluid and to compare the value with that obtained by calculation from the observed density of a fairly concentrated solution. The latter value was found to be much greater than the former.

As far as we have been able to ascertain, this is the first attempt to determine the density of proteins with the exception of gelatin. Quincke [1903] found the specific gravity of a specimen of the latter to be 1·368, while Lüdekings [1888] had previously given the value of 1·412, and had shown that the density calculated from that of jellies (14 to 35 % gelatin) was as high as 1·9.

The increase in density when proteins are dissolved in water is an instance of the phenomenon constantly associated with colloidal solution of the lyophile or emulsoid character. The intimate association of the protein particles with water leads to a shrinkage in total volume, so that the increase in density is more properly regarded as an attribute of the whole system than of the protein alone. Analogous cases are those of gum-tragacanth and starch. In the case of the latter Rödewald [1897] found the density in the dry state and that calculated from that of a solution to be 1·38 and 1·49 respectively.

In our experiment with caseinogen, a sample of the pure, dry, powdered protein was prepared by acidifying a solution in dilute sodium hydroxide, washing and drying the precipitate, and grinding to a fine powder. A known weight was inserted in a pyknometer bottle, covered with benzene of known

density, and all the air expelled by careful heating *in vacuo*. The bottle was then filled up with benzene in a thermostat at 25° C. and weighed. The weight obtained was compared with that of the bottle filled with benzene at the same temperature, and the density of the powder calculated. The value obtained was 1·318 as the mean of two determinations (see Table I). A solution of caseinogen (7·85 %) in dilute sodium hydroxide gave a density of 1·0240, from which the density of sodium caseinogenate was calculated to be 1·42.

In order to compare this value with that obtained by direct determination, it is necessary to apply a small correction, seeing that the solution consisted of sodium caseinogenate. The ash present composed 4·331 % of the dry weight and, assuming this to be sodium carbonate, 2 % of the dry weight would consist of sodium, and the density, for comparison with that of the powdered caseinogen, should be reduced by that amount. After this correction has been made the caseinogen in solution is still seen to be denser than the solid caseinogen in the proportion of about 1·39 to 1·318.

In addition to caseinogen three other pure proteins have been similarly investigated, viz.:

Crystalline serum albumin, prepared from horse-serum by the method of Hopkins and Pinkus [1898].

Serum globulin (pseudo-globulin). Three different samples were investigated, obtained from horse-serum by different methods. I and II were prepared by repeated precipitation with half-saturated ammonium sulphate, the euglobulin present in the serum being separated by the subsequent dialysis; in the case of III the latter was removed by preliminary precipitation with saturated brine, after which the pseudo-globulin was precipitated from the warm diluted filtrate by adding anhydrous sodium sulphate until the concentration equalled 20 % Na_2SO_4. The precipitate was thoroughly washed with a solution of sodium sulphate of the same strength.

Crystalline egg-albumin, prepared from egg-white, also by the method of Hopkins and Pinkus.

All the proteins were thoroughly dialysed in presence of toluene and filtered, and concentrated solutions were finally obtained. Determinations were then made of the protein-content and the density. The latter were obtained by pyknometer readings, carried out usually at 15° C. or 20° C., the density of water at the same temperature being taken as unity for the purpose of calculation. It was found that the proportionality between the density of water and that of the protein solution was, within our error of experimentation, maintained between 15° C. and 25° C.

Dry specimens of the proteins were obtained by evaporating the solutions to dryness and grinding the residue to a fine powder. The evaporation and drying were carried out *in vacuo* at room-temperature, and were continued until the weight remained constant, an operation requiring 2–6 weeks. It was feared that, if the samples were dried at a high temperature, the denaturation of the proteins might introduce a source of error. This would not appear to be so, for, in the case of serum albumin, the densities of two samples dried at 100°–110° and 20° respectively, were not found to differ by any significant amount (Table I). The densities of the various powders were determined by weighing in benzene of known density (water at 4° C. = 1·00) at 25°, as described above.

TABLE I.

Density of Protein in solution compared with that in the solid state.

Protein	*In solution*			*Dry State*
	Concentration of protein % (by weight)	Density of the solution	Calculated density* of the protein	Density* of the protein
Caseinogen	7·85	1·0241	1·39†	1·318
Egg-albumin (crystalline) ...	14·6	1·0401	1·359	1·269‡
Serum-albumin (crystalline)	22·15	1·0647	1·378	1·275§
				1·281‡
Serum-globulin I	15·33	1·0428	1·365	1·279§
Serum-globulin II	16 35	1·0466	1·374	1·289‡
Serum-globulin III	11·05	1·0316	1·384	1·312‡

* Density of water at 4° C. = 1·00.
† Corrected for presence of sodium.
‡ Dried at room temperature *in vacuo*.
§ Dried at 105°—110° C.

The results are given in Table I and show that the density of albumin and globulin is also increased when in the state of colloidal solution, and to a higher degree (6·8 %) than was found for caseinogen (5 %). Egg- and serum-albumin have an almost identical density in the dry condition, viz. 1·269 and 1·281, and this is increased in the same proportion on entering solution, viz. to 1·359 and 1·378 respectively. The three samples of globulin give 1·293 as mean value when dry and 1·374 when in solution.

In Tables II and III are given the variation of the density of solutions of serum-albumin and serum-globulin with alteration in protein concentration. If the latter is plotted against the former, straight lines are obtained. The curve for caseinogen, on the other hand, shows a slight convexity which

is rather more than can be explained by our experimental error, the contraction on entering solution being proportionally greater for dilute solutions. This is shown in the third column of Table II, where the value of the density of caseinogen calculated from that of its solution is seen to decrease progressively from 1·465 to 1·412 as the concentration of the protein is increased. In the case of the serum proteins, however, the value remains constant (Table III).

TABLE II.

Density of Caseinogen (Na Caseinogenate) solutions of varying concentration.

Concentration of caseinogen % (by weight)	Density of the solution*	Calculated density of sodium caseinogenate
9·39	1·0283	1·412
8·33	1·0250	1·409
7·52	1·0232	1·424
6·05	1·0190	1·437
4·35	1·0140	1·460
2·173	1·0070	1·465
1·086	1·0033	†

* Compared with water = 1·00 at the same temperature, 15° C.
† Solution too dilute for trustworthy calculation.

TABLE III.

Density of solutions of Horse-Serum Proteins of varying concentration.

Protein	Concentration % (by weight)	Density of the solution*	Calculated density of the protein
Serum-globulin	15·33	1·0428	1·365
,, ...	10·32	1·0290	1·374
,, ...	6·916	1·0190	1·365
,, ...	3·478	1·0096	1·370
Serum-albumin	22·15	1·0647	1·378
,, ...	15·16	1·0440	1·382
,, ...	7·725	1·0220	1·381

* Compared with water = 1·00 at the temperature of expt.

SUMMARY.

A comparison has been instituted in case of four proteins, viz. caseinogen, egg- and serum-albumin, and serum globulin, between the density directly determined with dry specimens and that calculated from the specific gravity

of concentrated solutions. The latter is found to be 5–8 % in excess of the former, showing the extent of shrinkage in volume taking place when these proteins enter colloidal solution.

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