THE "BOUND" WATER OF BIOLOGICAL COLLOIDS: A REPLY

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The interpretation of the paper by Greenberg and Greenberg (1) has recently been questioned by Bull (2). In the paper under question it was pointed out, on the basis of the assumption that "bound" water has lost its solvent properties, that the amount of water in this condition in colloidal solutions can be estimated by ultrafiltration experiments with the use of appropriate reference substances. From a series of such experiments it was decided that the amount of bound water associated with such substances as gelatin, casein, and the serum proteins must be quite small.

Bull points out that an adsorption of the solute along with the water molecules would invalidate the interpretation offered. After questioning the accuracy of the data, he goes on: "Assuming, however, for the moment that the experimental results describe completely the actual conditions, we make the further assumption that 5 per cent of the solute is adsorbed; *i.e.*, bound by the substrate—surely a modest estimate." ". . . . It can be seen that this small correction changes the final values in some cases by over 300 per cent . . . . ." By means of this assumption, Bull shows the possibility of considerable amounts of bound water being present, but not detected. In the original paper this possibility was considered. As was there pointed out, one of the conditions which may invalidate the method is the selection of a reference material which to some extent reacts with or is adsorbed by the colloid. To obviate this as an obscuring factor, a considerable number of reference substances were used in the original experiments. It seems quite a strain upon the assumption advanced by Bull that the reference substances, urea, glucose, KCl, NaCl, and Na_2SO_4, should all be adsorbed by gelatin to an extent just sufficient

to mask the presence of bound water. The adsorption of the solutes, assumed by Bull, however seemingly modest, would be more convincing if bolstered by an experimental demonstration rather than a mere opinion.

No such evidence being offered, a number of experiments were carried out by us designed to detect an adsorption of one of the refer-

TABLE I

Test for Bound Water, and the Adsorption of Glucose in Gelatin and Casein Solutions by Means of Varying Quantities of Glucose as Reference Substance

No.	Glucose in original protein-free solution per 100 ml.	Glucose in ultrafiltrate per 100 ml.	Bound water per gm. of protein
	5.0 per cent gelatin in water + glucose		
	mg.	*mg.*	*gm.*
1	100	101	0.20
2	200	199	−0.10
3	300	301	0.07
4	400	400	0.00
5	500	502	0.08
Average			0.05
	6 per cent casein in aqueous solution containing 5 millimols $NaHCO_3$ and 5 millimols NaCl per 100 ml.		
1	100	100	0.00
2	200	200	0.00
3	300	301	0.05
4	400	397	−0.12
5	500	503	0.10
Average less than			0.01

ence materials used, namely glucose, by the proteins, gelatin and casein. An examination of adsorption isotherms and their mathematical equations, makes it highly improbable that a constant fraction of the glucose would continue to be adsorbed as its concentration in the solution is varied. Determinations of the glucose ultrafiltered from a series of protein solutions with varying amounts of glucose then should demonstrate if it is adsorbed even though at some one point

the bound water exactly would counterbalance the glucose adsorbed. Such a series with the proteins, gelatin and casein, is given in Table I.

EXPERIMENTAL

To avoid uncertain corrections as to non-solvent volume of the proteins, the aqueous glucose solutions were first accurately prepared from pure recrystallized glucose and to measured volumes, weighed amounts of purified protein bone dried in an electric oven at 100°, were added. The gelatin was added to mixtures containing only water besides the glucose, but to bring the casein into solution, the aqueous media also contained 5 millimols of $NaHCO_3$ and 5 millimols of NaCl per 100 ml. The gelatin was dissolved by warming to 50°C. and the casein by vigorous agitation. Portions of these protein solutions were now ultrafiltered in the manner previously described (1). Samples of the ultrafiltrate and of the original aqueous solution were analyzed for glucose by the Hagedorn-Jensen method (3). The analyses were carried out in duplicate, often in triplicate. The estimated accuracy of the analysis is to better than 0.5 per cent.

DISCUSSION

The data tabulated in Table I show there was a complete recovery of the 100 mg. of glucose per 100 ml. of solution added to each increasing step in the series. Over a fivefold increase in the glucose concentration, there is no indication whatsoever of an adsorption of glucose by the proteins. The net amount of bound water per gram of each protein, calculated in the last column of the table, while positive in value, is very small in amount. From this test, the assumption of Bull does not appear tenable, and if any amount of glucose is adsorbed by either of the two proteins, it must be small indeed.

The probability of an adsorption or association of some water with the proteins and other lyophilic colloids has not been denied by us. However, our evidence indicates it to be far below the amount claimed. A rough picture of the probable order of magnitude of the bound water can be gained from an examination of the molecular dimensions of the proteins. From modern work on surface adsorption, initiated by Langmuir (4), no more than one or two monolayers of water molecules would be expected to be adsorbed by the proteins with such force as to lose their solvent properties. Furthermore, the whole of the protein surface would hardly be expected to be the seat of such an adsorption, but rather only the polar portions. If the protein hemoglobin is used

as a model (molecular weight 66,700; density 1.3), from Avogadro's number, there is calculated, assuming the protein to be spherical, the molecular diameter 54.5 Å. u. and the volume 85,000 Å. u.^{3} By a similar calculation, water molecules have a diameter of 3.85 Å. u. and a volume of 30 Å. u.^{3} If the water molecules are considered to be spheres packed over the spherical surface of the protein, then about 300 water molecules are required to give a complete surface layer of water 1 molecule thick. Assuming the water molecule to be a cube, about double this number can be so packed. The first consideration would lead to a value of 0.08 gm. of bound water per gram of protein for each monolayer of water, the latter about 0.16 gm. However, since, as has already been mentioned, the total protein surface can hardly be adsorptively active for water, these amounts must be reduced to some fractional values, a good deal less. Such a consideration leads to the view then that an adsorbed water layer a number of molecules thick on the proteins would hardly yield a detectable amount of bound water.

SUMMARY

The objection by Bull to the estimation of bound water by ultrafiltration, because of an assumed adsorption of the reference substance, has been found invalid for glucose. No adsorption of glucose by the proteins, casein and gelatin, could be detected.

The estimation of the bound water of proteins from the probable surface adsorption of water by proteins leads to only a small value.

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