

## SOME METHODOLOGICAL ERRORS WHICH MAY ARISE IN THE DETERMINATION OF BOUND WATER\*

By HENRY B. BULL

(From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul)

(Accepted for publication, June 21, 1933)

“Bound water” has been a subject of unusual interest to the physiologist (1) in recent years and many papers of merit have appeared upon the question.

These methods and concepts of the physiologist have been attended by a parallel development in the realm of pure physical chemistry. Thus Polanyi *et al.* (2) have developed the so called potential theory of adsorption and have studied the decrease in the adsorption potential as the distance from the particle is increased. This adsorption potential is calculated by means of the equation

$$A = RT \ln \frac{P_s}{P}$$

in which  $P_s$  is the vapor pressure of the liquid at the given temperature and  $P$  is the vapor pressure of the material in equilibrium with the adsorbed material. It is the adsorption potential which determines the extent to which the vapor pressure of a gas is to be reduced upon approaching a solid surface. On the immediate surface the potential is exceedingly high, the vapor pressure and the fugacity of the gas are low. Assuming for the moment that we are dealing with some liquid below its critical temperature, perhaps water, we can then say that its activity has been correspondingly reduced. As we increase the amount of water vapor present we eventually reach the saturation point. It is hard to see how the addition of further water could change the situation. In other words, if the substance with its adsorbed water were immersed in water the condition at the surface would not be changed. We should have under these conditions what the physiologist has been

\* Paper No. 1194, Journal Series, Minnesota Agricultural Experiment Station.

calling "bound water." Its activity has been decreased and its ability to dissolve substances has been correspondingly diminished. This, it seems to the author, is the physical picture of bound water.

If, however, we add to this system a small amount of some solute—perhaps sucrose—we upset the conditions. This same potential which contributed to the diminution of the vapor pressure and activity of the water now diminishes the activity of the sucrose which comes in the proximity of the solid surface. The sucrose has been adsorbed. The author is aware that Polanyi has endeavored to apply his adsorption theory to solutions with but small quantitative success. However, the author does feel that the theory is a sound one and that the lack of quantitative agreement is due to the extremely complicated situation. In any event, however, that the solute can be adsorbed by colloidal material has been demonstrated a great many times and is a fact, whatever may be the theory by which we account for the phenomenon.

It appears to the author that adsorption of the solute by the substrate may be a source of large errors and it may turn out that this is the principal cause of disagreement so generally noticed between the results of different workers. It is true that it has been recognized that adsorption might play a rôle, but there seems to be a lack of appreciation of size of errors which might result from a relatively small adsorption of the solute.

In a recent paper Greenberg and Greenberg (3) presented results obtained from a study of the concentration of the solute in the ultrafiltrate from certain colloidal materials. This concentration was compared with the concentration of the solute in the original colloid. They proposed the following equation

$$h = \frac{1}{P} \left( 1 - \frac{C_T}{C_u} \right)$$

where  $h$  is the bound water per gram of colloid,  $P$  is the amount of the colloid per gram of total water,  $C_T$  is the concentration of the reference substance per gram of total water in the system,  $C_u$  is the concentration per gram of water of the reference substance in the ultrafiltrate. They presented data from which the bound water might be calculated by the use of this equation. The author has used their

TABLE I

Gelatin concentration	Solvent composition		Reference substance	$(C_T)$	$(C_u)$	Bound water $h$	$C_T$ corrected	Bound water corrected
1	0.0165	0.01 N HCl	Urea	0.00109	0.00109	0.0	0.00104	3.03
2	0.0220	0.025 N HCl	"	0.00200	0.00205	1.109	0.00190	3.327
3	0.0267	0.10 N HCl	"	0.00129	0.00120	-2.808	0.001226	-0.798
4	0.0254	0.075 N HCl	"	0.00140	0.00125	-4.72	0.00133	-2.52
5	0.0274	0.26 N HCl	"	0.00138	0.00138	0.0	0.00138	1.825
6	0.0300	0.011 N NaOH	"	0.00099	0.00095	-1.40	0.0094	0.35
7	0.0370	0.005 N KCl	"	0.00113	0.001138	0.19	0.001074	1.62
8	0.0290	0.01 N NaCl	"	0.00211	0.00205	-1.008	0.002005	0.8413
9	0.0273	0.0075 N KCl	"	0.00157	0.00160	0.6886	0.001492	2.48
10	0.0300	H <sub>2</sub> O	"	0.001415	0.00139	-0.599	0.001344	1.10
11	0.0300	H <sub>2</sub> O	Glucose	0.001	0.00099	-0.337	0.00095	1.35
12	0.0338	H <sub>2</sub> O	"	0.001	0.001008	0.235	0.00095	1.701
13	0.0311	H <sub>2</sub> O	"	0.001	0.00100	0.0	0.00095	1.61
14	0.0263	0.1 N NaCl	"	0.001	0.000994	-0.228	0.00095	1.68
15	0.0316	0.0043 N HCl	"	0.001158	0.001159	0.275	0.00110	1.61
16	0.0500	H <sub>2</sub> O	Urea	0.00987	0.00980	-0.143	0.00937	0.816
17	0.0400	0.005 N NaOH	"	0.00987	0.00984	-0.076	0.00937	1.118
18	0.0400	0.091 N HCl	"	0.02670	0.02660	-0.0938	0.00254	1.127
		0.08 N NaCl						
19	0.0500	0.1 N KCl	"	0.02670	0.02680	0.0761	0.00254	1.044

TABLE II

Casein concentration	Solvent composition		Reference substance	$C_T$	$C_u$	Bound water $h$	$C_T$ corrected	Bound water corrected
(a)	0.0325	0.023 NaOH	Urea	0.001435	0.001425	-0.216	0.00136	1.34
(b)	0.0228	0.05 KOH + 0.01 KSCN	"	0.00145	0.00145	0.0	0.00137	2.21
(c)	0.0175	0.01 KOH + 0.02 KC <sub>2</sub> H <sub>3</sub> O <sub>2</sub>	"	0.00078	0.00079	0.725	0.000741	3.55
(d)	0.0118	0.0067 KOH + 0.03 KC <sub>2</sub> H <sub>3</sub> O <sub>2</sub>	"	0.00078	0.00078	0.0	0.000741	4.24
(e)	0.0346	0.030 KOH + 0.019 KC <sub>2</sub> H <sub>3</sub> O <sub>2</sub>	"	0.00061	0.00060	-0.479	0.00058	1.012
(f)	0.0144	0.012 KOH + 0.0145 K <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	"	0.00057	0.00059	2.35	0.00054	5.77
(g)	0.0266	0.0145 NaOH	Glucose	0.000979	0.000986	0.269	0.00093	2.135
(h)	0.0294	0.0154 NaOH	"	0.000882	0.000882	0.0	0.000838	1.697
(i)	0.0227	0.0119 NaOH	"	0.000909	0.000907	-0.0969	0.000864	2.088

data and their equation for bound water and has calculated the amount of water bound or associated with each gram of colloid. This has been done both for gelatin and casein. The results of these calculations are presented in Tables I and II.

The results can be seen to be extremely erratic. In some cases a tremendous amount of *negative* bound water is found (Table I, 0.0254 per cent gelatin, 0.075 N HCl, and 0.00140 gm./cc. urea). In five cases the amount of bound water is zero. In others a positive value is found. From these erratic results we can judge that the method is capable of only a small degree of accuracy. 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. We have then recalculated the data with the results as shown in Column 9 of Table I and Table II.

We have used in these calculations the modified formula

$$h = \frac{1}{P} \left( 1 - \frac{C_T - S}{C_u} \right)$$

where  $S$  is now the amount of the solute adsorbed per gram of total water.

It can be seen that this small correction changes the final values in some cases by over 300 per cent and in many cases the mysterious *negative* bound water becomes a more reasonable positive value. This represents a serious and drastic revision of the results and conclusions of Greenberg and Greenberg.

#### SUMMARY

The "bound water" hypothesis has an adequate theoretical basis. A relatively slight adsorption of the solute along with water molecules (bound water) will explain the failure of *certain technics* to demonstrate the existence of bound water in biochemical systems.

I wish to thank Dr. R. A. Gortner for his helpful criticism of this paper.

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