BOUND WATER IN MUSCLE

By J. BROOKS

(From the Food Investigation Board of the Department of Scientific and Industrial Research, and Low Temperature Research Station, Cambridge, England)

Rubner (1922) has defined the bound water in a colloidal system as the water which does not freeze at −20°C. On this basis Rubner (1922) and Thoenes (1925) obtained large values ranging from 0.8 to 2 gm. of bound water in muscle per gm. of dry solid. The same definition has been applied by other investigators to various biological systems; it has, however, been criticised by Briggs (1931) and Heiss (1933) on the ground that in systems containing crystalloids, unfrozen free water (*i.e.* water acting as normal solvent) may be present at −20°C.

It seems preferable to define bound water as water which does not act as solvent. On this basis low values at 0°C. were calculated from the vapour pressure isotherm of frog's muscle (Brooks, 1933–34). At an activity of free water of 0.994 it was found that 0.3 gm. of water was bound by 1 gm. of dry solid. Hill (1930) had previously obtained by another method a still lower figure; this may be due partly to the higher temperature employed, namely about 16°C. These results, when compared with the degree of hydration of various proteins in solution, indicate that the bound water in muscle is mainly, if not wholly, accounted for by the water of hydration of the muscle proteins. It would be expected that the degree of hydration would be influenced by the activity of free water and the temperature.

An attempt has been made to calculate the amount of free unfrozen water in muscle at different temperatures below the initial freezing-point from the vapour isotherm at 0°C. The results indicate that a significant amount of free unfrozen water is present at −20°C. and that the system invariant point is considerably below this temperature. The total amount of unfrozen water at −20°C. gives therefore only a maximum figure for the bound water at that temperature. The

783

large values obtained by Rubner and Thoenes can be attributed partly to this cause and partly, to an even greater extent in view of the later work of Heiss (1933) and Moran (private communication) on the freezing-point curve of muscle, to the considerable experimental difficulties of measuring unfrozen water at low temperatures.

The difference between the calculated amount of free unfrozen water in muscle at a given temperature and the amount of total unfrozen water experimentally determined by Heiss or Moran should give the bound water present. The values so obtained are of similar magnitude to those calculated from the vapour pressure isotherm at 0°C.

The Freezing-Point Curve of Muscle

In a "frozen" muscle in equilibrium at a temperature T' the activity of free water, a', is equal to the activity of ice at that temperature and is given by the equation (Lewis and Randall, 1923)

$$\log a' = -0.004211\, v - 0.0000022\, v^2 \tag{1}$$

where $v = 273 - T'$.

The amount of free water present in frog's muscle in rigor at 0°C. and at different activities of water has been calculated from the vapour pressure isotherm. The reference state of the muscle is taken as the water content at 0°C. (T'') and an activity of free water (a_0'') of 0.994. At this activity and temperature it was found that 100 gm. of muscle contained 19.9 gm. of dry solid, 74.3 gm. of free water, and 5.8 gm. of bound water. Let the weight of free water per 19.9 gm. of dry solid at the same temperature T'' and another activity of water a_1'' be m gm. Then if the activity of free water in a muscle is independent of the temperature the decrease of temperature v required to freeze out $(74.3 - m)$ gm. of free water from 100 gm. of muscle in the reference state is given by substituting a_1'' for a' in (1).

It cannot be assumed, however, that the activity of water in a muscle is independent of temperature. The relation between temperature and activity is given by the equation:

$$\frac{d\ln a}{dT} = -\frac{\bar{L}_1}{RT^2} \tag{2}$$

where $\bar{L}_1$ is the relative partial molal heat content of water in the

muscle. For most solutions $\bar{L}_1$ is negligible unless the solution is concentrated or has an exceptionally large heat of dilution. It would be expected therefore for high values of a_1'' that the activity of water in a muscle would not alter greatly with temperature.

Lewis and Randall (1923) take $\bar{L}_1$ as a linear function of temperature[1] and integrating (2) at constant composition obtain the following equation for the change in activity of water with temperature:

$$\log a''_1 - \log a'_1 = x \tag{3}$$

where

$$x = -\bar{L}_{1(T')} \cdot \frac{T'' - T'}{2.303 \, RT''T'} + (\bar{C}_{p_1} - \bar{C}^{\circ}_{p_1})\left(T'' \cdot \frac{T'' - T'}{2.303 \, RT''T'} - \frac{1}{R}\log\frac{T''}{T'}\right) \tag{4}$$

and $\bar{C}p_1$ and $\bar{C}^{\circ}p_1$ are partial molal heat capacities of water.

The substitution of (3) in (1) gives the following equation,

$$\log a''_1 + 0.004211 \, v + 0.0000022 \, v^2 = x \tag{5}$$

where x is given by (4). If therefore at one temperature, T'', the free water content and the partial molal heat terms for water in muscle at different activities of water, a_1'', are known the required values of v (where $v = 273 - T'$) can be obtained from (5).

The partial molal heat terms for water in muscle are unknown, but when the crystalloid constituents of muscle are considered,[2] it is reasonable to assume that they do not differ greatly from the heat terms for water in a solution of a uni-univalent electrolyte, e.g. sodium or potassium chloride, of equivalent concentration. The concentrations of solutions of sodium chloride with activities of water at $0°C$. corresponding to the activities used (down to $a_1'' = 0.798$) in the determination of the vapour pressure isotherm have already been calculated (Brooks, 1933–34). These values are given in Table I, together with the mean amounts of free and bound water in frog's muscle at $0°C$. and at different activities of water. The calculated

---

[1] This assumption, according to Young (1933), is not strictly correct. It is sufficiently accurate for the present purpose, however.

[2] The proteins in muscle would not be expected to influence $\bar{L}_1$ unless the total water content of the muscle was lower than approximately 0.3 gm. of water per gm. of protein (cf. Rosenbohm, 1914; Moran, 1932).

values of free water at $a'' = 0.85$ and 0.80 are less accurate than those at the higher activities but are probably of the correct order.

The partial molal heat terms for water at 0°C. in the solutions of sodium chloride given in Table I were obtained from the data of Randall and Bisson (Lewis and Randall, 1923) at 25°C. These values, together with the corresponding values of a''_1, were substituted in (5), giving v (or $273 - T'$). The first column of Table II contains the molality of sodium chloride and the second column the corresponding value of v from (5).

It will be seen that this particular use of (5) gives simply the freezing-point of the solutions of sodium chloride in the first column. For comparison therefore the third column contains the experimental

TABLE I

Dry Weight of Muscle = 19.9 Gm.

a''	Molality of sodium chloride	Equilibrium weight of muscle	Free water	Bound water
		gm.	*gm.*	*gm.*
0.9936	0.1969	100	74.3	5.8
0.9552	1.381	36.3	10.6	5.8
0.8904	3.138	30.5	4.7	5.9
0.8502	4.098	28.0	3.5	4.6
0.7984	5.215	26.8	2.8	4.1

values of the freezing-points interpolated from those given in the International Critical Tables.[3] The fourth column gives values of v obtained on the assumption that $\bar{L}_1$ is zero; *i.e.*, by the direct substitution of a''_1 in (1). As would be expected this assumption does not lead to a large error in the calculated freezing-point except at low activities of water.

The fourth column of Table I and the second (or third) column of Table II give the amounts of free unfrozen water remaining in 100 gm. of "frozen" frog's muscle (initially in the reference state) in equilibrium at v degrees below 0°C. These values are given below.

[3] International Critical Tables, New York, McGraw-Hill Book Co., Inc., 1928, 4, 258.

| v | 0.67* | 4.7 | 11.6 | 16.0 | 21.9 |
| Free water, gm | 74.3 | 10.6 | 4.7 | 3.5 | 2.8 |

* −0.67°C. is the calculated value for the initial freezing-point of frog's muscle in rigor; the freezing-point of living frog's muscle is −0.42°C. Hill (1930) gives the osmotic equivalent of frog's muscle in rigor as 0.2 molal sodium chloride; this solution has a freezing-point of −0.68°C.

If the total unfrozen water at −21.9°C. is considered as bound and expressed as gm. of bound water per gm. of dry solid it will be seen that the value so obtained is approximately 2.8/19.9 = 0.14 gm. too high.

The proportion of free unfrozen water in beef muscle (in rigor) at a given temperature will be greater than in frog's muscle as the initial

TABLE II

Molality of sodium chloride	v (from (5))	v (experimental)	$\frac{v}{(\bar{L}_1 = 0)}$
0.1969	0.674	0.674	
1.381	4.68	4.70	4.68
3.138	11.56	11.4	11.8
4.098	16.0	15.5	16.6
5.215	21.9	21.2	22.9

freezing-point is lower (−1°C. instead of −0.67°C.). Assuming that the water-binding capacity of the proteins in beef muscle at 0°C. is the same as in frog's muscle at 0°C. and making allowance for the differences in initial freezing-point and total water content (77 instead of 80 per cent) the amounts of free unfrozen water in 100 gm. of "frozen" beef muscle have been calculated; these are given below.

| v | 1.0 | 4.7 | 11.6 | 16.0 | 21.9 |
| Free water, gm | 70.3 | 15.1 | 6.6 | 5.1 | 4.0 |

The total amount of unfrozen water in beef muscle (total initial water content 77 per cent) at different temperatures has been determined by Heiss (1933). Values interpolated from his results are given in the second column of Table III. The fourth column is the difference between total (experimental) and free water (calculated) at each temperature and gives the amount of bound water at that temperature.

It will be seen that the values for bound water are of the same order as those for frog's muscle at 0°C. It is not believed that any quantitative conclusion can be drawn from the values in the last column regarding the effect of decrease in temperature and activity of water on the degree of hydration of the muscle proteins. They show, however, that the results from the vapour pressure isotherm and from the freezing-point curve are both in agreement with a low value for the amount of bound water in muscle. A similar conclusion is reached when the results of Moran (private communication) for the freezing-point curves of both frog and beef muscle are compared with the calculated values of v. It should be pointed out that even if the total amount of unfrozen water at $-20°C$. (determined by Heiss) is

TABLE III

Weight of muscle = 100 gm.
Dry weight of muscle = 23 gm.

Temperature	Total water	Free water	Bound water
*C.	gm.	gm.	gm.
−1	77.0	70.3	6.7
−4.7	19.5	15.1	4.4
−11.6	11.4	6.6	4.8
−16.0	9.2	5.1	4.1
−21.9	7.8	4.0	3.8

considered as bound the value is considerably smaller than those given by Rubner or Thoenes.

The System Invariant Point

The temperature at which all the free water in frog's muscle is frozen out can only be estimated very roughly. From the vapour pressure isotherm it can be seen that the equilibrium weight of a muscle is practically constant below $a'' = 0.5$ (down to $a'' = 0.1$). It can be concluded therefore that only a negligible amount of free water is present over this range, *i.e.* the drying-up point of the muscle at 0°C., as regards free water, occurs in the region of $a'' = 0.5$. Substitution of $a'' = 0.5$ in (1) gives the system invariant point as $-69°C$. This temperature is too low as L_1 is not negligible at this activity of water. The insolubility of sodium chloride below $a'' = 0.798$ at 0°C. does not

allow the calculation of v from (5) for the case of $a'' = 0.5$, but a rough idea of the effect of the heat terms can be obtained by extrapolation.

If values of $(\log a_1'' - \log a_1')/\log a_1''$, obtained from (3) for solutions of sodium chloride down to $a'' = 0.798$, are plotted against log a_1'' an approximately straight line is obtained. From this graph the hypothetical value of a_1, when $a_1'' = 0.5$, is 0.553; this figure substituted in (1) gives $v = 59$. As the drying-up point at $0°C$. is not known at all accurately it can only be stated that the system invariant point as regards free water probably lies below $-40°C$. and above $-60°C$. It is interesting that Heiss (1933) obtained a value of $-62°$ to $-65°C$. for the system invariant point from the temperature-time cooling curve of beef muscle. The probable effect of such a low temperature and activity of water on the water of hydration of the proteins is unknown, but the presence of bound water in frog's muscle (at $0°C$.) at values of a'' well below 0.5 suggests that even lower temperatures would be required to freeze completely this water of hydration.

SUMMARY

1. The amount of free unfrozen water, *i.e.* water acting as normal solvent, in frog's muscle at temperatures below the initial freezing-point has been calculated from the vapour pressure isotherm of the muscle.

2. Significant amounts of free water are present at $-20°C$. The total amount of unfrozen water at $-20°C$. cannot, therefore, be taken as a measure of the bound water in muscle.

3. The calculated values of free water, when compared with experimentally determined values of total unfrozen water, indicate that the amount of bound water in muscle at various temperatures is small.

4. A temperature considerably below $-20°C$., roughly between $-40°$ and $-60°C$., is required to freeze completely the free water in muscle.

REFERENCES

Briggs, D. R., 1931, Colloid symposium monograph, New York, The Chemical Catalog Co., Inc., **9**, 367.

Brooks, J., 1933–34, *Proc. Roy. Soc. London, Series B*, **114**, 258.

Heiss, R., 1933, *Z. ges. Kälte-Ind.*, **40**, 97, 122, 144.

Hill, A. V., 1930, *Proc. Roy. Soc. London, Series B,* **106,** 477.

Lewis, G. N., and Randall, M., 1923, Thermodynamics, New York, McGraw-Hill
    Book Company, 85–95, 284, 349.

Moran, T., 1932, *Proc. Roy. Soc. London, Series A,* **135,** 411.

Rosenbohm, E., 1914, *Kolloidchem. Beihefte,* **6,** 177.

Rubner, M., 1922, *Abhandl. preuss. Akad. Wissensch., phys.-math. Kl.,* No. 1.

Thoenes, F., 1925, *Biochem. Z.,* Berlin, **157,** 174.

Young, T. F., 1933, *Chem. Rev.,* **13,** 103.