SOME CRYOSCOPIC AND OSMOTIC DATA.[1]

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Subsequent to the publication in 1905 of data on " The Osmotic Pressure of Sea Water and the Blood of Marine Animals, etc.," (1) the author has had occasion in the course of his other investigations, to make numerous determinations of the freezing point of various sea waters, solutions and bloods; this method having been used to check up other methods of obtaining solutions of known osmotic pressures. Some of the data thus acquired have been correlated, and although somewhat fragmentary, they are published in hopes that they may facilitate the work of other biologists.

The determinations have been made with the Beckmann apparatus and a differential thermometer, which could be read accurately to $0.005°$ C. When it is remembered that the depression of the freezing point (Δ) of a gram-molecular solution of a non-electrolyte is (theoretically at least) $1.85°$ C. below zero, that this depression corresponds with an osmotic pressure of 22.4 atmospheres (at $0°$ C.), and that the osmotic pressures vary directly with the depression of the freezing point, it is seen that the osmotic pressure of any solution may be calculated from the simple formula: osmotic pressure $= 22.4$ a. $\Delta/1.85$.

SEA WATERS.

Sea waters are not solutions of absolutely fixed chemical composition, nor have they a constant concentration. While the ratios of certain salts are quite constant, there are other variations such as the content of absorbed oxygen and carbon dioxide and even of the fixed carbonates. J. Loeb (2) has called attention to the fact that the free alkalinity, *i. e.*, the number of HO ions, is distinctly higher in the sea water at Woods Hole than at Pacific Grove.

[1] From the Physiological Laboratory of Washington University, St. Louis.

The figures for the depression of the freezing point (Δ) given in Table I. indicate the wide range in concentrations in sea waters of different localities.

TABLE I.

Sea Water from:	Δ — °C.	Observer.	Reference.
Naples...............	−2.29	Bottazzi	*Arch. ital. de biol.*, 1897, XXVIII., 61.
Arcachon............	−1.89	Rodier	Trav. des Lab. d'Archachon, 1899.
Pacific Grove, Cal.....	−1.925	Greene	Bull. U. S. Bureau Fisheries, 1904. XXIV., 429.
Pacific Grove, Cal.....	−1.90	Garrey	BIOL. BULL., 1905, VIII., 257.
Woods Hole..........	−1.81	"	BIOL. BULL., 1905, VIII., 257.
Beaufort, N. C........	−2.04	"	1911.
Helgoland............	−1.90	Dakin	Bio-Chem. Jour., 1908, 269.
In the Kattegat.......	−1.66	"	
Open Baltic Sea.......	−1.30	"	
Kiel harbor..........	−1.093	"	

In the following sections further details obtained by the author, by means of the cryoscopic method, are given for sea waters of some American localities.

(*a*) *Woods Hole.*—Determinations made during the summer of 1904 have been previously reported (*loc. cit.*, pp. 258–259) showing the freezing point to be slightly variable between − 1.805 and − 1.84° C. The average of determinations made the latter part of July of six different years gave an average Δ = − 1.81° C., with which, as will be seen from succeeding data, the following solutions are isosmotic: Sodium chloride, 0.52 *m*; Magnesium chloride, 0.29 *m.*, cane sugar, 0.73 *m.* "Van't Hoff's solution," made from *m*/2 stock solutions, had a freezing point of − 1.84° C., this is so slightly in excess of the concentration of Woods Hole sea water that it may be considered isosmotic with it. This "Van't Hoff's solution" was made up from half molecular solutions according to the formula given by J. Loeb, (3) viz: 100 molecules NaCl, 2.2 molecules KCl, 1.5 molecules $CaCl_2$, 7.8 molecules $MgCl_2$ and 3.8 molecules $MgSO_4$. The traces of bicarbonate and phosphate were omitted from the solution, but when added in optimum amounts (*e. g.*, 1 c.c. N/20 $NaHCO_3$ per 100 c.c. solution, as in the procedure of Loeb, p. 35), the solution becomes exactly isosmotic with Woods Hole sea water.

(*b*) *Pacific Grove.*—Green in 1904 made freezing point deter-

minations of the Pacific Grove sea water and found that $\Delta =$ — 1.924° C. Garrey in 1905 made determinations, obtaining a slightly lower value for Δ viz., — 1.905° C. On the basis of either of these figures, it is seen that the sea water in this locality is about 5 per cent. more concentrated than at Woods Hole and that a correction for this amount must be made if the osmotically equivalent solutions are to be calculated from the figures given in the previous section (a).

(c) *Beaufort, N. C.*—Working in the laboratories of the U. S. Bureau of Fisheries during the summer of 1911, the author made the following observations of the freezing point of sea water obtained at different localities in that vicinity and under different conditions as described in Table II.

TABLE II.

1911.	Beaufort, N. C.	$\Delta = °C.$	Remarks.
June 8	Open sea, outside "Sea Buoy"	—2.043	
" "	Wharf of U.S.F.C..........	—1.987	11:40 a.m.—tide low, N.E wind, previous showers.
.	Wharf of U.S.F.C..........	—2.015	4:00 p.m. tide high
July 19	Wharf of U.S.F.C..........	—2.038	
" "	Open sea, at "Sea Buoy".....	—2.03	
" 20	Wharf, U.S.F.C............	—2.07	9:15 a.m., tide low
" 25	Wharf, U.S.F.C............	—2.06	3:00 p.m., tide high
" "	Bogue Sound..............	—2.073	
" "	Wharf U.S.F.C.............	—2.079	Tide low, strong south wind
Sept. 6	Sea Buoy.................	—2.05	
" 8	Wharf, U.S.F.C............	—2.052	Tide low
" "	Newport River, at "Cross Rocks"................	—1.707	

From these figures it is seen that the open sea water off Beaufort has a $\Delta = - 2.04°$ C., and is 12 per cent. more concentrated than at Woods Hole.

(d) *Diluted Sea Water.*—The constant necessity for the use, in biological investigations on marine forms, of diluted sea water and corresponding concentrations of pure salts, has led the author to make the determinations found in Table III. Various dilutions of Woods Hole sea water were made and the freezing points determined. In most cases the densities at these dilutions have also been determined by the pycnometer method. The figures given in the table are all from actual determinations made

by the author; when not given they may be approximated by interpolation.[1]

<div align="center">TABLE III.</div>

Dilution:			$\Delta = °C.$	Densities of Sea Water Dilutions at 21.5° C. (Ref. H_2O at 21.5° C.)	NaCl with same Gms. in 100 cc. of Solution.
Woods Hole Sea Water c. cm.	+	Distilled Water c. cm.			
Undiluted		0	−1.81	1.02426	3.04
85 c.c.		15 c.c.	−1.54		2.6
75 "		25 "	−1.35		2.275
$66\frac{2}{3}$ "		$33\frac{1}{3}$ "	−1.20		2.00+
60 "		40 "	−1.09		1.81
50 "		50 "	−0.915	1.0123	1.58
45 "		55 "	−0.82		1.4
40 "		60 "	−0.73	1.0096	1.21
35 "		65 "	−0.64		1.07
$33\frac{1}{3}$ "		$66\frac{2}{3}$ "	−0.61	1.008	1.02
32 "		68 "	−0.595		1.00
30 "		70 "	−0.547	1.0073	0.91
25 "		75 "	−0.460	1.0062	0.76
20 "		80 "	−0.37	1.0046	0.60
10 "		90 "	−0.187	1.0023	0.30

<div align="center">CANE SUGAR.</div>

Attention should be directed to a fact to which Jones (4), Morse and Fraser and Berkeley and Hartley have called attention, viz., that cane sugar solutions show osmotic pressures considerably in excess of what theory would lead one to expect. Loeb has shown the importance of this fact for biological work (5). From purely theoretical considerations one would expect a molar (gram-molecular) solution to show an osmotic pressure only slightly in excess of that of Woods Hole sea water. Loeb found that it caused a shrinkage of the eggs of the echinoderms even of the Pacific, and his experiments caused him to select 6/8 m. cane sugar as the proper concentration for the development of Strongylocentrotus purpuratus. The osmotic pressure of Woods Hole sea water by calculation from the freezing point is 21.9 a. (at 0° C.), a figure which is almost identical with that obtained by

[1] The determinations of Gerlach for NaCl and KCl (*Chemiker-Kalender*, 1914, I., p. 261) and of Schiff for MgCl and CaCl₂ (*ibid.*, p. 265) show, that, for concentrations of solutions of the magnitudes with which we are dealing and in which these salts are present in sea water, the densities are a linear function of the concentration. A plat of our determinations shows the same to be true for both densities and freezing points of dilutions of sea water.

calculation for 0.75 gram molecular solutions of cane sugar, using the measurements of Berkeley and Hartley.[1] Some of our determinations of the freezing point of solutions of cane sugar illustrate their peculiar osmotic behavior.[2]

For a gram molecular solution of cane sugar (342.2 grams per liter *of solution*) we found $\Delta = - 2.775°$ C.; for ¾ mol. (256.6 grams per liter) $\Delta = - 1.855°$, $- 1.86°$ C.; and for ½ mol. (171.1 grams per liter) $\Delta = - 1.15°$, $- 1.155°$ C. For these three solutions the theoretical depression of the freezing point would be to $- 1.85°$, $- 1.387°$ and $- 0.925°$ C., respectively. Comparison of these figures shows how much in excess of the theoretical osmotic pressure, that of these solutions really is. Morse and Fraser have pointed out that the correspondence with the theoretical expectations is greater, if "weight normal" solutions are used, *i. e.*, if the substance is present in a liter of the solvent, instead of this volume of the solution. This does not account, however, for the full amount of the discrepancy found. To illustrate this: It was found in our experiments that in making a gram-molecular solution by dissolving 17.11 grams of cane sugar in 50 c.c. of the solution (15° C.), it was necessary to add only 39.4 c.c. of distilled water; Δ was $- 2.775°$ C. Had 50 c.c. of solvent been used to make the corresponding "weight normal" solution, Δ would have been $- 2.187°$ C.[3] This figure exceeds the theoretical Δ ($- 1.85°$ C.) by $0.337°$ C., which is probably to be accounted for by hydration of the sucrose molecule (Callendar (6)).

In the figures given above it is to be noted that the Δ of .75 mol. solution of sucrose ($- 1.855°$ C.) is that which theory expects of a gram molecular solution, and its osmotic pressure lies between that of the sea water at Woods Hole and Pacific Grove. By

[1] The measurements of Berkeley and Hartley were made using other concentrations. The original figures of these workers as also those of Morse and Fraser et al. are given in the " Physikalisch-chemische Tabellen," Landolt, Börnstein and Roth, 4th ed., Table 179, p. 787. Their original papers are referred to, *ibid.*, p. 790.

[2] The sugar used in these experiments was free of all reducing sugars and had been twice recrystalized from glass-distilled water with subsequent drying in vacuo.

[3] Calculation of the freezing point of a molecular "weight normal" solution, based upon Morse's figure for the observed osmotic pressure (24.8 a. —0° C.) gave a slightly lower figure, viz.: $\Delta = - 2.048°$ C.

extrapolation we obtain the following figures for the concentration of sucrose; isosmotic with sea water of:

1. Woods Hole = 0.73 m. ($\Delta = -$ 1.81° C.)
2. Pacific Grove = 0.765 m. ($\Delta = -$ 1.90° C.)
3. Beaufort   = 0.81 m. ($+$) ($\Delta = -$ 2.01° C.)

SALT SOLUTIONS.

(a) *Sodium Chloride.*—In addition to the freezing points of solutions given in Table III., the following have been deter-

TABLE IV.

NaCl, Molecular Concentration.	Made by	$\Delta = °$C.	Remarks.
0.65	L.	−2.255	
0.65	G. 13° C.	−2.35	
0.65	G. 22° C.	−2.50	Made in a flask standardized to 15° C.
0.60	T.	−2.11	
0.58	G.	−2.03	Isosmotic with Beaufort Sea water
0.54	G.	−1.90	Isosmotic with Pacific Grove Sea water
0.54	L.	−1.895	
0.52	G.	−1.81	Isosmotic with Woods Hole sea water.
0.50	L.	−1.735	
0.50	L.	−1.74 −	
0.50	G.	−1.745	
0.50	F.	−1.75	
0.50	L.	−1.765	
0.50	G.	−1.745	
0.444	G.	−1.54	

mined by the author on solutions made up by different competent workers. The concentrations chosen were somewhat

TABLE V.

MgCl$_2$ concentration.[1]	$\Delta = °$ C.	Remarks.
0.50 molecular	−2.845	
0.36 '	−2.03	Isosmotic with sea water at Beaufort.
0.35 '	−1.985	
0.31 "	−1.895	Isosmotic with sea water at Pacific Grove (or 0.32 m. according to Greene's determination).
0.30 "	−1.85	
0.29 "	−1.815	Isosmctic with sea water at Woods Hole.
0.10 "	−0.495	Dissolved in 100 c.c. of distilled water (not of solution).

[1] Concentration referred to volume of solution, not of solvent.

to either side of those isosmotic with the sea waters of our coast laboratories.

(b) *Magnesium Chloride.*—In solutions of this salt some of the molecules are dissociated into three ions, which accounts for the fact that the osmotic pressure is greater and consequently the depression of the freezing point is lower, than that of equimolecular solutions of sodium chloride. The following commonly employed solutions have been tested (cf. Table V.).

ANIMALS.

In addition to data previously published by the author (*loc. cit.*, p. 263), several determinations have been made on the blood of animals of the waters of the American coast, and inland rivers.

(a) *Limulus polyphemus.*—In the work referred to it was shown that under experimental conditions the blood of this animal, like that of other marine invertebrates varies to conform in concentration to that of the external medium. We have since found that this is true in the natural habitat of these animals, thus at Woods Hole (1904) the blood of Limuli depressed the freezing point like sea water, to − 1.82° C. At Beaufort, N. C., July 20, 1911, the water of the Fisheries "pound" froze at − 2.03° C. The blood of four Limuli taken from this water, in which they had been kept for several weeks, showed the following freezing points, respectively, − 2.025°, − 2.03°, − 2.04° and − 2.35° C. In the case of another *Limulus* captured at "Cross Rocks" in the Newport River, near Beaufort, September 8, 1911, the blood Δ = − 1.71° C. while the water at that place depressed the freezing point to − 1.707° C. Such readings taken from animals under natural conditions established the absolute identity of osmotic pressure of the external and internal media despite the differences in their composition.

(b) *Elasmobranchs.*—A shark seven feet in length (not identified) was captured in the Fish Commission nets at Beaufort, July 27, 1911; the sea water froze at − 2.02° C.; cryoscopic readings of the blood from the heart and portal vein, withdrawn immediately after death, were identical, within 0.01° C., and showed Δ to be − 2.182° C., which again is practically identical with that of the Beaufort sea water taken in the neighborhood of the fish trap on that date.

The Δ for this elasmobranch is larger than for those tested at Woods Hole by the author and later by Scott (7). The figures indicate an adjustment to the greater concentration of the sea water at Beaufort, a fact which is also borne out by determinations made on the blood of "sting rays," the blood of four of which at Beaufort gave $\Delta = -1.98°$, $-2.04°$, $-2.03°$, $-2.07°$ C., respectively. These depressions are not greater than that of the sea water from which the animals were taken although both the author and Scott found a slightly greater depression for the blood of the dog fish (*Mustelus canis*) of Woods Hole than for the sea water of the laboratories; this water is, however, somewhat less concentrated than the water outside the heads.

(c) *Marine turtles.*—The defibrinated blood of three species of marine turtles[1] caught at Beaufort in 1911 was frozen and the Δ thus determined for each individual is as follows:

Chelonia mydas $\Delta = -0.675°$ C.
Colpochelys kempi $\Delta = -0.687°$, $-0.70°$, $-0.70°$ C.
Caretta caretta $\Delta = -0.69°$, $-0.69°$, $-0.685°$ C.

In the cases of two carettas obtained at Woods Hole in 1913, the Δ found was identical with that given above. These depressions (Δ) are, in all cases, greater than those obtained by Bottazzi for "Thalassochelys caretta" ($\Delta = -0.61°$) although it is worthy of mention that the waters from which our animals were taken were, if one can judge from Bottazzi's writings, less concentrated than that from which his specimens were obtained; if any adjustment to aqueous media were to take place it would be in the direction opposite to that indicated by the above figure. It is certainly true, however, that the blood of fresh water and land turtles shows a depression of the freezing point which is distinctly less than that of marine turtles; Bottazzi found Δ for *Emys europa* $= -0.463°$ to $-0.485°$ C., while for *Pseudemys elegans* of the Mississippi Valley we obtained a depression in which $\Delta = -0.48°$ C. These figures are so much below those obtained with the blood of marine turtles that they would seem to indicate the possibility of some degree of adjustment to the concentration of the external medium; on the other hand it is a

[1] The author is indebted to Mr. Hay for the identification of these animals.

fact that the land turtles do not show a more concentrated blood than do those which live mainly in fresh water.

This point was put to the experimental test upon the marine turtles, *Colpochelys kempi* and *Caretta caretta*; the last figure in the previous data given above for each of these species was obtained, with the blood of a specimen which had been kept for two months in a tank containing fresh water. There was absolutely no change in the concentration of the blood of these individuals, and we feel justified in concluding that adjustments of the nature of those under consideration do not take place in these forms at least not within the duration of our experiments.

(*d*) *Fresh Water Fish.*—Preliminary to a study of the effects of osmotic and saline media upon fresh water fish (soon forthcoming) it was desirable to know the osmotic pressure of the blood of forms taken from the Mississippi river. The following list contains some forms peculiar to this region on which no data have hitherto been given.

1. *Polyodon spathula* $= -0.492°, -0.486°, -0.50°$ C.
2. *Scaphirhynchus platyrhynchus* $= -0.505°, -0.507°, -0.503°$ C.
3. *Lepidosteus osseous* (L.). ("Gar") $= -0.487°$ to $-0.52°$ C.
4. *Amia calva* (L.) (land locked) $= -0.508°$ C.
5. *Catostomus teres* $= -0.51°$ to $-0.52°$ C.
6. *Perca fluviatilis* $= -0.498°$ to $-0.51°$ C.

Fresh water ganoids are seen to have blood which is identical in concentration with that of fresh water teleosts. All have blood less concentrated than that of any of the marine fishes and it is conceivable that in the case of these animals some adjustment to environment has taken place; such adjustments are known for marine fish as has been shown by the author (1) and others (Frédericq, Bottazzi, Dekhuysen (8), Dakin, *loc. cit.*).

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Note.—For other literature consult the papers by Garrey[1] and by Scott;[7] also Bottazzi, Ergebnisse der Physiologie, 1908, VII., p. 162; cf. also Table I. of this paper.