THE COMPOSITION, ESPECIALLY THE HYDROGEN ION CONCENTRATION, OF SEA WATER IN RELATION TO MARINE ORGANISMS.

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Since the extensive analyses of Førchhammer and Dittmar, the relative concentration of the chief salts in sea water has been accurately known, the differences in the results of different investigators only slightly exceeding the differences in the values of the atomic weights that they used. Omitting regions affected by land drainage and the melting of the polar ice caps, ocean surface salinity (gm. of total salts per kilo) varies only from about 33 to 38, the most dilute region being the North Pacific. The known ocean currents affect only the surface layers, but there is probably a drift of the bottom water chiefly from the South and less from the North Polar regions toward the equator, which could take place at a depth of 4,000 meters without obstruction. The evidence for this is the fact that the depths of the oceans have a remarkably uniform salinity of nearly 35 and temperature of about 2° which is much above the temperature of maximum density, and are fairly well oxygenated. It seems safe to infer, therefore, that all surface water has comparatively recently come from the depths under the equator.

Dittmar showed that there is proportionately 0.44 per cent more CaCO₃ at the bottom than at the surface, due to the solution of pelagic shells by means of the CO₂ from organic decomposition. It is evident, therefore, that the CaCO₃ brought from the depths and from the land is precipitated in surface water, especially in the tropics. Drew supposes that this is not done entirely by the formation of shells and skeletons, but also by the action of denitrifying bacteria which increases the alkalinity of the water by removal of nitrates until the solubility product for CaCO₃ is exceeded. Palitzsch has shown that the alkalinity of the sea varies inversely with the depth, but this is probably largely the result of changes in CO₂ content due to the synthesis of organic matter at the surface and its decomposition in the depths, and less to the changes in nitrates, especially since nitrates and ammonia vary in the same direction. The effect of denitrifying bacteria is probably greatest where the sea is diluted with fresh water. I
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found that CaCO₃ began to precipitate on the glass when the $P_H (= - \log H \cdot$ concentration) was kept at 8.26, although the addition of CaCl₂ causes no precipitation.

In selecting a place to study sea water, the coast laboratories are to be avoided, owing to the drainage of the land. Therefore it was decided to go to Tortugas, Florida, where the water may be considered ocean water since it is a deep blue with an average salinity of 36 (35.23 to 36.09) (Dole) and is remarkably free from plankton or sediment. The scarcity of life is probably due to the lack of fixed nitrogen as I found 0.0085 mg. of NH₃ in a liter and hardly a trace of nitrates in 2 liters. Since Tortugas is about 500 miles from the mouth of the nearest large river, the accession of fixed nitrogen from the land is negligible, and its marine flora is dependent on that coming up from the depths or down with the rain, which escapes the action of the denitrifying bacteria.

The temperature of the Tortugas laboratory (which is practically in the sea) showed a diurnal rhythm of about 25-31°, and a general drift of about 2° during the season. Most of the experiments were done at a temperature of 29.75-30.25°. The temperature of the sea was not taken regularly, but it was always near 29°.

The special Leeds and Northrup potentiometer (reading to 0.5 millivolt), Weston cell (of the unsaturated type), and weights (used in making the solutions) had just been standardized by the United States Bureau of Standards. The calomel electrodes were made as follows: A round-bottomed 100 cc. cylinder, with a side neck attached near the top and bending down to the bottom, was supplied with a large platinum electrode fused in the bottom. Redistilled mercury, purified by shaking in a shaking machine for 30 minutes with each of several changes of 3 per cent nitric acid, was dissolved in nitric acid, and this mercurio-nitrate was used in making the mercury by electrolysis. The electrode was filled with the mercurio-nitrate and the side neck dipped into a vessel of mercurio-nitrate containing a platinum anode. A mercury anode was tried and discarded since I could not find a method of preventing the formation of a less soluble mercury salt on its surface. As much current was passed, through a resistance, from the 110 volt direct current circuit as was possible without undue heating of the side neck, until the platinum was plated over its whole surface with a thick layer of mercury, and mercury commenced to drop off of it in considerable amount. The KCl was recrystallized by solution in hot distilled water in fused quartz and cooling (and throwing away the mother liquor) five times, and dried 3 months over CaCl₂. The solution was made as needed by weighing out 7.456 gm. and dissolving it in
distilled water in a liter flask. A fresh portion of mercurio-nitrate was purified by electrolysis, redissolved, and precipitated with Baker's analyzed HCl, and the precipitated calomel washed by decantation several times with distilled water and many times with the \(0.1 \text{ N KCl}\), always being in contact with excess Hg which was finally shaken to form a gray mixture with the calomel, and washed into the electrode with KCl solution until the platinum was deeply covered. The end of the side neck was closed with an ungreased ground glass cap and passed through the rubber stopper of a cylinder filled with \(0.1 \text{ N KCl}\). The electrode was filled with \(0.1 \text{ N KCl}\) and closed by fusing the glass in a flame. The second cylinder was connected by means of a syphon closed with an ungreased ground glass cap to a third cylinder filled with a saturated solution of KCl. The electrode vessel and part of the side neck were painted black to prevent the reduction of the calomel by light.

The only course left for change of this electrode is change in average size of grain of calomel, due to the greater solubility of the smaller grains, but it seems to be a general opinion that such a process is very slow. The electrodes measured zero against one another when first made up and when they were periodically tested later. They gave the expected E. M. F. against electrodes of several other types. Since I aim at an absolute accuracy of 1 millivolt (=0.016 \(P_H\)), their drift of potential during the season cannot be considered serious.

The combined tonometer and hydrogen electrode and the improved hydrogen electrode previously described (McClendon and Magoon) were used, with the exception that the latter was made with the large compartment about 11.5 cc., owing to the fact that the buffer value of sea water is less than that of blood. After passing the hydrogen bubble over into the 1.5 cc. compartment the end cock (d) was opened before closing the middle cock (c) in order to restore the pressure that had been reduced by the solution of some hydrogen. Several forms of titration hydrogen electrodes were tried but the one shown in Fig. 1 is to be recommended, owing to the absence of rubber bulb, piston, or mercury funnel to suck up the liquid into the electrode. It is simply immersed in the liquid far enough so that when the cock is properly turned the liquid enters the electrode and drives some of the hydrogen out through the trap T. It is likewise preferable to other non-sucking titration electrodes in being more protected from diffusion of \(O_2\) from the fluid outside.

The hydrogen electrode proper in each of these instruments was made of a gold disc (welded to platinum wire) cleaned with a saturated solution of potassium bichromate in concentrated sul-
furic acid, washed, and platinized with 2 per cent platinic chloride containing a trace of basic lead acetate. It was not cleaned by electrolyzing \( H_2SO_4 \), since it was never used as anode and the possibility of the formation of \( Cl_2 \) was excluded. It was replatinized a few seconds after each dozen determinations. Since this process would finally make the platinum black too thick, the life of the electrode may be prolonged by the use of palladium, which can be dissolved off (Clark and Lubs; Ostwald and Lurther).

![Diagram of hydrogen electrode for titration]

**Fig. 1.** Hydrogen electrode for titration. Insert a drop of Hg and a copper wire in W. The bulb, D, is immersed in the fluid in a beaker and hydrogen is bubbled through it. The cock, C, is turned so that the bulb fills (by gravity) with fluid which drives hydrogen out at T. Hydrogen is bubbled through again and the fluid allowed to rise until it just touches the platinized gold disc (black), when the reading is taken.

The hydrogen was generated from zinc and \( H_2SO_4 \) and washed with (1) alkaline permanganate, (2) \( HgCl_2 \), and (3) alkaline pyrogallol, in all glass, stopperless wash bottles. The \( CO_2 \) was generated from marble and HCl and washed with NaHCO3 solution.

Owing to the high electrical resistance in the circuit caused by the 0.1 n KCl in the two ground joints in the calomel electrode (which were always closed) and the sea water in the ungreased stopcock of the hydrogen electrode, which was also closed, the
ordinary capillary electrometer was not sufficiently sensitive to read down to 0.5 millivolt. The capillary tube of the electrometer was drawn out in the flame to about 0.01 mm. bore and until the wall was thin enough (even after pasting a cover-glass on it with Canada balsam) to admit the use of an 8 mm. objective and very high ocular in the reading microscope. By bending back the bulb of the capillary electrometer it was made to fit on the stage of a microscope tilted back and held by rubber bands. In order to prevent shaking, the table legs were passed through the floor into the ground without touching the floor. The use of the capillary electrometer saves much time.

The millivolt readings after correction for H₂ density, according to Clark and Lubs, were converted into Pₙ values by means of the conversion table previously published (McClendon, 1916) which was extended sufficiently for the purpose. This change makes all the values in the table about 1 millivolt too low, and therefore 1 millivolt was arbitrarily subtracted from each of Clark and Lubs' corrections. Clark and Lubs find a different theoretical value for the E. M. F. of the normal hydrogen electrode against the 0.1 N calomel electrode from that used in making the conversion table. Since much of the older work was done with HCl, and since Ellis has shown that its curves for dissociation calculated (1) from electrode potential and (2) conductance ratio are different even at considerable dilution, the discrepancy may be due to the use of the conductance ratio to denote the dissociation of HCl at dilutions at which it differs from that calculated from electrode potential.

Since my solutions were near neutrality and the reading was taken immediately after making junction with saturated KCl solution, I do not think that the diffusion potential vitiates my results.

For preliminary tests a series of "nonsol" glass tubes (1 cm. bore) filled with Sörensen's phosphate mixtures plus phenolsulfonephthalein from Pₙ (= \(- \log H^+\) concentration) = 7-8, and a similar set of tubes filled with Sörensen's borate buffer mixtures plus thymolsulfonephthalein¹ (Lubs and Clark) from Pₙ = 8-10, and sealed in the flame, were used. The phosphate and borate mixtures were calibrated with the hydrogen electrode, but owing to the salt action on the indicator 0.3 must be subtracted from the Pₙ of sea water as determined with the tubes in order to obtain the same result as with the hydrogen electrode. Although the Pₙ of sea water was determined with the hydrogen electrode out to the second place of decimals

¹ For the convenience of Dr. Rowntree, Dr. A. G. Mayer, and others I have standardized solutions or samples in relation to the phenolsulfonephthalein and thymolsulfonephthalein tubes for determining hydrogen ion concentration kept in stock for sale by Hynson, Westcott and Co., Baltimore.
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(0.5 millivolt = 0.008 $P_n$), the estimation of the salt action on the indicator could not be carried this far because the tubes read only to the first place of decimals. Palitzsch defines the $P_n$ to the second place of decimals, but this must be an estimate since his borate mixtures record only to the first decimal place. The apparently remarkable coincidence that the correction for salt action (at 35 to 36 salinity) should be the same for phenolsulfonephthalein and thymolsulfonephthalein is probably due to the similar chemical constitution of these indicators. The advantage of these indicators over $\alpha$-naphtholphthalein and phenolphthalein is that each shows two strikingly different colors, and errors due to dye concentration cannot be hidden.

These tubes of thymolsulfonephthalein are to be especially recommended for determinations at sea in oceanographic work and all determinations of the $P_n$ of sea water in which an accuracy of 0.1 is sufficient. They were used by Dr. L. R. Cary to measure the CO$_2$ excreted by medusae (see also Haas). For a greater accuracy the tubes would have to be made larger or of extremely uniform bore. In making the small tubes the glass should be carefully selected. The bore should be 10 mm. = 0.35 mm. They should be filled with 3 cc. of the buffer mixture plus 0.2 cc. of 0.1 per cent thymolsulfonephthalein in 70 per cent alcohol, measured with a graduated pipette. A test-tube of 10 mm. bore should have an etched mark at 3.25 cc. for use with sea water, since the addition of the sea water after the indicator helps mix the latter. By retaining the 0.2 cc. pipette used in filling the tubes, the necessity of the calibration of one for making the tests is avoided.

The $P_n$ of Tortugas sea water (within 8 miles of Loggerhead Key or 82° 52' to 82° 58' W. and 24° 30' to 24° 38' N.) from the surface to a depth of 35 meters; as well as in the moat of Fort Jefferson, was found to vary from 8.1 to 8.22, which is about the average for ocean water (7.95 to 8.25, Palitzsch; $P_n$ of 8.06 just colors phenolphthalein, at 35 salinity). Since there was no general drift of $P_n$ during the season, it is probable that the limits of variation during the entire year are not very different. No relation between $P_n$ and location, time, or tide was found.

Since calcium is the only non-volatile base in sea water that has been shown to vary independently of the salinity, it is possible that variations in the calcium content due to the activity of corals and other organisms might affect the $P_n$ but this question must be reserved for future study. Since I found only 8.5 parts of ammonia per billion, this factor must be excluded. The effect of denitrification is probably small, since I found only minute traces of nitrates in the water. The amount of nitric acid brought down by the rain in the Barbados was estimated by Harrison and Williams at 2.75 kg. per hectare per annum, but this was accompanied by 1.13 kg. of ammonia, which would mean a mixture of NH$_3$NO$_3$ and NH$_4$CO$_3$. After
thunder showers over the sea this fixed nitrogen is probably taken up very quickly by algae and denitrifying bacteria. Although the evaporation is rapid, it would not be sufficient to concentrate the fixed nitrogen before it mixed with the sea to a great depth, since Dole could detect no immediate influence of rain on the surface salinity.

That variations in the amount of $H_3PO_4$ in the sea water could account for variations in the $P_H$ seems improbable, since I found about 4 mg. in 20 liters that had been acidified with HCl and evaporated to a small quantity. Although some phosphates may have been occluded in the salts crystallizing out, this fraction was probably small, owing to the strongly acid reaction which was constantly maintained during the evaporation.

Since dilution of the sea water must dilute the carbonates and bicarbonates responsible for its reaction, it would theoretically affect the $P_H$. Solutions of soda were aerated by drawing moist air through them vigorously for 72 hours in order to make their CO$_2$ tension equal that of the air. The $P_H$ of the 0.1 $N$ solution was 9.8; that of the 0.01 $N$, 9.26; and of the 0.001 $N$, 8.3. We thus see a marked change in the $P_H$ with dilution, but the effect of diluting a bicarbonate solution is much less if it is not aerated; we cannot say that the sea is thoroughly aerated, and, furthermore, the presence of neutral salts in the sea affects the dissociation of the alkali. In order more nearly to imitate the effect of rain falling in the sea, a portion of sea water of $P_H = 8.1$ was diluted with an equal volume of conductivity water of $P_H = \pm 6$, and the $P_H$ of the mixture was found to be about 8.09 in the hydrogen electrode. Perhaps if the CO$_2$ tension of the conductivity water had been exactly that of the air, a greater change would have been noted, but the experiment indicates that a dilution of the most concentrated Tortugas sea water (salinity = 36.09) to the most dilute (salinity = 35.23) would not account for the variation in $P_H$ (from 8.22 to 8.1).

There remains to be considered the change in CO$_2$ content and its effect on the $P_H$ of the sea water. No direct measurements of the CO$_2$ content of the sea water were made, but the change of $P_H$ with change of CO$_2$ tension is shown in Fig. 2. In making mixtures containing less than 1 per cent CO$_2$, a double dilution is necessary. For instance, in making 0.02 per cent CO$_2$ a 1 per cent mixture is made in the combined tonometer and hydrogen electrode as previously described (McClendon and Magoon), then the apparatus is shaken (without disconnect-
ing the mercury funnel) so as to mix the CO₂ thoroughly with the H₂. By raising the mercury funnel, all but 2 per cent (2 cc.) of the gas mixture is expelled, then hydrogen is admitted in the usual manner so that the resulting mixture contains 0.02 per cent CO₂.

![Graph showing the relationship between pH and total CO₂ content in sea water at 30°.](http://www.jbc.org)

Fig. 2. Table for converting pH of sea water (35 salinity) into CO₂ tension and total CO₂ content at 30°. The dotted line represents cc. of total CO₂ per liter.

CO₂. After shaking 1.5 cc. of sea water with 10 cc. of a gas mixture in the tonometer, the CO₂ content of the gas mixture may be perceptibly changed, so it was necessary, after determining
the $P_H$, to expel the gas mixture and make a new one like it, and repeat this process until a constant $P_H$ was reached.

The change in $P_H$ with a change in CO$_2$ tension is shown in the continuous line in Fig. 2. The dotted line shows the total CO$_2$ content extrapolated from data given by Fox, and is 40 cc. at 0.015 per cent CO$_2$ tension, 41.9 at 0.02 per cent, 44.5 at 0.03 per cent, 46.25 at 0.04 per cent, and 47.5 at 0.05 per cent.

Fig. 2 shows that within the normal limits for sea water a very small change in the CO$_2$ tension or CO$_2$ content causes a comparatively large change in the $P_H$, and the changes that have been observed in the $P_H$ of uncontaminated ocean water could probably be explained by the changes in CO$_2$ tension produced by the action of organisms. I found that a jellyfish pulsating in sea water in a closed vessel changed the $P_H$ from 8.2 to 8 in a very short time, indicating an increase of about 6.5 cc. of CO$_2$ per liter. Algae in the sunlight would change the $P_H$ to 8.25 in less time. It seems almost certain that the CO$_2$ tension is the chief factor in determining the $P_H$ of the oceans. If this is true, the water from a depth of 2,000 meters in the Atlantic, which Palitzsch found to have a $P_H$ of 7.95, should have a CO$_2$ tension of about 0.06 per cent at 30°, and Tortugas water should have a CO$_2$ tension of about 0.03 to 0.043 per cent. In one experiment water drawn directly from the sea into the electrode had a $P_H$ of 8.15. It was then passed into the tonometer and its $P_H$ at different CO$_2$ tensions determined, with the result that it had a $P_H$ of 8.15 at 0.04 per cent CO$_2$ tension, from which I conclude that this was the CO$_2$ tension of the sea where the sample was taken.

It would be interesting to know the CO$_2$ tension of the air at Tortugas to see how it compared with the sea. An attempt was made to determine this by bubbling air through the water until equilibrium was reached and then determining the $P_H$ of the water. The first experiments were valueless, owing to the assumption that equilibrium would be reached within a few hours. It was found necessary to pass a strong stream of moist air through a small quantity of sea water at least 15 hours in order to reach equilibrium, and during this time the temperature changed about 5° if the aeration was all done continuously. In one experiment starting with $P_H=8.1$, equilibrium was reached at the end of 15 hours' aeration with the final temperature 30°, and $P_H=8.16$. After 24 hours' more aeration (which was stopped at a lower temperature), the $P_H$ was about the same (8.15).
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We may assume, therefore, that the CO₂ tension of Tortugas air on that
day was about 0.033 per cent. Legendre found the air off the coast of
France to contain 0.022 to 0.087 per cent CO₂, and Lewy found ocean air
in the Antilles to contain from 0.038 to 0.053 per cent CO₂ (average 0.046
per cent). He found ocean air to average 0.053 per cent in the day and
0.035 per cent at night, presumably due to absorption of CO₂ by the water
when cooled at night.

Since the sea contains more than thirty times as much CO₂ as the air,
it must regulate the average concentration of this gas in the air. Krogh
supposes that CO₂ is being increased in the air by the combustion of coal
and hence the CO₂ tension of the air is greater than that of the sea. He
calculates the carbon in the air as 600 billion tons, and since the annual
consumption of coal is now more than a billion tons, the increase might
become measurable if it were not absorbed by the sea. Chamberlin sup-
poses that 440 million tons of carbon are withdrawn annually from the air
by the weathering of rocks.

If the CO₂ content of the Gulf Stream water remains approximately
constant as it flows northward, its CO₂ tension must decrease, owing to
the increase in the absorption coefficient for CO₂ and the decrease in the
dissociation of bicarbonates with fall in temperature. But if equilibrium
with the air is partially reached, the CO₂ content and H⁺ concentration
should be greater in the North, which seems to be the case. Palitzsch
found the P₇ in the Atlantic equal to 8.1 in the far North, and 8.25 nearer
the equator. If the sea locally affects the CO₂ content of the air, we might
expect northern air to be poor in CO₂, but Krogh found from 0.025 to 0.07
per cent in the air of Greenland, whereas the average for the world is taken
at 0.03 per cent. Benedict found, however, from 0.01 to 0.034 per cent
between Boston and Genoa, and 0.003 to 0.027 per cent between Montreal
and Liverpool, with the same apparatus. It would be easier to determine
whether CO₂ is being absorbed or given out by the sea by determining the
CO₂ tension of the sea and air simultaneously at the same place, as I hope
to do next year.

The sea water is so complex a mixture that it would be difficult
to apply the law of mass action to it, but owing to the remark-
able constancy in the relative amounts of the chief neutral salts
in it, we may say that the P₇ depends on the excess of non-vola-
tile base over acid and also on the CO₂ tension, at a given tem-
perature. This excess of base was found by Dole to be 0.00237
to 0.00257 N, but this determination seemed of sufficient impor-
tance to see whether the same result is obtained by other methods
of titration as follows: The titrations were made with 0.1 N HCl.
The sea water (100 cc.) was placed in a wide-mouth bottle covered
by a paraffined cardboard, perforated in three places for the in-
troduction of the burette tip, the hydrogen electrode used as
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an indicator, and a rubber tube of 1 mm. bore filled with saturated KCl solution and closed at the end with a wooden plug, for connection with the calomel electrode. After dropping in a small quantity of acid a vigorous stream of hydrogen was passed through the electrode into the sea water, which was allowed to fill the electrode and was blown out with hydrogen three times; it was then allowed to rise only far enough to touch the platinized disk, and the reading was taken. This process was repeated, and the curve of $P_h$ and cc. of acid was plotted. The angle of the curve was taken to denote the acid necessary to neutralize the excess of base over non-volatile acid. I found the excess base in sea water to be 0.0023 to 0.0025 N. Titrations with 0.05 N $H_2SO_4$ gave the same results, but the angle seemed not to be as sharp as with HCl. That the excess base had been neutralized at the angle in these titration curves was confirmed by adding the indicated amount of acid to 100 cc. of the same sample of sea water and determining the $P_h$ after expelling the $CO_2$ more or less completely. After bubbling air through for 12 hours, the $P_h$ was about the same as that of an NaCl solution that had been exposed to the air. After boiling it was nearly neutral, and after boiling down to half volume it was also about neutral, and the same was true after bubbling hydrogen through it for a long time. In no case was it alkaline. Artificial sea water made of absolutely neutral salts plus 24 cc. of 0.1 N $NaHCO_3$ to the liter reached a $P_h$ of 8.2 after aeration for about 6 hours, whereas if only 23 cc. were used the aeration could be continued for a slightly longer period without danger of making it too alkaline. Since artificial sea water containing only 23 cc. of the soda solution reacted to aeration more nearly like natural sea water, this amount of soda (added as bicarbonate) is recommended in making artificial sea water, which should be aerated sufficiently to bring the $P_h$ to about 8.15.

Although Herbst maintained the life of sea urchin eggs for some time in artificial sea water, and the experiments of many investigators have shown that the exact proportions of the salts are immaterial in many physiological experiments, Dr. A. G. Mayer informed me that no artificial sea water had been found that would maintain the jellyfish, Cassiopeia, in a normal condition. It pulsates intermittently (after the excitement has passed)
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when put into artificial sea water and continues to do so until removed or until death ensues. It seemed important, therefore, to attempt to make a more successful sea water in order to determine, if possible, the full physiological significance of this medium. The principal salts are so well known that errors in their concentration would not be looked for in locating the trouble. The minor constituents are probably more variable, and the older analyses not so reliable, but some more accurate analyses have been made by Raben, Matthews, and others. It is possible that organisms may suffer from lack of some of these minor constituents, and it is also possible that organisms may suffer from an excess of them when they occur as impurities in the principal salts or in the distilled water. These constituents are supposed to occur as follows, in parts per million:\textsuperscript{2}

\begin{tabular}{llll}
\text{NH}_3 & . & . & 0.0085-0.15 \\
Li & . & . & Trace. \\
Rb & . & . & 11-15(?) \\
Cs & . & . & Trace. \\
Sr & . & . & " \\
Ba & . & . & " \\
Mn & . & . & " \\
Zn & . & . & 0.002 \\
Fe & . & . & 0.9-3.0 \\
Co & . & . & Trace \\
Ni & . & . & " \\
Pb & . & . & 0.1(?) \\
Cu & . & . & 0.012(?) \\
Ag & . & . & 0.01-0.169 \\
Au & . & . & 0.005-0.085 \\
Ra & . & . & 0.00000017(?) \\
F & . & . & 0.3-0.8 \\
I & . & . & 0.2-1.9 \\
NO_4+NO_2 & . & . & 0.18-1.1 \\
PO_4 & . & . & 0.2-2.2 \\
SiO_2 & . & . & 0.2-1.4 \\
Al_2O_3 & . & . & 0.2(? ) \\
B & . & . & Trace. \\
As & . & . & 0.01-0.08 \\
\end{tabular}

If the purest reagents are used for the principal salts in making sea water, the mixture would be supposed to contain, in parts per million, about: 0.03 Al_2O_3; 0.3 SiO_2; 0.2 Fe; 0.05 I; traces of Ba and NH_3; traces of As and heavy metals; 0.001 NO_4; and 0.0003 PO_4. Warburg found enough Cu in reagents that had been crystallized in copper dishes to increase the oxidation greatly in sea urchin eggs. Warburg found further that the addition to sea water of 0.001 Ag or 0.002 Au or 0.0006 Cu (parts per million) increased oxidation in unfertilized sea urchin eggs about 600 per cent.

\textsuperscript{2} For references to the literature on this subject see the works of Clarke, Krümmel, Raben, Matthews, Roth, Murray and Hjort, Forchhammer, and Dittmar, quoted in the list of references at the end of this paper.
In order to test whether there might be enough heavy metals in "reagent," "analyzed," and "für Analyse" salts to affect organisms, sea water was made from them and compared with that made from further portions of the same salts after solution in water redistilled in quartz (since Locke found Cu in distilled water in a toxic concentration) and recrystallization in "pyrex" glass. The NaCl was precipitated by admitting HCl gas into its saturated solution. Instead of NaHCO₃, recrystallized Na₂CO₃ plus an excess of CO₂ was used. The results were equally favorable with the two sea waters. The addition of as much as five parts per million of PO₄ was detrimental to animals.

Since H₂O is the chief constituent of sea water, particular attention was paid to it. It was found that conductivity water made in Hulett's laboratory and sealed in pyrex flasks by fusing the glass accomplished no better results than Merck's distilled water. Pyrex glass contains arsenic, but very little should have dissolved in the water since the glass is extremely insoluble. It is said to contain no lead. The only positive result from experiments with different qualities of distilled water was that aeration improves it. The aeration necessary to bring the artificial sea water to the required Pₐ is not sufficient from other standpoints. It was not determined whether the beneficial effect of aeration was the addition of some element (O₂, for instance), or the elimination of gaseous impurities. Merck's distilled water was free from NH₃ but had a taste that was not lessened by redistillation in quartz. The conductivity water had this taste in a much smaller intensity, and it required 72 hours of vigorous aeration to reduce the taste (of a liter) of Merck's water to equal that of conductivity water. Since the conductivity water had purposely been kept from the air as much as possible, its lack of taste may have been due to the oxidation of all organic impurities. If this was so, the taste of Merck's water must have been due to some volatile substance other than NH₃ or CO₂.

In making the artificial sea water, the salts were made up in normal solutions (0.5 M of bivalent salts), and the following number of cc. were used to make a liter, according to an analysis of Tortugas sea water of salinity 35.49 (Clarke) or 35.41 (Dole). The proportions for isotonic solutions are also given. The
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Isotonic solution of NaHCO₃ is approximately isotonic after aeration, but it should be noted that these solutions cannot be exactly isotonic since mixing them affects the dissociations.

<table>
<thead>
<tr>
<th>Normal solutions.</th>
<th>Isotonic solutions.</th>
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<td>cc.</td>
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<tr>
<td>CaCl₂</td>
<td>0.5 M</td>
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<tr>
<td>MgCl₂</td>
<td>0.5 M</td>
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<tr>
<td>MgSO₄</td>
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<td>KCl</td>
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<td>NaCl</td>
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<td>NaBr</td>
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<td>NaHCO₃</td>
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<td>H₂O</td>
<td></td>
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<td></td>
<td>1,000.00</td>
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</tbody>
</table>

The mixture should be aerated until it has a Pₗ of about 8.15.

This artificial sea water was tested with all the delicate marine organisms available. The lagoon jellyfish, Cassiopeia xamachana, lived in it indefinitely and its pulsations were normal. Some died in 6 weeks if the water was allowed to evaporate, but the prevention of evaporation or the occasional addition of distilled water to restore the volume was all that was necessary to keep them alive (without food). Eggs of the Atlantic Palolo worm, Eunice fucata, developed in it and produced two pairs of setæ. Some were alive at an age of 13 days, although they had never been fed.

Many animals apparently live at a great depth in the sea where the water is cold, and when brought to the surface in warm regions die sooner or later, and very rapidly when brought into laboratories. Since these animals are considered to be the most delicate, I placed them in the artificial sea water. Sagitta lived 7 days, various pelagic medusae, ctenophores, and siphonophores lived about 12 hours when crowded in small vessels, and Salpa only a few hours under the same conditions. If the Pₗ was changed beyond the limits, 6 to 8.26, death of all of the animals occurred sooner.

The Pₗ of sea water is rapidly changed by the presence of animals or eggs, even in uncovered dishes. For this reason it seemed
of interest to determine the $P_N$ of the fluids inside invertebrates, which do not differ markedly from sea water in the salts they contain, and are often very poor in proteins. The $P_N$ of the body fluid of the sea urchin, *Toxopneustes (Lytechinus) variegatus,* was found to vary from about 7.7 to 7.8. The blood of the conch, *Strombus gigas,* was about 7.5.

Some salts may be considered nutritive, especially to plants. The growth of marine Diatoms, but not algae, was favored when I added 2 cc. of $\text{Ca(NO}_3\text{)}_2$ per liter. But most of the salts, or more correctly, ions in sea water, are to be considered protective rather than nutritive, especially to animals. Since the work of Ringer, O. Loew, Loeb, Mayer, and others, the idea that one ion protects the organism from the toxic action of another ion has constantly gained ground. I have shown that certain ions increase the permeability of sea urchin and fish eggs (McClendon, 1910, 1914; McClendon and Mitchell). Since these ions are present in sea water they must be antagonized by other ions in sea water. This work was greatly extended by Osterhout who showed that certain ions increase the permeability of plant cells and others inhibit their action. If the action of all ions is of this nature there must be only two classes of ions, those that increase permeability and those that inhibit this change. The work of Ringer, Mines, and others shows the antagonistic action of ions on the heart. In order to test this hypothesis of the two classes of ions the action of ions of sea water on the pulsations of Cassiopeia and the heart of the conch was studied. I had found the freezing point of a sample of Tortugas sea water in 1910 to be $-2.03^\circ$ when corrected for undercooling, which is the freezing point calculated from a salinity of 37, whereas that of salinity 35.41 is $-1.937^\circ$.

Isotonic solutions were made as follows:

$$A = 1.937^\circ: 0.565 \text{ n NaCl}, 0.577 \text{ n KCl}, 0.378 \text{ M CaCl}_2, 0.364 \text{ M MgCl}_2$$
$$A = 2.03^\circ: 0.59 \text{ n NaCl}, 0.6 \text{ n KCl}, 0.395 \text{ M CaCl}_2, 0.38 \text{ M MgCl}_2$$

Whole Cassiopeias when paralyzed by pure $\text{MgCl}_2$ solution begin to beat after the addition of a little KCl or more NaCl, but not by CaCl$_2$. When paralyzed by a pure CaCl$_2$ solution they may begin to beat if sufficient NaCl or KCl is added before this toxic

*$^a$ Krummel, page 241.
solution has had time to injure the animal severely. This action of Na is inhibited by an increase in the concentration of H\textsuperscript{+}. The rate of pulsation is above normal in pure NaCl solution, but is reduced (finally to zero) by increase in the H\textsuperscript{+} concentration. If the P\textsubscript{n} of sea water is changed beyond the limits, 7.5 to 8.25, the pulsation rate is finally reduced, but a long period may intervene before the pulsations respond unless the change in P\textsubscript{n} is great.

The pulsation rate is increased by the addition of KCl to sea water, and its action is inhibited by increase in H\textsuperscript{+}. If the Cassiopeia is paralyzed by a marked increase in the H\textsuperscript{+} concentration of sea water it may beat after the addition of KCl. When paralyzed by MgCl\textsubscript{2} or CaCl\textsubscript{2} it may be caused to beat by increasing the OH\textsuperscript{-} concentration.

The heart of the conch will beat if perfused with sea water. In these experiments the auricular wall was used to make connection with a glass reservoir, and a hydrostatic pressure of about 2 cm. of water was substituted for the auricular action; hence only the ventricular action was studied. It stops in systole if the P\textsubscript{n} of the sea water is changed to 9.7, and in diastole if the P\textsubscript{n} is 5.6. Sea water of P\textsubscript{n} = 8.26 deposits CaCO\textsubscript{3} on glass, and a precipitate occurs throughout the solution if the alkalinity is increased much further, but it may remain in a supersaturated condition, as regards CaCO\textsubscript{3} for some time. It is improbable that a precipitation of CaCO\textsubscript{3} occurs in the tissue before it appears generally, since the tissue is constantly reducing the alkalinity by the production of CO\textsubscript{2}. Within the limit of P\textsubscript{n} = 9.5, an increase of OH\textsuperscript{-} merely increases the rate of pulsation. The effect of the ions may be summarized: Increase in concentration of OH\textsuperscript{-}, Na\textsuperscript{+}, or K\textsuperscript{+} increases the rate and finally stops the heart in systole; whereas H\textsuperscript{+}, Mg\textsuperscript{2+}, and Ca\textsuperscript{2+} decrease the rate and may stop the ventricle in diastole. This action of Ca\textsuperscript{2+} is only seen when it is applied suddenly in great excess, since the discovery of Ringer that Ca\textsuperscript{2+} favors systolic contractions holds true for the conch ventricle. When placed in equal parts of sea water and isotonic CaCl\textsubscript{2} solution, the ventricle stops in diastole but soon shrinks up and cannot be revived. In the following tabulation, the numbers express the volumes of neutralized sea water (n. s. w.) to which one volume of isotonic chloride solution is
added, and the effect produced, followed by the effect of the addition of another solution.

4 n. s. w. + Na increases rate, + HCl to make 0.001 N, stops in diastole.
11 " + K " " " + HCl " " 0.001 N, " " "
4 " + Mg stops in diastole, + OH-, starts again.
1 " + Ca " " " then passes into rigor.

We may sum up these experiments on Cassiopeia and the conch ventricle in the light of electrical conductivit,y experiments on animals and plants by assuming that Na-, K-, and OH- increase the permeability of the plasma membrane and that Ca-, Mg-, and H- inhibit their action, thus causing decrease in permeability when the antagonistic ions are present. It has been shown that muscular contraction is accompanied by increase in permeability (McClendon, 1912) which may explain the fact that Na-, K-, and OH- favor systole, and Mg- and H- favor diastole, but the experiment must be performed in a certain way in order to show that Ca- may produce diastole. The anomaly that a small amount of Ca- favors the systolic contractions produced by a large excess of Na- may be explained by the assumption that the optimum ratio of each pair of antagonistic ions is different for each part of an organism and that more than one of these hypothetical parts are concerned in any one of the experiments. For instance, suppose the optimum ratios are as follows, Ca: Na = 1 : 100 for muscle, 1 : 50 for nerve fiber, 1 : 25 for motor end plate, 1 : 10 for nerve cell body, it is clear that no ratio of Ca to Na would be especially favorable for an action in which all of these structures take part. In sea water we have a summation of antagonisms in which Na-, K-, and OH- are combined against Ca-, Mg-, and H-, and the proof that it is favorable for all of these structures is that it works. It should be added that some of the minor constituents of sea water take part in these antagonisms, and that their effect is large in comparison to their concentration.

Since the statement was made by Mines that the heart of a species of Pecten would not beat in sea water unless it is neutralized or slightly acidified, it seems worth noting that this is not general even for molluses of this particular group. The heart of Pectenella will beat in normal, neutral, acid, or hyperalkaline sea water, and even in hyperalkaline NaCl solution.
Concentration of Ions in Sea Water

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THE COMPOSITION, ESPECIALLY THE HYDROGEN ION CONCENTRATION, OF SEA WATER IN RELATION TO MARINE ORGANISMS

J. F. McClendon


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