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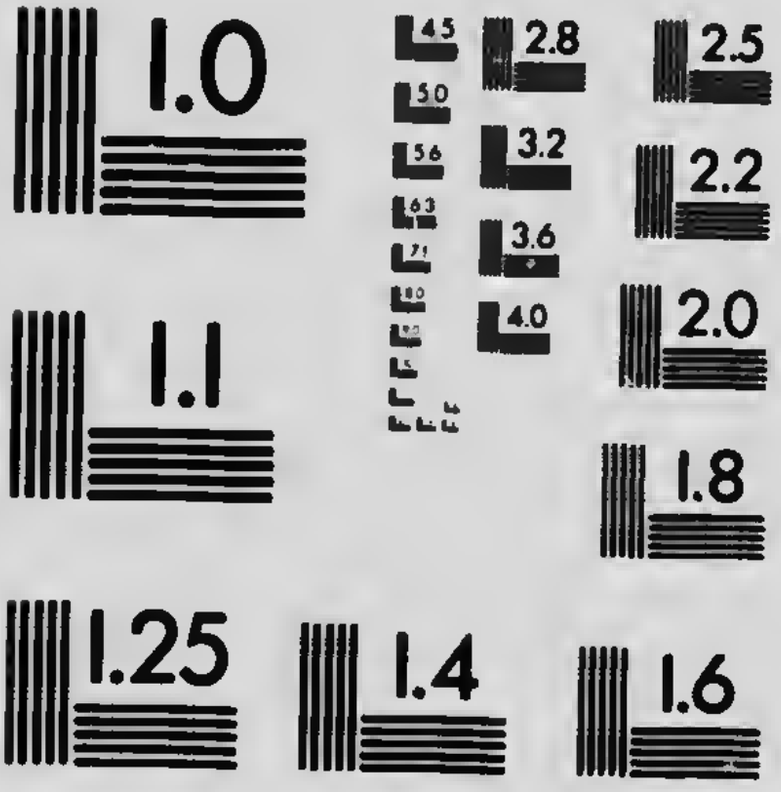


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# THE ALKALI RESERVE OF MARINE FISH AND INVERTEBRATES

## THE EXCRETION OF CARBON DIOXIDE

BY
J. B. COLLIP

(From the Marine Biological Station, Departure Bay, Canada)

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# THE ALKALI RESERVE OF MARINE FISH AND INVERTEBRATES.

## THE EXCRETION OF CARBON DIOXIDE.

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(*From the Marine Biological Station, Departure Bay, Canada.*)

(Received for publication, September 3, 1920.)

### INTRODUCTION.

The results of an investigation to determine the carbon dioxide content of the blood and body fluids of such marine forms as were available for study at or in the vicinity of the Marine Biological Station, Departure Bay, Vancouver Island, British Columbia, are herein reported. As the Van Slyke-Cullen (1) apparatus furnishes a convenient and ready means of determining the carbon dioxide content and capacity of blood and thereby the alkali reserve of the same, it was used exclusively in this investigation.

### *Methods.*

The representative forms studied were, for the most part, collected personally. Great care was taken to insure that the individual specimens, the blood or celomic fluid of which was to be examined, should be bled while in a fresh condition. As will appear more fully in a subsequent communication this is a very essential point especially as regards various molluscan types. An endeavor was made to secure specimens representative of as many of the invertebrate phyla and orders of the Pisces as was possible.

The methods employed in obtaining blood or celomic fluid from the forms studied will now be detailed.

*Cœlenterata.*

Two types of Medusæ and the sand anemone were examined. The results recorded for these specimens can only be taken as approximate since the methods here adopted were of a special character.

*Medusæ.*—Several specimens were caught in a dip net, transferred to a glass container filled with fresh sea water, and carried at once to the laboratory. They were first drained free of sea water by suspending over a wide funnel by means of a double fold of cheese-cloth. They were then gently macerated and passed through the cheese-cloth. The jelly-like mass was collected in a clean, dry test-tube. This was shaken vigorously for 3 minutes, air being admitted on several occasions. 1 cc. of the fluid was then transferred to the Van Slyke-Cullen apparatus and the analysis made in the usual manner. The evolved carbon dioxide was absorbed by the use of 10 per cent sodium hydroxide. The volume of gas was reduced to cubic centimeters of carbon dioxide at 0°C. and 760 mm. pressure held by 100 cc. of fluid.

Owing to the difficulties in the way of collecting blood or celomic fluid from marine forms without loss of carbon dioxide it was thought best to examine all specimens under uniform conditions. The various samples were therefore shaken for 3 minutes in the test-tube or 25 cc. Luer syringe with atmospheric air which was renewed on several occasions. The samples thus equilibrated with atmospheric air were submitted at once to analysis. In many instances concurrent samples were also equilibrated with alveolar air of the normal human subject after the manner suggested by Van Slyke and Cullen (2). Such specimens were then transferred by means of an Ostwald pipette to the apparatus for analysis without appreciable loss of carbon dioxide.

*Sea Anemones.* The specimen was dug from a sand flat at low water and carried to the laboratory in a container filled with fresh sea water. The animal was then allowed to contract in a dry open vessel. A slit was next made in the side by means of a sharp scalpel and the cavity drained free of the fluid. The soft parts were macerated in a mortar and finally suspended in an equal volume of fresh distilled water. 2 cc. of the suspension

were analyzed and the results interpreted as being approximately indicative of the carbon dioxide content of the tissues of the animal.

*Brachiopoda.*

The specimens were collected at low water from the rocks closely adjacent to the laboratory and examined immediately. It was found that from 1 to 2 cc. of fluid could be obtained from these small forms by aspirating into a Luer syringe from the celomic cavity through a needle passed through the soft tissue exposed at the hinge.

*Echinodermata.*

*Starfish.*—These were collected near the laboratory at low water and examined immediately. The celomic fluid was aspirated from the celomic pouch of one of the arms. This was entered dorsally and laterally.

*Sea Urchins.*—The celomic fluid was aspirated from a celomic pouch in much the same manner as in the case of the starfish.

*Arthropoda.*

*Crustacea.*—Several species of Crustacea were studied. Blood was obtained from the individual specimens by introducing a hypodermic needle into the pericardium and aspirating into a Luer syringe. In the case of the larger forms such as *Echidnocerus formatus* and *Cancer magister* a small hole was first trephined through the carapace just dorsal to the pericardium. The needle was then introduced through this opening. It was found that the needle could be passed directly through the carapace of small crabs and shrimps. Blood was obtained from the pericardial sinus of the eagle barnacle (*Balanus aquilla*) after a small hole had been drilled through the calcareous shell just dorsal to the sinus.

*Mollusca.*

*Pelecypoda.*—Many forms representative of different species of the pelecypod Mollusca were examined. The celomic fluid or "clam juice" was obtained in all instances by aspirating from the

pericardial cavity. The needle was introduced into the sinus through the soft tissue in the mid-line dorsal to the latter.

*Gastropoda.*—Four different species were obtained for study. Celomic fluid was obtained from the pericardial sinus of the two shell forms *Polynices* (Lunatia) *lewisii* and *Thais lamellosa*, after a small opening had been drilled in the shell adjacent to the sinus. The fluid exuded from the foot of *Polynices lewisii* was also analyzed. A direct puncture in the dorsal region allowed the aspiration of body fluid from the nudibranch *Anisodoris*.

*Amphineura.*—The large form *Cryptochiton* was the only representative of this class examined. Fluid was obtained from the pericardial cavity by aspirating through a needle introduced into the latter between two adjacent valves.

*Cyclostomata.*

The lamprey *Entosphenus tridentatus* was obtained while ascending a creek to spawn. Blood was obtained by severing the dorsal aorta posterior to the last gill slit. The blood was oxalated as collected.

*Pisces.*

Blood was collected from various specimens by severing the caudal vessels. It was oxalated as obtained and examined at once.

*Reptilia.*

Two garter snakes were caught while feeding at the water's edge. They were bled from the caudal artery. The analysis of this blood was made for comparative purposes only.

## RESULTS AND DISCUSSION.

The results of the analyses of the various samples are shown in Table I.

As might be anticipated the carbon dioxide content of the blood or celomic fluid of marine forms is relatively very low as compared with the blood of mammals. The carbon dioxide content of sea water in the region from which the specimens were

secured is of a widely varying quantity. The influx of a large volume of fresh water emanating from the water-sheds of the coast ranges, the varying effects of tides, winds, and currrents all contribute towards a constantly changing density of the sea water in this vicinity. In Table II is shown the density corrected to 15°C. of sea water collected daily from the surface off the landing stage of the Marine Station, Departure Bay, for a consecutive period of 33 days, also the density of depth samples taken as indicated by means of the Nansen water bottle. There are also shown the temperature and the alkalinity of the various samples in terms of cubic centimeters of 0.01 N sodium hydroxide required just to discharge the pink color imparted to 100 cc. of sea water by phenolphthalein and the buffer value of Sörensen (3) or the reactivity of Moore and Wilson (4). This latter factor is the amount of 0.01 N acid required to carry 100 cc. of sea water from the phenolphthalein to the methyl orange point. Moore, Prideaux, and Herdman (5) found that the reactivity of sea water taken at Port Erin did not show seasonal variation. It is evident, however, as the titration figures indicate that the buffer value of sea water in the vicinity of Departure Bay varies directly as the density. Moore and coworkers (5) state that this buffer effect is due in the main to dissolved magnesium bicarbonate and not calcium bicarbonate as usually stated. The slight variation of the alkalinity of the water from day to day is of interest. It is probably associated, as Moore, Prideaux, and Herdman (5) have pointed out, with the varying intensity of photosynthesis by microscopic organisms. Surface and depth tows made daily during the summer months by Mounce[1] showed great variation in the relative amounts, and in the distribution of diatoms. The falling off of the degree of alkalinity of sea water with increasing depth is suggestive in this connection. Sörensen (3) found that deep sea water was less alkaline than that at the surface. The total carbon dioxide content of surface samples of sea water obtained in Departure Bay during the summer of 1920 was found to vary between 2 and 4 volumes per cent, the amount varying directly with the specific gravity. It is therefore of interest to find that the amount of carbon dioxide in the blood or

[1] Unpublished results quoted by courtesy of Miss Irene Mounce.

TABLE I.

| Specimen. | $CO_2$ content of 100 cc. of fluid equilibrated with atmospheric air. | $CO_2$ content of 100 cc. of fluid equilibrated with alveolar air. | Remarks. |
|---|---|---|---|
| | *cc.* | *cc.* | |
| Jellyfish. | | | |
| 10 *Phyllidium*..... | 4.6 | | Specimen drained, then passed through cheese-cloth. |
| 3 *Aurelia flavidula*..... | 5.1 | | " " " " " " |
| Sea anemone. | | | |
| *Holocampa* (sand anemone)..... | 6.7 | | Specimen drained, macerated, and mixed with an equal volume of distilled water. Reading × 2. |
| Brachiopod. | | | |
| *Terebretella transversa*..... | 6.8 | | Obtained at low water off rocks of Jesse Island. |
| Starfish. | | | |
| *Pisaster ochracea*..... | 6.0 | | " " shore of Departure Bay. |
| " " ..... | 6.0 | | " " " |
| " " ..... | 5.0 | | " " " |
| " " ..... | 4.2 | | " " " |
| " " ..... | 4.1 | 9.8 | " " " |
| " " ..... | 6.5 | | " " " |
| *Dermasterias imbricata*..... | 5.5 | | " " " |
| " " ..... | 5.5 | 8.8 | " " " |
| *Evasterias troschelii*..... | 6.0 | | " " " |
| Sea urchin. | | | |
| *Strongylocentrotus drobachiensis*..... | 10.2 | | Obtained at low water off shore of Departure Bay. |
| " " ..... | 10.2 | | " " " |
| " " ..... | 7.4 | | " " " |

| | | | |
|---|---|---|---|
| Crustacea. | | | |
| *Cancer magister* | 24.0 | | Obtained at low water off shore of Departure Bay. |
| " " | 18.0 | | " " " |
| " " | 27.9 | | " " " |
| " " | 18.4 | | " " " |
| " *productus* | 20.3 | | " " " |
| " " | 23.0 | | " " " |
| " " | 24.2 | | " " " |
| *Echidnocerus formatus* | 20.0 | | Obtained in cod nets off Nanoose Bay. |
| " " | 22.5 | 28.0 | " " " |
| *Hemigrapsus nudus* | 24.2 | | Obtained off shore of Departure Bay. |
| *Epialtus productus* | 10.2 | | " near " " " |
| *Upogebia pugettensis* (sand shrimp) | 10.2 | | " in sand at low water in Departure Bay. |
| *Balanus aquilla* | 11.2 | | " off reef " False Narrows. |
| Mollusca. | | | |
| *Schizothoerus nuttalli* | 8.1 | | Obtained at low water from sand at Protection Gap. |
| " " | 8.3 | | " " " " |
| " " | 9.9 | 14.4 | " " " " |
| 2 " " | 9.0 | | " " " " |
| *Saxidomus gigantea* | 8.1 | | Obtained at low water from the shore at Departure Bay. |
| " " | 10.2 | | " " " " |
| 6 " " | 8.5 | 14.0 | " " " " |
| 15 " " | 7.8 | | " " " " |
| *Paphia staminea* | 27.9 | | " " " " |
| " " | 27.0 | | " " " " |
| 8 " " | 29.0 | | " " " " |
| 11 " " | 12.8 | | " " " " |

TABLE I—*Continued.*

| Specimen. | $CO_2$ content of 100 cc. of fluid equilibrated with atmospheric air. | $CO_2$ content of 100 cc. of fluid equilibrated with alveolar air. | Remarks. |
|---|---|---|---|
| Mollusca—*Continued.* | *cc.* | *cc.* | |
| *Mya arenaria* | 10.2 | | Obtained at low water from the shore at Hammond Bay. |
| " " | 8.3 | | " " " " |
| 14 " " | 8.0 | 13.0 | " " " " |
| " " | 8.3 | | " " " " |
| 10 " " | 6.5 | 13.0 | Kept in running sea water several days. |
| 12 " " | 6.5 | | " " " |
| 11 " " | 6.5 | | " " " |
| " " | 6.5 | | Bled as dug at Protection Gap. |
| *Macoma secta* | 11.2 | | Obtained at low water from shore, Hammond Bay. |
| *Entodesma saxicola* | 9.1 | | Obtained at low water off reef, False Narrows. |
| *Penitella penita* | 22.8 | | " " " " " " " " |
| *Mytilus edulis* | 9.0 | | Obtained at low water from shore at Departure Bay. |
| " " | 8.1 | | " " " " |
| " " | 9.9 | | " " " " |
| *Modiola modiolus* | 11.0 | | Obtained at low water from Protection Gap. |
| *Cryptochiton* | 6.8 | | " off False Narrows Reef at low water. |
| " | 9.0 | | " from Jesse Island at low water. |
| *Cardium corbis* | 6.4 | | " at low water off Departure Bay. |
| " " | 7.9 | | " " " " " " " |
| 6 " " | 7.5 | 15.0 | " " " " " " " |
| 5 " " | 8.4 | | " " " " " Protection Gap. |
| 5 " " | 9.3 | | " " " " " " " |

| | | | |
|---|---|---|---|
| **Mollusca**—*Concluded.* | | | |
| *Anisodoris nobilis* | 7.4 | | Obtained at low water off Jesse Island. |
| " *troschelii* | 7.0 | | " " " " " " " |
| *Polynices* (Lunatia) *lewisii* | 21.5 | | " " " " " Protection Gap. |
| " " " | 18.1 | | " " " " " Departure Bay. |
| " " " | 18.6 | | " " " " " " " |
| " " " | 19.5 | | " " " " " " " |
| " " " | 17.5 | | " " " " " " " |
| " " " | 12.5 | | " " " " " Protection Gap. |
| " " " | 21.0 | | " " " " " Departure Bay. |
| Fluid from foot of above | 16.5 | | |
| *Polynices* (Lunatia) *lewisii* | 20.7 | | " " " " " " " |
| Fluid from foot of above | 17.1 | | |
| *Polynices* (Lunatia) *lewisii* | 17.1 | | " " " " " " " |
| Fluid from foot of above | 17.1 | | |
| *Thais lamellosa* | 18.5 | | " " " " " Jesse Island. |
| *Puncturella* | 13.5 | | " " " " " reef at False Narrows. |
| **Lamprey.** | | | |
| *Entosphenus tridentatus* | 9.4 | | Plasma. Obtained in mill creek, Nanaimo. |
| **Dogfish.** | | | |
| *Squalus sucklii* | 5.6 | 13.5 | " " " " nets, Nanoose Bay. |
| " " | 5.6 | | " " " " " " |
| " " | 5.6 | | Whole blood " " " " " |
| " " (dead) | 3.7 | | " " " " " " " |
| **Shark** (mud shark, weight 800 lbs.) | 9.3 | 16.2 | Plasma. Obtained in net, Nanoose Bay. |

TABLE I—*Concluded.*

| Specimen. | $CO_2$ content of 100 cc. of fluid equilibrated with atmospheric air. | $CO_2$ content of 100 cc. of fluid equilibrated with alveolar air. | Remarks. |
|---|---|---|---|
| Ratfish. | *cc.* | *cc.* | |
| 3 *Hydrolagus colliei* (sluggish)..... | 3.7 | 11.2 | Whole blood. Obtained in nets, Nanoose Bay. |
| " " " ..... | 4.8 | | Plasma. " " " " " |
| Teleosts. | | | |
| *Enophrys bison*..... | 12.6 | | Whole blood. Obtained from Departure Bay. |
| " " ..... | | 24.0 | " " " " " " |
| " " ..... | 12.6 | | " " " " " " |
| " " ..... | | 19.6 | " " " " " " |
| " " ..... | 11.4 | | " " " " " " |
| *Anoplarchus atropurpureus* (sluggish)..... | 7.0 | | " " " at reef, False Narrows. |
| *Phanerodon furcatus*..... | 10.8 | | " " " " Protection Gap. |
| 2 *Porichtys notatus*..... | 10.8 | | " " " " " " |
| *Sebastodes* (sluggish)..... | 8.3 | | " " " from Nanoose Bay. |
| " ..... | 10.2 | | " " " " Departure Bay. |
| " ..... | 11.2 | | Plasma. " " " " |
| 3 *Clupea harengus*..... | 11.2 | | " " " " " |
| *Platichthys stelatus*..... | 10.2 | | " " " " " |
| Garter snake. | | | |
| *Thamnophis*..... | | 45.0 | Whole blood. Obtained at Hammond Bay. |
| " ..... | 35.0 | | " " " " Departure Bay. |

TABLE II.

| Date. | Source of sample. | Temperature. | Density at 5°C. | Alkalinity. | Reactivity. |
|---|---|---|---|---|---|
| 1920 | | °C. | | cc. 0.01 N NaOH | cc. 0.01 N $H_2SO_4$ |
| June 27 | Off landing stage at station. | 15.9 | 1.0178 | 1.0 | 17.5 |
| " 28 | " | 17.6 | 1.0146 | 1.1 | 15.0 |
| " 29 | " | 19.5 | 1.0140 | 1.3 | 13.8 |
| " 30 | " | 18.0 | 1.0159 | 1.0 | 15.7 |
| July 1 | " | 18.2 | 1.0161 | 0.7 | 14.8 |
| " 2 | " | 18.0 | 1.0167 | 1.3 | 15.1 |
| " 3 | " | 17.6 | 1.0186 | 1.2 | 16.5 |
| " 4 | " | 17.6 | 1.0181 | 1.4 | 13.9 |
| " 5 | " | 17.3 | 1.0187 | 1.7 | 15.6 |
| " 6 | " | 18.0 | 1.0196 | 1.5 | 17.5 |
| " 7 | " | 18.8 | 1.0198 | 1.6 | 17.4 |
| " 8 | " | 20.5 | 1.0112 | 1.3 | 13.0 |
| " 9 | " | 20.5 | 1.0122 | 1.2 | 13.5 |
| " 10 | " | 19.5 | 1.0133 | 1.4 | 13.8 |
| " 11 | " | 17.9 | 1.0187 | 1.0 | 17.0 |
| " 12 | " | 15.1 | 1.0194 | 1.5 | 17.5 |
| " 13 | " | 14.7 | 1.0208 | 0.4 | 19.0 |
| " 14 | " | 17.2 | 1.0094 | 0.5 | 12.0 |
| " 15 | " | 19.7 | 1.0108 | 0.7 | 12.5 |
| " 16 | " | 19.9 | 1.0097 | 1.0 | 12.3 |
| " 17 | " | 21.0 | 1.0133 | 0.7 | 13.6 |
| " 18 | " | 19.9 | 1.0142 | 1.0 | 13.5 |
| " 19 | " | 17.0 | 1.0187 | 0.7 | 17.2 |
| " 20 | " | 17.3 | 1.0199 | 1.7 | 16.5 |
| " 21 | " | 17.1 | 1.0192 | 1.3 | 16.7 |
| " 22 | " | 18.5 | 1.0088 | 0.3 | 11.3 |
| " 23 | " | 17.4 | 1.0114 | 1.3 | 13.0 |
| " 24 | " | 16.1 | 1.0150 | 1.5 | 14.7 |
| " 25 | " | 17.0 | 1.0142 | 1.5 | 14.7 |
| " 26 | " | 18.2 | 1.0119 | 1.3 | 13.5 |
| " 27 | " | 19.3 | 1.0115 | 1.0 | 13.0 |
| " 28 | " | 17.8 | 1.0115 | 1.0 | 13.0 |
| " 29 | " | 18.7 | 1.0120 | 1.0 | 13.7 |
| " 5 | Surface sample off Five Fingers. | 17.2 | 1.0203 | 1.6 | 19.0 |
| " 5 | At 5 fathoms off Five Fingers. | 15.4 | .0215 | 1.5 | 19.0 |
| " 5 | At 10 fathoms off Five Fingers. | 10.00 | 1.0225 | 0.2 | 20.5 |
| " 5 | At 20 fathoms off Five Fingers. | 8.98 | 1.0252 | 0.1 | 20.8 |

TABLE II—*Concluded.*

| Date. | Source of sample. | Temperature. | Density at 15°C. | Alkalinity. | Reactivity. |
|---|---|---|---|---|---|
| 1920 | | °C. | | cc. 0.01 N NaOH | cc. 0.01 N $H_2SO_4$ |
| July 5 | At 50 fathoms off Five Fingers. | 7.92 | 1.0267 | ... | 21.0 |
| " 5 | At 100 fathoms off Five Fingers. | 8.01 | 1.0267 | −0.1 | 21.0 |
| " 12 | At 1 fathom off Departure Bay. | 14.3 | 1.0214 | 2.0 | 18.5 |
| " 12 | At 2 fathoms off Departure Bay. | | 1.0217 | 1.5 | 18.6 |
| " 12 | At 3 fathoms off Departure Bay. | | 1.0220 | 1.4 | 18.5 |
| " 12 | At 5 fathoms off Departure Bay. | | 1.0222 | 1.0 | 18.6 |
| " 12 | At 10 fathoms off Departure Bay. | | 1.0233 | −0.3 | 21.8 |
| " 12 | At 20 fathoms off Departure Bay. | | 1.0255 | −1.4 | 24.0 |

celomic fluid of the various marine forms investigated is invariably greater than the maximum value for total carbon dioxide in sea water. The amount of combined carbon dioxide in the body tissues of Medusæ and sea anemones, the celomic fluid of brachiopods, starfish, certain sea urchins, and a number of mollusks and the blood of the dogfish and the ratfish is relatively low, but in all instances is higher than that in sea water. Certain types of Crustacea have a relatively high carbon dioxide content, such forms as *Cancer magister*, *Cancer productus*, *Echidnocerus formatus*, and *Hemigrapsus nudus* being included in this group. The kelp crab *Epialtus productus*, the sand shrimp *Upogebia pugettensis*, and the eagle barnacle *Balanus aquilla* are on the contrary comparatively low in the fixed carbon dioxide in the celomic fluid but they are in this respect somewhat analogous with the teleosts examined.

A somewhat similar phenomenon exists in the case of the Mollusca. While the majority of the forms studied have a carbon dioxide factor falling within the range between 6.3 and 11 volumes per cent certain species such as *Paphia staminea*, and *Penitella penita* of the pelecypod type and *Polynices lewisii*

and *Thais lamellosa* of the gastropod class have a fixed carbon dioxide content which in nearly all instances is considerably higher than that observed in the former group.

As pointed out earlier in the paper it is essential that the blood be obtained while the animal is perfectly fresh. For instance it was noted that specimens of *Mya arenaria* bled immediately after they had been dug gave a fixed carbon dioxide factor of 6.5 volumes per cent while other specimens carried to the laboratory in fresh sea water gave a factor of 8.3 volumes per cent for combined carbon dioxide. The fixed carbon dioxide of the mollusk tends to rise rapidly when the animal is not kept in a large volume of fresh water while the opposite effect was noted in the case of the fish examined. Exposure to air causes the carbon dioxide content to fall while in the dead fish the latter may be near to that of sea water. If a fish is allowed to remain hooked but left in the open water for some little time the carbon dioxide content of the blood falls. The fish are in their reaction to injury much like the mammals as far as the alkali reserve of the blood is concerned.

The relatively low figure for carbon dioxide in the blood of the elasmobranch *Squalus sucklii* and the holocephalan *Hydrolagus colliei* stands in sharp contrast with that for the teleostian types studied.

As the hydrogen ion concentration of sea water is in most instances lower than that obtaining in the blood of marine forms and as the bicarbonate content of the latter is much higher than that of the former it is evident that the amount of the dissolved carbon dioxide in the blood or body fluids of marine forms must be considerably greater than that occurring in sea water. The tension of carbon dioxide in the blood of marine forms must also be proportionately higher than that in sea water. This brings up an interesting point. Does a process of simple diffusion furnish an adequate explanation of the mode of elimination of carbon dioxide from the blood or body fluids of marine forms to the surrounding sea water? The maintenance of a definite acid-base balance in the blood and tissues of marine forms is no doubt quite as prime an essential as in the case of higher forms. In the mammal this is largely effected by the concerted action of the respiratory and renal apparatus. The gaseous exchange

between the blood and the atmosphere takes place almost entirely in the alveolar sacks where the tensions of the gases concerned are such that a process of physical diffusions seems to furnish a sufficient explanation for the passage of the oxygen inward and of the carbon dioxide outward (6). In the case of the fish conditions are somewhat different. While the respiratory and renal apparatus here as in mammals is apparently closely associated with the regulation of the reaction of the blood yet the interchange of gases between the blood and sea water taking place as it does largely through the medium of the gill filaments is on a somewhat different basis from the exchange in the mammal at the lung surface. The tension of dissolved gases in sea water to which the gill filaments are exposed cannot differ greatly from the tensions obtaining in the enveloping medium, unless it is possible that the respiratory movements are so adjusted that the tension of gases in the water of the gill cavities is kept in equilibrium with the tension of gases in the blood, in which case a process of physical diffusion would be a sufficient explanation of the mode of gaseous exchange in the fish. That this latter condition should hold seems plausible. It may also be possible that the permeability of the gill membranes to carbon dioxide is such as to allow a steep pressure gradient to exist between the dissolved carbon dioxide in the blood on the one side, and in the sea water on the other. Further experiment only can furnish a full explanation of this phenomenon.

In the case of arthropod types, such as *Cancer magister, Echidnocerus formatus* and others, the combined carbon dioxide is at a much higher concentration than it is in any of the Pisces examined. A relatively greater difference must therefore exist between the tension of carbon dioxide in the blood of these forms and sea water than in the case of the Teleostei. If one is to explain the mode of carbon dioxide excretion in these forms by a process of physical diffusion one must assume that the permeability of the gill filaments to carbon dioxide is of a very low order allowing a very steep pressure gradient to be maintained between the two sides of the medium for gaseous exchange. Certain of the Mollusca have a relatively high concentration of bicarbonate in the body fluids but it may possibly be due to the anatomical features occurring here for the carbon dioxide in the sea water

which is bathing the organisms to exist at a higher tension than in the open sea.

Such a difference does not exist between the bicarbonate content of the body fluids and tissues of Echinodermata, Brachipoda, and Cœlenterata, and that of sea water as has been noted in the forms above mentioned. The existence, however, of a definite pressure gradient for carbon dioxide between the tissue and the sea water suggests that a definite mechanism exists for regulating the tension of this gas in the body fluids and tissues.

SUMMARY.

1. The carbon dioxide content of the blood and the celomic fluids of various marine forms has been determined.

2. The carbon dioxide content of the blood and celomic fluid of marine forms examined equilibrated with atmospheric air is in all instances higher than the carbon dioxide content of sea water.

3. The carbon dioxide content of certain of the Arthropoda and Mollusca is relatively very high.

4. The carbon dioxide content of the blood of marine Teleostei is approximately 10 volumes per cent.

5. The carbon dioxide content of the elasmobranch *Squalus sucklii* and the holocephalan *Hydrolagus colliei* is relatively very low.

6. The alkalinity and the reactivity of several samples of sea water have been determined.

7. Surface samples of sea water in the vicinity of Departure Bay are invariably alkaline to phenolphthalein.

8. It is held that in order to maintain the constant reaction of the blood or body fluids of marine forms the carbon dioxide tension must be considerably higher in the blood and body fluids than it is in sea water.

9. The question of carbon dioxide excretion is discussed.

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