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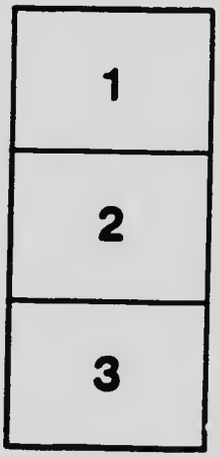

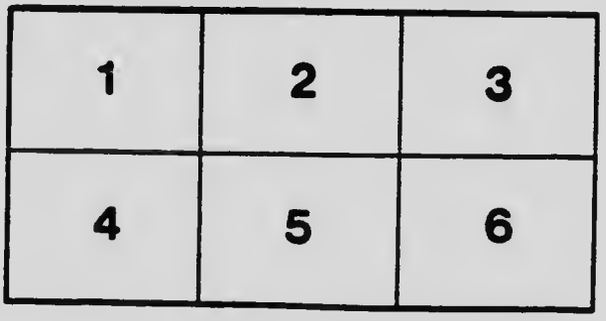

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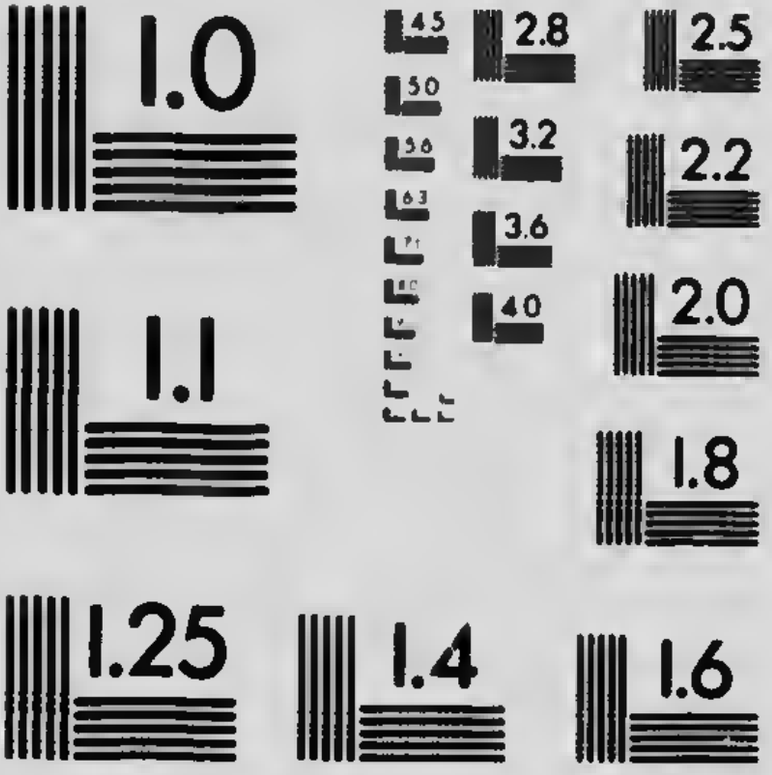

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STUDIES ON MOLLUSCAN CELOMIC FLUID

I. EFFECT OF CHANGE IN ENVIRONMENT ON THE CARBON DIOXIDE CONTENT OF THE CELOMIC FLUID

ANAEROBIC RESPIRATION IN MYA ARENARIA

BY

J. B. COLLIP

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

Reprinted from
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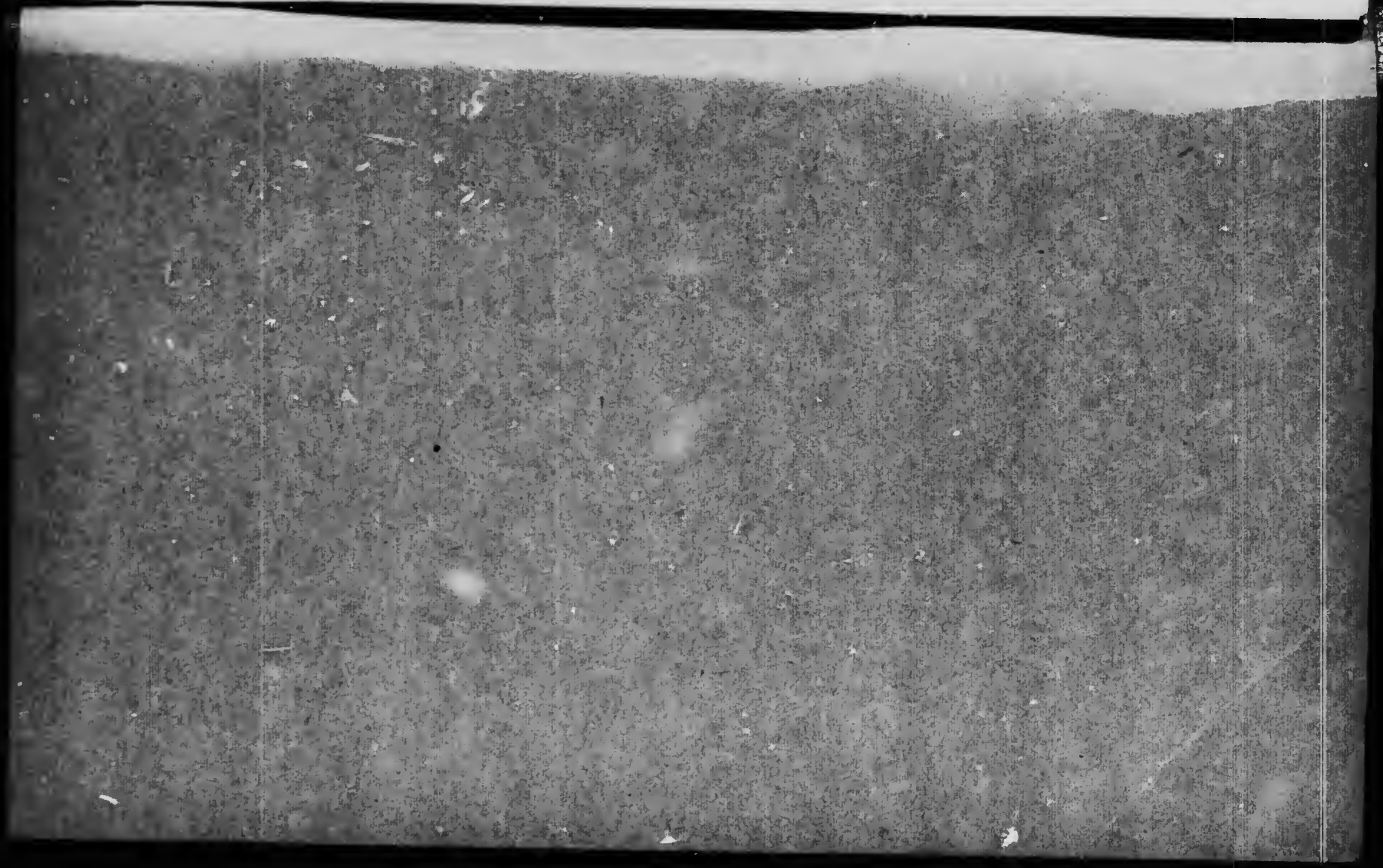

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STUDIES ON MOLLUSCAN CELOMIC FLUID.

EFFECT OF CHANGE IN ENVIRONMENT ON THE CARBON DIOXIDE CONTENT OF THE CELOMIC FLUID.

ANAEROBIC RESPIRATION IN MYA ARENARIA.

BY J. B. COLLIP.

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

(Received for publication, September 10, 1920.)

INTRODUCTION.

It was noted in a previous communication (1) that the content of combined carbon dioxide of molluscan celomic fluid tends to rise when the animals are removed from their natural environment whereas a fall was noticed in this factor in the case of fish removed from their natural habitat. In order to determine what was the cause of this peculiar effect in the molluscan forms a series of experiments was undertaken, the results of which are herein reported.

EXPERIMENTAL.

Effect of Exposure to Atmospheric Air on the Combined Carbon Dioxide Content of the Celomic Fluid.

The method of securing samples of celomic fluid or "clam juice" from the various specimens was the same as that detailed previously (1). Specimens of seven species of pelecypod Mollusca were exposed to atmospheric air in a closed glass container for varying periods of time. One species of the Amphineura and two species of the Gastropoda were similarly studied. Several non-molluscan forms were also exposed to atmospheric air under similar conditions. These included the calcareous shelled arthropod *Balanus aquilla*, the common brachiopod *Terebretella transversa*, various Crustacea of the decapod type, starfish, sea urchins, and certain varieties of marine fish. The container

TABLE I.

No. used.	Specimen.	Time out of water.	CO_2 content of 100 cc. celomic fluid equilibrated with atmospheric air.	Remarks.
		hrs.	*cc.*	
1	*Macoma secta*	0	11.2 } one experiment.	
1	" "	6	48.6 }	
1	*Mya arenaria*	0	11.2 } one experiment.	
1	" "	17	27.3 }	
1	" "	1	19.9	
1	" "	4	19.5	
1	" "	0	10.2 } one experiment.	
1	" "	24	32.3 }	Same specimen as above.
1	" "	24	28.2	
9	" "	0	8.0	Kept for some days in floating carriage attached to landing stage.
11	" "	28	36.0	Kept for some days in floating carriage attached to landing stage.
3	" "	25	34.8	
3	" "	24	28.2	
4	" "	24	38.0	Kept in sealed container placed in sea water off landing stage.
4	" "	120	55.0	
12	" "	90	44.0	
12	" "	0	6.5	Kept for some days in floating carriage attached to landing stage.
10	" "	48	65.0 } one experiment.	Kept in sealed container placed in sea water off landing stage.
11	" "	96	71.5	Kept in sealed container placed in sea water off landing stage.

6	*Mya arenaria*	0	6.5	one experiment.	Kept for some days in floating carriage attached to landing stage.
4	" "	24	38.0		Kept in sealed container placed in sea water off landing stage.
10	" "	48	69.0		Kept in sealed container placed in sea water off landing stage.
8	" "	72	78.2		Kept in sealed container placed in sea water off landing stage.
2	" "	96	105.0		Kept in sealed container placed in sea water off landing stage.
11	*Paphia staminea*	0	12.8		
1	" "	0	27.9		
3	"	6	39.0		
1		6	34.5		
	"	16	40.0		
1	"	44	59.0		
8	"	28	39.5		
1	*Schizothaerus nuttalli*	0	8.3		
1	" "	12	23.2		
3	" "	32	30.7		
1	*Saxidomus gigantea*	0	8.1		
1	" "	12	24.2		
1	" "	6	29.0		
1	" "	24	40.0		
8	" "	30	44.0		
1	" "	30	53.0		

TABLE 1.—*Concluded*

No. used	Specimen.	Time out of water	CO_2 content of 100 cc. celomic fluid equilibrated with atmospheric air	Remarks.
		hrs.	*cc.*	
1	*Cardium corbis*.....	0	7.9	
5	" "	2	12.6	
1	" "	6	14.9	
1	" "	14	16.5	
1	" "	24	18.6	
8	" "	24	16.3	
5	" "	30	20.0	
4	" "	32	18.0	
1	*Mytilus edulis*.....	0	8.1	
1	" "	0	9.9	
1	" "	30	27.3	
1	*Cryptochiton*.....	0	9.0	
1	"	16	18.0	
1	*Polynices* (Lunatia) *lewisii*. ...	0	12.5	
1	" " "	24	31.0	
1	" " "	30	77.4	
1	" " "	52	61.2	
1	" " "	52	63.0	
1	*Thais lamellosa*.....	0	18.5	
1	" "	10	28.2	
1	" "	24	45.8	
1	*Balanus aquilla*.....	0	11.2	
1	" "	9	17.7	Same specimen as above.

1	*Terebretella transversa*.....	0	6.8	
3	" "	30	12.7	
1	*Strongylocentrotus drobachiensis.*	0	10.2	
1	" "	4	14.8	Same specimen as above.
1	" "	0	10.2	
1	" "	8	6 6	Same specimen as above.
1	*Pisaster ochracea*.....	0	6.5	
1	" "	8	6 5	Same specimen as above.
1	" "	32	7 0	
1	" "	0	7.0	
1	" "	24	10 0	Same specimen as above.
1	" "	0	6 0	
1	" "	24	7 0	Same specimen as above.
1	*Cancer magister*.....	0	18.0	
1	" "	5	18 0	Same specimen as above.
1	" "	0	18.4	
1	" "	8	19 5	Same specimen as above.
1	*Platichtys.* plasma.....	0	10 2	
1	" whole blood.....	9	3 7	Dead some hrs.
1	*Sebastodes*.....	0	11 2	
3	"	3	7.3	
1	"	0	9 3	On line ½ hr.
1	*Squalus sucklii*.....	0	5 6	
1	" "	4	3.7	

used was of good size and a full supply of oxygen was assured. It was kept covered to prevent loss of water by evaporation. Filter paper moistened in sea water was frequently placed in the container with the specimens. Specimens which were exposed to air were kept in certain instances at a fairly constant temperature by immersing the container in sea water while in others they were kept in the laboratory and were thus subject to the temperature changes of the latter. Table I illustrates the effects of exposure to air for various periods upon the combined carbon dioxide content of the celomic fluid of the different species investigated. The rapid increase in the carbon dioxide content of the celomic fluid of the molluscan forms and the arthropod *Balanus aquilla* is very striking. That this increase is due to bicarbonate is evident since the samples were equilibrated with atmospheric air before being submitted to analysis. The decapod crustaceans examined failed to show this reaction, while a very slight increase was in some instances manifested in the echinoderms. As the latter reaction was not uniform it is of very doubtful significance.

The survival time for specimens exposed to atmospheric air varied greatly. It was early noted that *Mya arenaria* was peculiarly resistant to long exposure to atmospheric air at the temperatures which prevailed in the surface water and the air at Departure Bay during the summer months. It was for this reason used extensively in later investigation. It is regretted that no facilities were available which would enable one to keep specimens at a low as well as constant temperature. The results obtained will therefore have to be considered in the light of this condition. The greatest increase in the carbon dioxide content of the blood was in two specimens of *Mya arenaria* which had been exposed for 96 hours. The increase here was from 6.5 volumes per cent in the controls kept in sea water to 105 volumes per cent in the specimens exposed to atmospheric air at the temperature of surface sea water. The container used was a 6 liter cylindrical glass museum jar and it was opened daily both for the purpose of removing specimens for examination and to allow a change of air. The temperature of the sea water in the vessel which was taken on the 4 consecutive days during which these specimens were exposed was 19.8°, 18.8°, 19.3°, and 18.9°C. Slightly over a

sixteenfold increase in the combined carbon dioxide in the blood was noticed in this instance.

The increase in the carbon dioxide in the small pelecypod *Macoma secta* from 11.2 to 48.6 volumes per cent in 6 hours is noteworthy, as is also that observed in the gastropod *Polynices lewisii* following 30 hours exposure. The combined carbon dioxide rose in this latter instance from 12.5 volumes per cent in the control to 77.4 volumes per cent in the exposed specimen. There were few forms which would survive an exposure to atmospheric air of more than 24 hours at the prevailing land and surface water temperatures. The amphineuran *Cryptochiton*, the cockle *Cardium corbis*, and the eagle barnacle *Balanus aquilla* were very sensitive to exposure. The horse clam *Schizothoerus nuttalli*, and the butter clam *Saxidomus gigantea* withstood an exposure of 24 to 48 hours. The little neck clam *Paphia staminea* and the edible form *Mya arenaria* were very resistant to exposure and in some instances survived as long as 5 days when placed in the air, the evaporation of water being practically excluded. The nudibranch *Anisodoris* was most sensitive of all forms, dying shortly after being brought into the laboratory. The absence of a calcareous shell is probably associated with the lack of resistance to exposure to air in this form although the temperature factor must also be of great importance.

Effect of Exposure to Atmospheric Air on the Carbon Dioxide Capacity of the Celomic Fluid.

Several samples of celomic fluid taken from specimens exposed to atmospheric air for varying periods were analyzed in the Van Slyke apparatus (2) when equilibrated with atmospheric air and also when equilibrated with alveolar air of the normal subject after the manner described by Van Slyke and Cullen (3). Table II illustrates the results obtained in this series of experiments. It will be noted that in every instance the carbon dioxide capacity of the sample was considerably in excess of the carbon dioxide content of the same when equilibrated with atmospheric air.

TABLE II

No. used	Specimen.	CO_2 content of 100 cc. celomic fluid. Equilibrated with atmospheric air.	Equilibrated with alveolar air.	Remarks.
		cc.	cc.	
1	*Echinarachnius formatus*	22.5	28.0	3 hrs. out of water.
1	*Pisaster ochracea*	4.1	9.8	Fresh.
1	" "	7.4	11.2	9 hrs. out of water.
1	" "	7.0	12.2	32 " " " "
1	*Strongylocentrotus drobachiensis*	7.4	9.8	Fresh.
1	*Dermasterias imbricata*	5.5	8.8	"
1	*Schizothaerus nuttalli*	9.9	14.4	"
3	" "	30.7	39.8	30 hrs. out of water.
1	*Balanus aquilla*	11.2	18.6	Fresh.
3	*Mya arenaria*	34.8	39.4	25 hrs. out of water.
10	" "	42.0	50.0	60 " " " "
4	" "	17.6	23.3	24 " in boiled sea water in sealed container.
11	" "	36.0	40.0	28 hrs. out of water.
3	" "	28.2	33.0	24 " " " "
10	" "	6.5	13.0	Fresh.
2	" "	18.8	25.4	25 hrs. in desiccator over alkaline pyrogallol.
8	*Paphia staminea*	39.5	45.0	28 hrs. out of water.
1	*Cardium corbis*	12.6	18.0	2 " " " "
1	*Saxidomus*	39.6	43.2	30 " " " "
1	*Polynices lewisii*	77.4	82.8	30 " " " "
1	" " (fluid from foot)	16.5	21.5	Fresh.

Effect of Exposure to Atmospheric Air on the Total Nitrogen Content of the Celomic Fluid.

In order to determine if the increase in the carbon dioxide content of mollusean celomic fluid brought about by exposure to atmospheric air was in any way due to an increase in its protein content the total nitrogen was determined in from 25 to 100 cc. of composite samples of celomic fluid taken from several specimens. The estimation was made by the usual Kjeldahl method. The results are expressed in Table III. As there was a slight

TABLE III.

No. used.	Specimen.	Total nitrogen per 100 cc. of fresh material.	Total nitrogen per 100 cc. after exposure to atmospheric air.	Time in air.
		mg.	*mg.*	*hrs.*
1	*Schizothoerus nuttalli*.....	37.5		0
2	" "	34.0		0
3	" "		34.4	32
15	*Saxidomus gigantea*.....	50.3		0
8	" "		51.8	30
23	*Paphia staminea*.....	70.0		0
8	" "		61.6	28
6	*Cardium corbis*.....	37.5		0
6	" "		50.4	30
14	*Mya arenaria*.....	33.6		0
11	" "		40.7	28
1	*Polynices lewisii* (fluid from foot)...		9.5	52

increase in two species, *Cardium corbis* and *Mya arenaria*, practically no change in *Saxidomus gigantea*, and a slight decrease in *Schizothoerus nuttalli* and *Paphia staminea*, it is very unlikely that protein plays any appreciable part in the increase in the combined carbon dioxide or alkali reserve of the celomic fluid of the mollusk which has been exposed to atmospheric air.

Effect of Exposure to Atmospheric Air upon the Calcium and Magnesium Content of the Celomic Fluid.

Calcium and in a few instances magnesium were determined in composite samples of celomic fluid taken from several specimens which had been exposed to atmospheric air for varying

TABLE IV.

No. used.	Specimen.	CO_2 per 100 cc. of fluid equilibrated with atmospheric air.	Ca per 100 cc.	Mg per 100 cc.	Alkalinity.	Reactivity.	Remarks.
		cc.	*mg.*	*mg.*	*cc. 0.01 N NaOH*	*cc. 0.01 N H_2SO_4*	
	Experiment I.						
10	*Mya arenaria*.....	6 5	35 4	49.0	−9.0	99.0	Fresh. Kept some days in floating carriage off landing stage.
4	" "	38.0	92 0	79 0			24 hrs. in air at temperature of sea water.
10	" "	69 6	186 0	82 0	−24 0	720.0	48 " " " " " " " "
8	" "	78.2	220 0		−60.0	1,080.0	72 " " " " " " " "
2	" "	105 0	240 0	90 0	−130 0	1,140.0	96 " " " " " " " "
	Experiment II.						
10	*Mya arenaria*.....	6 5	30 0	41.5			Fresh. Kept some days in floating carriage attached to landing stage.
10	" "	65.0	135.0	48.0			48 hrs. in air at temperature of sea water.
11	" "	74.5	223 0	57.3			96 " " " " " " " "
	Experiment III.						
2	*Mya arenaria*.....	7.5	38 0		−6 0	76.0	Fresh.
3	" "	38.7	140 0		−18.0	450.0	24 hrs. in air at temperature of sea water.
4	" "	61.2	208.0		−30.0	690 0	48 " " " " " " " "
3	" "	72.5	231.0	75.0	−24.0	860.0	72 " " " " " " " "
1	" "	84.0					96 " " " " " " " "
	Experiment IV.						
5	*Cardium corbis*....	9.3	48.0	78.0	−17.6	140.0	Fresh from Sand Beach.
8	" "	16 3	84.0	78.0	−32.0	200.0	Kept 24 hrs. in air in laboratory.
	Experiment V.						
5	*Cardium corbis*....	8.4	48.0	100.8	−16 0	95 0	Fresh from Sand Beach.
7	" "	19 0	98 0	100 2	−30.0	220.0	Kept 20 hrs. in air in laboratory.

periods of time. The method of McCrudden (4) was followed, the calcium being estimated by the titration of the oxalate with 0.1 N potassium permanganate. 25 cc. of celomic fluid were used in most instances. This was evaporated to dryness on the water bath, fused, dissolved by the aid of concentrated hydrochloric acid, and the calcium finally precipitated as the oxalate. The results are shown in Table IV. It will be noted that, whereas magnesium increases only very slightly in the blood of an exposed mollusk, the calcium increases to a great extent and also the increase in this latter constituent is more or less parallel with the increase in the combined carbon dioxide. It is therefore evident that the great increase in the alkali reserve of the blood of molluscan forms when exposed to atmospheric air is due to an increase in the concentration of bicarbonate which is balanced for the most part by an increase in the concentration of the calcium ions. Myers (5) has reported finding 307 mg. of calcium calculated as oxide in the blood of *Saxidomus nuttalli* and 197 mg. in *Schizothoerus nuttalli*. He has also commented on the very high calcium content of molluscan blood. I have failed to find that the calcium content of the celomic fluid of fresh molluscan forms differs materially from that of sea water. It is only after exposure to air that the calcium content becomes high.

Effect of Exposure to Atmospheric Air upon the Total Alkalinity and the Buffer Value or Reactivity of the Celomic Fluid.

Lacking the means of determining the hydrogen ion concentration, a most important factor in these experiments, a method was employed to determine approximately the total alkalinity of the blood and also its buffer value. The method adopted was similar to that previously described (1) based on the principle made use of in the method of double titration for bicarbonates by Brown and Escombe (6). The presence of small amounts of protein would of course introduce an error but as has been shown the protein content of the celomic fluid does not vary to any appreciable extent and therefore approximately the same degree of error would exist in all the titrations. The alkalinity was determined by noting the amount of 0.01 N alkali required to produce a just noticeable pink tint when phenolphthalein had

been added to the celomic fluid. The reactivity of Moore and Wilson (7) or the buffer value of Sörensen (8) was determined by titrating from the phenolphthalein to the methyl orange point using 0.02 sulfuric acid. 0.2 N acid was used in those instances where the reactivity was of large proportion. The results are shown in Table IV. It will be noted that the rate of increase in the reactivity of the celomic fluid is in close agreement with the rate of increase of calcium and also of the combined carbon dioxide content of the same.

Effect of Exposure to Air Followed by Submersion in Fresh Water.

Table V illustrates that, whereas exposure to air causes a rapid increase in the alkali reserve of the celomic fluid, the subsequent immersion in fresh sea water causes a return to approximately the normal value for this factor.

TABLE V.

Specimen.	CO_2 content of 100 cc. of fluid equilibrated with atmospheric air.	Remarks.
	cc.	
Mya arenaria . .	8.2	Fresh.
" " . .	32.0	72 hrs. in glass container in laboratory.
" " . .	23.3	Submerged 4 hrs. in fresh sea water.
" " . .	14.9	" 16 " " " " "
" " . .	9.2	" 64 " " " " "

Effect of Submersion in Sea Water in a Sealed Container.

Several fresh specimens of *Mya arenaria* were immersed in a relatively small volume of sea water in a cylindrical glass container which was then tightly sealed. After varying periods of time the celomic fluid of the specimens was examined. An analysis of the sea water which was used in the experiment was also made. The results are expressed in Table VI. A few experiments were carried out in which boiled out sea water was used in place of fresh sea water. In one instance sea water, the buffer value of which had been greatly increased by the addition of 5 gm. of basic sodium phosphate per liter, was used. It will

be noted that the behavior of *Mya arenaria* kept in a sealed container in either boiled or fresh sea water is very similar to that observed when the specimens were kept in the air. The alkali reserve and the calcium content of the celomic fluid mount steadily until the animal dies. The increase in the carbon dioxide and calcium content of sea water is also considerable. The values for these two factors in sea water are, however, lower in the case of the celomic fluid except after long submersion of the specimens, in which instance there is a tendency for the concentrations of these latter substances in the celomic fluid and sea water to equalize. The addition of basic sodium phosphate to the boiled sea water used in one experiment did not materially alter the results. A dense precipitate was formed in the sea water in this experiment which consisted for the most part of calcium phosphate.

If one considers the increase in the combined carbon dioxide in the sea water used in an experiment one finds that there is very little difference in the rate of increase in this factor in specimens exposed to air and in specimens submerged in a relatively small volume of sea water. Thus in Experiment I, Table VI, an increase of 29.3 volumes per cent was noted in the combined carbon dioxide content of the celomic fluid, while an increase of fully 20 volumes per cent took place in the sea water. The total bulk of the eight specimens used in this experiment was 550 cc. while the volume of sea water used was 750 cc. There was therefore an increase of 150 cc. of combined carbon dioxide due to the activity of the specimens over and above the increase noted in their celomic fluid. The shells of the eight specimens displaced 85 cc. of water. Not allowing for the water entrapped in the mantle cavities, there were 465 cc. of clam tissue present in this experiment so that the increase of 150 cc. of carbon dioxide found in the sea water would mean that 32 cc. of this carbon dioxide had resulted from the activity of each 100 cc. of clam tissue. This added to the carbon dioxide content of 100 cc. of celomic fluid indicates that approximately 61.3 cc. of combined carbon dioxide resulted from the activity for 46 hours of 100 cc. of clam tissue, an amount which is in close agreement with the observed increase of the volume per cent of carbon dioxide in the celomic fluid of similar specimens exposed to atmospheric air in a closed vessel for a corresponding period of time (Table IV).

TABLE VI.

No. used.	Specimen.	CO_2 per 100 cc. equilibrated with atmospheric air.	Ca per 100 cc.	Mg per 100 cc.	P_2O_5 per 100 cc.	Alkalinity.	Reactivity.	Remarks.
		cc.	*mg.*	*mg.*	*mg.*	*cc. 0.01 N NaOH*	*cc. 0.01 N H_2SO_4*	
	Experiment I.							
10	*Mya arenaria*.....	7.5	38.0			−6.0	74.0	Fresh.
8	" " (550 cc.).....	36.8	149.0			−25.0	550.0	46 hrs. in 750 cc. sea water in sealed container at temperature of sea water.
	Sea water (750 cc.).....	23.4	102.0	95.0		−13.5	140.0	8 *Mya arenaria* in this 46 hrs.
	Experiment II.							
	Boiled sea water..	2.2	33.0			3.0	11.3	
10	*Mya arenaria*.....	6.5	35.4	49.0		−9.0	90.0	Fresh control.
1	" "	31.5						One of fifteen in boiled sea water in sealed container 42 hrs. All alive.
12	" "	51.0	121.0					90 hrs. in boiled sea water; 3 dead, 12 living; analysis of latter only.
	Boiled sea water (700 cc.)....	51.0	121.0			−21.4	350.0	15 *Mya arenaria* (600 cc.) in this 90 hrs.
	Experiment III.							
	Boiled sea water.....	2.2			273.0	−30.0	160.0	5 gm. of Na_2HPO_4 added per liter.

11	*Mya arenaria*.....	6.5	29.0			−11.2	80.0	Fresh control.
11	" "	26.6	125.0		51.0	−70.0	480.0	All alive (600 cc.). 44 hrs. in boiled sea water as above.
	Boiled sea water (700 cc.)....	26.6	50.0	50.0	50.0	−82.0	265.0	11 *Mya arenaria* in this 44 hrs.
	Experiment IV.							
	Boiled sea water.....	3.7				4.0	16.7	
10	*Mya arenaria*.....	6.5	56.0	66.0		−9.0	90.0	Fresh control.
4	" "	17.5						24 hrs. in boiled sea water (250 cc.). All alive.
	Boiled sea water (250 cc.)....	9.4				−5.5	54.0	4 *Mya arenaria* in this 24 hrs.

TABLE VII.

No. used.	Specimen.	CO_2 per 100 cc. equilibrated with atmospheric air.	Ca per 100 cc.	Mg per 100 cc.	Cl	Alkalinity.	Reactivity.	Remarks.
		cc.	*mg.*	*mg.*	*per cent*	*cc. 0.01 N NaOH*	*cc. 0.01 N H_2SO_4*	
	Experiment I.							
12	*Mya arenaria*..	6.5	35.4	49.0		−9.0	90.0	Fresh control.
20	" " ..	25.0	84.0	28.5		−24.0	330.0	45½ hrs. in distilled water in sealed container. 10 alive, 1 dead. Bulk of clams 390 cc. Water 400 cc.
	400 cc. water...	13.0	30.0	15.0		−11.0	95.0	11 *Mya arenaria* in this 45½ hrs.
	Experiment II.							
6	*Mya arenaria*..	7.5	38.0		1.301	−9.0	90.0	Fresh. Cl in sea water 1.303 mg.
6	" " ..	19.5	77.6	24.0	0.747	−9.0	250.0	24 hrs. in fresh distilled water in sealed container. Bulk of clams 530 cc. Water 770 cc.
	770 cc. water...	4.0	15.0	10.0	0.211	−3.5	38.0	6 *Mya arenaria* in this 24 hrs.
	Experiment III.							
2	*Mya arenaria*.	20.5			0.802			20 hrs. in distilled water in sealed container. Bulk of clams 200 cc. Water 1,100 cc.
	1,100 cc. water.				0.132			2 *Mya arenaria* in this 20 hrs.

Effect of Submersion in Distilled Water.

The results of some experiments in which specimens of *Mya arenaria* were submerged in fresh distilled water in a glass container and kept at the temperature of surface sea water appear in Table VII. The combined carbon dioxide and calcium content of celomic fluid rise after much the same manner as was observed when specimens were placed in either fresh or boiled sea water. Experiments I and II, Table VII, are of interest in that they show that a fall had taken place in the concentration of magnesium in the celomic fluid of the specimens, while a rise occurred in the concentration of calcium. There is also a marked difference in the total alkalinity and in the reaction of the water and the celomic fluid. The chlorine content of the celomic fluid and of the water in which the specimens of *Mya arenaria* were submerged was determined in Experiments II and III, Table VII. The manner in which the concentration of the chloride in the celomic fluid is kept at a relatively high level under the circumstances obtaining in these experiments is remarkable. Meigs (9) has shown that the adductor muscle of the clam *Venus mercenaria* is peculiarly resistant to hypertonic solutions of sodium chloride and to double strength sea water, the concentration of sodium chloride rising to only about one-half that of the surrounding medium. He also found that the mantle is almost impermeable to sodium chloride. The ability of *Mya arenaria* to withstand immersion in water, the osmotic pressure of which is very low, is of interest in the light of the observations of Meigs.

Effect of Exposure to a Hydrogen Atmosphere.

Several specimens of *Mya arenaria* were kept in a stream of sea water for 2 hours in order that the oxygen content of their tissues should be reduced to a low level. One specimen was then bled as a control, the others were placed in a hydrogen atmosphere over alkaline pyrogallic acid, two separate containers being used. One group of three was exposed for 26 hours; the other for 48 hours. The results of the analyses of the celomic fluids of these specimens are shown in Table VIII. The maximum and minimum temperatures for the 2 days during which

TABLE VIII.

No. used.	Specimen.	CO_2 per 100 cc. of fluid equilibrated with atmospheric air.	Ca per 100 cc.	Mg per 100 cc.	Alkalinity.	Reactivity.	Remarks.
		cc.	*mg.*	*mg.*	*cc. 0.01 N NaOH*	*cc. 0.01 N H_2SO_4*	
1	*Mya arenaria*....	8.2					Kept in small volume of sea water 2 hrs.
3	" "	26.4	156.0	75.0	−16.0	41[illegible]	Kept in hydrogen atmosphere over alkaline pyrogallol 26 hrs.
3	" "	45.5			−22.0	690.0	Kept in hydrogen atmosphere over alkaline pyrogallol 48 hrs.
4	" "	53.0			−45.0	950.0	Kept in hydrogen atmosphere 56 hrs.

this experiment was performed were on the 1st day 24.4° and 10.9°C., on the 2nd day 23.9° and 11.4°C. It will be noticed that the increase observed in the concentration of bicarbonate in the celomic fluid is very marked. It is not, however, so great as that which is found when the specimens are exposed to atmospheric air. The increase in the calcium and magnesium is comparable to that observed for bicarbonate.

Effect of Exposure to a Nitrogen Atmosphere.

Three specimens of *Mya arenaria* were placed in a small desiccator containing a concentrated solution of pyrogallic acid in 40 per cent sodium hydroxide. A glass tube was so attached

TABLE IX.

No. used.	Specimen.	CO_2 per 100 cc. of fluid equilibrated with atmospheric air.	Remarks.
		cc.	
3	*Mya arenaria*.	8.0	Fresh control.
3	" " .	34.8	25 hrs. in glass container in laboratory.
3	" " .	17.8	25 " " desiccator over alkaline pyrogallol.

to the exhaust cock that as oxygen was absorbed the air which entered first bubbled through the alkaline pyrogallol in the bottom of the desiccator. Three other specimens of like size were placed at the same time in a glass container which was kept at the same temperature as the desiccator for the duration of the experiment. After 25 hours the specimens were bled and an analysis was made of the composite samples of celomic fluid. The results are expressed in Table IX. It will be observed that the combined carbon dioxide of the celomic fluid did not increase to the same extent in the specimens which were kept in the desiccator over alkaline pyrogallol as it did in the controls which were kept in the air.

TABLE X.

Specimen.	Fluid examined.	CO_2 per 100 cc. equilibrated with atmospheric air.	Remarks.
		cc.	
Mya arenaria.....	Celomic fluid from sinus.	8.3	Fresh.
" "	Fluid from exhalent siphon.	4.6	" Same specimen as above.
" "	Celomic fluid from sinus.	39.6	25 hrs. out of water.
" "	Fluid from mantle cavity.	36.0	25 " " " " Same specimen as above.
Cryptochiton.....	Celomic fluid from sinus.	9.0	Fresh.
"	Fluid from foot.	3.6	" Same specimen as above.
Polynices (Lunatia) *lewisii*.	Celomic fluid from sinus.	20.7	Fresh.
" " "	Fluid from foot.	17.1	" Same specimen as above.
" " "	Celomic fluid from sinus.	21.0	"
" " "	Fluid from foot.	16.5	" Same specimen as above.
" " "	Celomic fluid from sinus.	14.9	"
" " "	Fluid from foot.	12.5	" Same specimen as above.
" " "	Celomic fluid from sinus.	31.0	24 hrs. out of water.
" " "	Fluid from foot.	31.0	24 " " " " Same specimen as above.
" " "	Celomic fluid from sinus.	12.1	Fresh.
" " "	Fluid from foot.	12.1	" Same specimen as above.
" " "	Celomic fluid from sinus.	65.7	52 hrs. out of water.
" " "	Fluid from foot.	63.0	52 " " " " Same specimen as above.
" " "	Celomic fluid from sinus.	77.4	30 " " " "
" " "	Fluid from foot.	59.4	30 " " " " Same specimen as above.
" " "	Celomic fluid from sinus.	61.2	52 " " " "
" " "	Fluid from foot.	37.8	52 " " " " Same specimen as above.

Comparison of the Carbon Dioxide Content of Celomic Fluid and Other Fluids Obtained from Mollusca.

A comparison was made between the carbon dioxide content of the celomic fluid and other fluids of different Mollusca. The results of this study are shown in Table X. It was found that the carbon dioxide content of the fluid which exuded from the exhalant siphon of fresh *Mya arenaria* was just slightly higher than that of sea water. When specimens of this species were exposed to atmospheric air for some time the bicarbonate content in the fluid of the mantle cavity closely approximated that in the celomic fluid. Somewhat similar observations were made on the amphineuran form *Cryptochiton*. The fluid in the foot of the large gastropod *Polynices lewisii* bears somewhat different relation to the blood and celomic fluid of this form as far as the bicarbonate content is concerned from that which holds in the fluid between the mantle cavity and the blood and celomic fluid of a pelecypod such as *Mya arenaria*. The combined carbon dioxide content of the fluid of the foot of *Polynices lewisii* approximates the value observed for the celomic fluid. The drawing in, therefore, of the foot in this form causes a considerable decrease in the total alkali reserve of the animal. It is of interest in this connection to note that this animal does not withdraw its foot unless subjected to rather violent irritation.

Effect of Submersion of Dead Specimens of Mya arenaria in Sea Water.

The results of two experiments are shown in Table XI. It is evident that the bicarbonate content of sea water in which dead clams are immersed rises quite rapidly once decomposition has set in. It will be noted, however, that there is little change during the first 24 hours submersion. The behavior of pelecypod mollusks exposed to air or submerged in a relatively small volume of water is therefore quite distinct from that of dead clams which are undergoing decomposition.

TABLE XI.

Specimen.	Total CO_2 per 100 cc.	Remarks.
	cc.	
Experiment I.		
4 *Mya arenaria* (175 cc.)....		Placed in 275 cc. of boiling sea water.
Sea water (boiled).....	3.3	
" " "	4.7	After 24 hrs. No decomposition of clam tissue.
" " "	42.0	After 48 hrs. Decomposition clearly manifested.
" " "	55.0	After 72 hrs.
" " "	67.0	" 96 "
" " "	72.0	" 144 "
Experiment II.		
10 *Mya arenaria* (500 cc.)....		Placed in 700 cc. of boiled sea water containing 9.5 per cent alcohol.
Sea water (boiled).....	2.8	
" " "	3.3	After 24 hrs.
" " "	11.7	" 48 " Milky, decomposition evident.
" " "	35.0	After 96 hrs.

DISCUSSION.

As has already been indicated the marked increase in the bicarbonate content of the celomic fluid, and therefore in all probability of the blood and tissues of the calcareous shelled pelecypod mollusks and the arthropod *Balanus aquilla* on exposure to air is quite opposite to the effect observed in fishes when they are removed from their natural habitat. This phenomenon is undoubtedly associated with the presence of a calcareous shell the calcium carbonate of which furnishes an alkali reserve which is added to that of the body fluids and tissues, and which it appears can be readily utilized.

As specimens of *Mya arenaria* appear to remain practically normal even after long exposure to atmospheric air there is no reason to suppose that any material change has been effected in their metabolic processes as a result of the change in environment. If one assumes, therefore, that combustion still proceeds in the

exposed specimens, then the increase in the bicarbonate content of the body fluids can be explained according to the equation

$$CO_2 + H_2O + CaCO_3 \rightleftharpoons Ca(HCO_3)_2$$

The carbon dioxide resulting from the respiratory process would, by slightly increasing the hydrogen ion concentration, dissolve calcium carbonate from the shell and the concentration of the calcium ions and of bicarbonate ions would therefore steadily rise as combustion in the tissues proceeded. The amount of carbon dioxide actually excreted from the specimens in the gaseous form was not determined. If this factor were known one could calculate the intensity of metabolism in these forms by considering the amount of carbon dioxide excreted in addition to the amount retained as bicarbonate. It would appear that 50 per cent of the increase observed in the carbon dioxide content of the celomic fluid is due to carbon dioxide formed by combustion in the tissues, while the remaining 50 per cent results from the solution of calcium carbonate of the shell.

The degree of alkalinity of the celomic fluid determined by titration is by no means an indication of the hydrogen ion concentration, but the ratios observed between the alkalinity figures and the reactivity values suggest that no marked increase in the hydrogen ion concentration takes place during the early part of the exposure at least. The reactivity or buffer value is, in nearly every instance, in close agreement with the calcium content and the carbon dioxide concentration of the celomic fluid.

The increase in the alkali reserve as indicated by an increase in bicarbonate concentration in specimens exposed to atmospheric air is due for the most part to increase in the calcium content. Magnesium, which in the normal animal in its natural habitat exceeds calcium in the degree of its concentration, and therefore balances a greater proportion of bicarbonate ions than does calcium, increases only slightly as compared with calcium when a specimen is exposed to air. It is probable that the relative increase in calcium and magnesium concentrations under these circumstances is somewhat similar to the relative amounts of these substances in the shell from which solution of bicarbonate is taking place. It is of interest to note here that no increase

was observed in the concentration of magnesium in the cockle (*Cardium corbis*) on exposure to air.

As specimens submerged in boiled sea water and kept in a sealed container continue to develop an increased carbon dioxide content, calcium concentration, and buffer value, after much the same manner as specimens exposed to atmospheric air, and since a similar effect is manifested by specimens kept in an atmosphere of hydrogen or nitrogen, one is led to ask the question "Can anaerobic respiration be manifested by these forms?"

If one considers the results of an experiment recorded in Table VIII, one finds that after 48 hours in a hydrogen atmosphere the combined carbon dioxide rose from 8.2 to 45.5 volumes per cent, or an observed increase of 37.3 volumes per cent. If one assumes that aerobic respiration was taking place and that carbohydrates were being burned, then a volume of oxygen equivalent to the volume of carbon dioxide produced would be required. If 50 per cent of the observed increase in the combined concentration of carbon dioxide is indicative of the amount of this substance produced due to combustion then an amount of oxygen equivalent to 50 per cent of 37.3 volumes per cent, or 18.65 volumes per cent, would be required. As the specimens used in this experiment were kept in a small volume of sea water for 2 hours before they were transferred to a hydrogen atmosphere, one fails to see how any appreciable amount of oxygen could be contained in the tissues of the specimens. As there is no apparent source for 18.65 volumes per cent of oxygen in these clams it is therefore evident that they must be respiring anaerobically or else the increase in the carbon dioxide, calcium, and buffer value of the celomic fluid is due to some other cause than that suggested earlier in the paper. Decomposition of the clam tissue can be excluded since the specimens were very active, responding to stimulation like normal animals, after they were removed from the hydrogen atmosphere.

There is the possibility of the solution of the calcium carbonate of the shell due simply to the solvent action of the tissue fluids containing free carbon dioxide. This would result in the formation of calcium carbonate the solubility of which is considerably increased by an excess of carbon dioxide in the water (10). In dealing with a closed system, however, such as the individual

clam in an atmosphere of hydrogen, the solution of calcium carbonate due to the solvent action of the free carbon dioxide would require a constant supply of the latter if the process is to continue; otherwise equilibrium would be established between the dissolved bicarbonate, the calcium carbonate of the shell, and the free carbon dioxide, and no further increase would be manifested unless this balance were disturbed. It will be noted that the rate of increase in the carbon dioxide content and the calcium concentration of the blood is for a considerable period practically constant. If respiratory activity accounts for the increase in bicarbonate concentration of the tissue fluids, then this uniformity in the rate observed would fit in well with the fairly constant rate of metabolism which might be expected under such circumstances, anaerobic respiration being possible.

The fact that the rate of increase in the bicarbonate concentration, the calcium content, and the buffer value is greater in air than it is in either hydrogen or nitrogen would indicate that absence of oxygen does exert an influence on the intensity of the metabolic processes but by no means causes a complete cessation in the respiratory function.

The extreme sensitivity of the cockle and the horse clam to exposure to air at the prevailing summer temperatures made experiments with these forms of the same type as were conducted with *Mya arenaria* temporarily impossible. Neither of these forms is normally submitted to the same degree of low oxygen tension as is *Mya arenaria*. It is hoped that experimental work of a similar nature to that carried out with *Mya arenaria* may be done on other forms at a more favorable time of the year.

It has long been known that animals show a very unequal resistance to lack of air. Bunge (11) in his work upon respiration of intestinal parasites and mud-dwelling organisms showed that parasites in the intestine of warm blooded animals must live practically in the absence of oxygen, while worms living in the mud were also subject to similar conditions, decomposition processes, with the formation of reducing substances, keeping the oxygen absent. Packard (12) found that worms and mud-dwelling Crustacea are resistant to the lack of oxygen for some time.

The ability of an animal to resist a lack of oxygen may or may not be connected with an anaerobic respiratory mechanism. If one finds complete evidence of metabolism in an animal exposed to anaerobic conditions, then anaerobic respiration would be indicated. Such seems to be the condition in the case of *Mya arenaria*. Crustacean types which were exposed to the air or submerged in boiled sea water died within a few hours. The carbon dioxide content of the blood was, however, practically unaltered by such procedures. These forms do not use the calcium carbonate of their carapace as a protective measure when removed from their normal habitat.

In the light of the results of the experiments which have so far been conducted upon *Mya arenaria* the writer has tentatively to conclude that individuals of this species behave as facultative anaerobic organisms. It is realized, however, that in these preliminary experiments absolutely anaerobic conditions were not secured. It is the intention to continue this work at another time when it is hoped to determine the hydrogen ion concentration of the celomic fluid and the rate of oxygen consumption under various conditions.

SUMMARY.

1. Calcareous shelled pelecypod Mollusca and the arthropod *Balanus aquilla* have in the calcium carbonate of their shells a potentially great alkali reserve.

2. Exposure of these forms to atmospheric air causes a marked increase in the combined carbon dioxide of the celomic fluid.

3. There is under these circumstances a parallel increase in the calcium concentration and the buffer value of the celomic fluid.

4. Various other marine forms studied did not so react.

5. There is no increase in the total nitrogen of the celomic fluid of the pelecypod Mollusca exposed to atmospheric air.

6. *Mya arenaria* is particularly resistant to long exposure to atmospheric air.

7. When specimens of *Mya arenaria* are placed in a relatively small volume of fresh sea water, boiled sea water, distilled water, or in a hydrogen or a nitrogen atmosphere much the same reaction is observed as when specimens are exposed to atmospheric air.

8. The rate of increase in the content of carbon dioxide, the calcium concentration, and the buffer value of the celomic fluid under all the above conditions is, during the first period of several hours, constant.

9. The rate of increase in the rate of combined carbon dioxide, the concentration of calcium, and the buffer value is not so great in a hydrogen or nitrogen atmosphere as it is in air.

10. It is suggested that *Mya arenaria* is a facultative anaerobic organism which continues to produce carbon dioxide under anaerobic conditions.

In conclusion I wish to express my thanks to Dr. C. McLean Fraser, the Curator of the Biological Station, Departure Bay, British Columbia, for his cooperation during the carrying out of the experimental work reported in this communication.

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