Float Gases, Gas Secretion and Tissue Respiration in the Portuguese Man-of-War, *Physalia*[1,2]

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The first analysis of the float gases of *Physalia* was reported by Schloesing and Richard (1896). In this paper it was suggested that the gases were of atmospheric origin, since the oxygen content was reported as 12.2–15.1%, the carbon dioxide as 0.0–1.7%, with the balance being nitrogen and argon. It is now known from the work of Wittenberg ('58) that in addition to the atmospheric gases, the floats also contain carbon monoxide. As a result of more extensive analyses, Wittenberg ('60) reported that the carbon monoxide averaged about 2% of the total gases in the float. He demonstrated that the carbon monoxide originates in the gas gland tissue as a product of serine metabolism. He also suggested that the carbon monoxide secretion serves to inflate the pneumocyst, and that it is slowly replaced by air through diffusion and exchange. More recently, Clark and Lane ('61) presented additional data on the composition of the float gases. Finally, correlative aspects of the behavior and physiology of the Siphonophora are given by Jacobs ('37) and Lane ('60).

The present experiments verify and extend these observations, including additional measurements of the secretion rate, of carbon monoxide. Further evidence is also presented which helps to explain the origin and final content of the various gases.

Finally, the tissues of the float were examined for possible sensitivity to carbon monoxide and several other inhibitors of respiration. The data given here indicate that the tissues are sensitive to carbon monoxide and other cytochrome inhibitors; however, the carbon monoxide produced by the animal apparently does not reach inhibiting concentrations in the normal aerated environment of the animals.

MATERIAL AND METHODS

Gas analyses were made by two methods, depending upon the type of data required. When it was necessary to determine the principal gases in a large number of samples, a recording gas chromatograph (Beckman GC-1) was used. When using a molecular sieve column, this method yields values for oxygen, nitrogen and carbon monoxide, while the use of a silica gel column allows an estimate of the carbon dioxide. For more complete analyses, the mass spectrometer was used (contract analyses by the Mass Spectrometry Laboratory of the Department of Chemistry, The University of Texas). From this technique, values were obtained for oxygen, nitrogen, argon, carbon monoxide and carbon dioxide.

The samples for gas chromatography analysis were drawn into 5 ml syringes fitted with 22 ga syringe needles. The float was punctured in an area that was normally below the water level. The puncture immediately sealed allowing sequential sampling if desired. The gases for analysis by mass spectrometry were obtained by introducing the 5 ml samples into special evacuated tubes which were supplied with the appropriate fittings for introducing the samples into the mass spectrometer.

The effects of inhibitors on the respiration of the excised float tissues were determined by the Warburg manometric method according to Umbreit et al. ('57). Minced tissue preparations of 0.3–0.5 g were suspended in 3 ml of sea water containing 3×10^{-3} M sodium succinate. The

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sea water was filtered (Millipore, H. A., 0.45 μ) and aerated before use. It was 31 parts per thousand in salinity and had a pH of 8.4. The experiments were carried out at 25°C while the flasks were agitated at 120 strokes per minute. The carbon monoxide gas mixtures were prepared using two 100 ml syringes fitted together by a three-way syringe stopcock. The mixed gases, which totaled 100 ml, were flushed through the 12 ml flasks eight to ten times with shaking to obtain equilibration. The control flasks were gassed with O_2-N_2 mixtures having the same oxygen concentration as the experimental flasks. After equilibration, the mixtures were analyzed by gas chromatography. The carbon monoxide experiments were carried out in complete darkness.

In addition to carbon monoxide, experiments were also carried out using CN^-, N_3^- and $S^=$. The effects of malonate were determined in order to assess the importance of the succinoxidase system, while oxidative phosphorylation was demonstrated by the response of the tissues to 2,4 dinitrophenol.

The animal material was collected near Port Aransas, Texas, from the surf or the beach rather than the open ocean and, as a result, many of them were dead or damaged when found. Only the best specimens were chosen for study. They were kept in shaded tanks supplied with running sea water. In almost all cases, they were used the same day they were collected, since in spite of considerable care, it was not possible to maintain them in good condition for more than two or three days.

RESULTS

Composition of float gases

The principal gases found in the floats of 32 animals are presented in table 1. These analyses were made by gas chromatography using the molecular sieve column. The argon values are included with the oxygen since the two gases are eluted from the column simultaneously. The average and range of values for the gases are as follows: oxygen plus argon, 17.4% (11.6–21.8%); nitrogen, 74.1% (66.1–84.3%); and carbon monoxide, 8.5% (0.5–20.8%). Table 2 presents the results of more complete analyses of float gases made by mass spectrometry. The data agree very well with those obtained by gas chromatography, but indicate also the concentrations of some of the minor components. The average and range of values are as follows: oxygen, 14.4% (7.2–18.8%); nitrogen, 74.4% (67.0–84.4%); argon, 1.1% (0.8–2.7%); carbon monoxide, 8.9% (2.3–19.3%); and carbon dioxide, 0.4% (0.0–0.9%).

Table 3 shows the results of analyses of the gases of four dead animals. It should be noted that the carbon monoxide is relatively low compared to values obtained for the normal animals, while the oxygen and nitrogen is usually present in about atmospheric concentrations.

TABLE 1

Composition of float gases (gas chromatography)

Specimen no.	Size	Oxygen and argon	Nitrogen	Carbon monoxide
	inches	%	%	%
1	8.0×2.0	19.6	79.2	1.2
2	6.0×4.0	18.6	80.9	0.5
3	9.0×4.0	21.8	76.6	1.6
4	6.0×2.5	15.3	80.1	4.6
5	3.5×1.5	16.7	68.5	14.8
6	5.0×1.5	15.4	66.1	18.5
7	4.5×2.0	19.2	76.6	4.2
8	6.0×1.7	16.3	69.8	13.9
9	4.7×2.0	16.7	71.0	12.3
10	5.0×2.0	16.9	73.7	9.4
11	5.5×1.2	18.0	72.7	9.3
12	7.0×2.5	18.7	71.7	9.6
13	4.2×1.5	19.7	75.2	5.1
14	5.5×1.7	20.1	74.9	4.9
15	4.0×2.0	16.8	70.8	12.4
16	5.5×1.8	14.9	69.6	15.5
17	4.5×1.5	20.0	74.0	6.0
18	5.2×1.5	18.3	74.4	7.3
19	7.0×2.0	20.5	77.2	2.2
20	6.0×1.5	20.0	77.1	2.9
21	5.5×2.0	19.7	75.0	5.3
22	1.3×0.8	15.7	68.9	15.4
23	4.0×1.0	14.6	84.3	1.0
24	3.5×0.7	18.8	70.2	11.0
25	4.0×1.0	17.3	71.6	11.1
26	1.5×1.2	13.7	74.0	12.3
27	2.5×1.0	12.7	73.9	13.4
28	2.5×1.5	15.7	78.5	5.8
29	4.2×2.0	18.0	76.4	5.6
30	4.0×2.0	18.0	77.1	4.9
31	3.0×1.5	16.9	73.5	9.6
32	5.0×1.3	11.6	67.6	20.8
Average		17.4	74.1	8.5

TABLE 2

Composition of float gases (mass spectrometry)

Specimen no.	Size	Oxygen	Nitrogen	Argon	Carbon monoxide	Carbon dioxide
	inches	%	%	%	%	%
1	7.0×4.0	8.3	84.4	2.7	4.2	0.4
2	4.3×2.5	7.2	83.2	2.1	7.1	0.4
3	[1]	18.2	77.7	0.9	3.0	0.2
4	[1]	18.8	76.4	0.9	3.0	0.9
5	[1]	17.0	75.5	0.9	6.3	0.3
6	7.0×2.0	18.6	77.9	0.9	2.3	0.3
7	5.5×2.0	18.3	75.6	0.9	5.0	0.2
8	4.5×1.3	15.6	70.3	0.8	13.0	0.3
9	3.5×1.0	9.5	73.3	0.9	5.6	—
10	8.0×2.0	14.9	71.4	0.9	12.4	0.4
11	3.0×0.7	12.4	67.0	0.9	19.3	0.4
12	7.0×1.7	13.8	68.9	0.9	16.0	0.4
13	3.0×1.5	15.4	70.6	0.9	12.8	0.3
14	5.0×2.0	13.3	70.2	0.9	15.2	0.4
	Average	14.4	74.4	1.1	8.9	0.4

[1] Samples pooled from many small specimens averaging 0.4 x 0.7 inches.

TABLE 3

Composition of float gases of dead animals

Specimen number	Size	Oxygen and argon	Nitrogen	Carbon monoxide
	inches	%	%	%
1	6.0×2.4	18.6	80.7	0.6
2	6.5×3.0	17.2	82.4	0.4
3	2.5×1.5	13.1	84.0	2.9
4	5.0×2.0	21.9	77.9	0.2

Secretion of carbon monoxide and float permeability

Upon complete removal of the float gases, the animals sank and failed to reinflate. If the float gases were partially removed, they only maintained their diminished volume, and the relative concentrations of the gases remained essentially unchanged. It was not feasible, therefore, to attempt to measure the secretion rate by these procedures. An estimate of secretion rate was obtained, however, by replacing the float gases with either pure nitrogen or oxygen and following the subsequent changes in composition with time. A record was made of the float volume, secretion time and the rise in carbon monoxide content. From these values, it was estimated that the carbon monoxide is secreted at a rate varying from 7.5–120 µl/hr. per animal (table 4). These values are somewhat higher than the figures of 5–20 µl/hr./gas gland reported by Wittenberg ('60) for excised whole gas glands.

TABLE 4

Secretion rates for carbon monoxide

Specimen number	Carbon monoxide in replaced gas	Carbon monoxide after secretion	Total volume	Sampling time	Carbon monoxide secretion
	%	%	*ml*	*hr.*	*µl/hr.*
Diluted with N_2					
1	0.71	1.18	25	7	17.0
2	0.10	1.94	14	6	43.0
3	0.07	0.32	18	6	7.5
4	0.11	0.46	43	4	38.0
5	0.37	0.64	35	4	24.0
6	0.00	0.20	240	4	120.0
Diluted with O_2					
1	0.00	0.16	70	1	112.0
2	0.00	0.30	100	4	75.0

One would expect that the final composition of the gases in the floats should be strongly influenced by the rates of diffusion of gases across the float wall as well as by the rate of carbon monoxide secretion itself. An attempt has been made, therefore, to determine the rate of diffusion of the gases, the diffusion coefficients relative to oxygen, and also the rate of carbon monoxide loss from the float. The procedure was as follows: The existing gases were replaced with pure CO, since this treatment provided a pressure difference across the float membranes for all the gases present. The expected partial pressure difference for each gas was calculated from the analysis obtained after CO replacement, assuming an atmosphere outside the float of 760 mm Hg and composed of 78.03% N_2 and 20.99% oxygen. The change in composition was recorded after four hours, during which time the gases diffused inward and outward to a sufficient extent to allow us to measure reasonably accurately the changes in composition due to diffusion. Since the cross-sectional area and the diffusion distance was identical for all the gases, and since the rates were reduced to equivalent pressure differences, the data become comparable to relative diffusion coefficients. The results of such experiments are shown in table 5. Column 8, table 5 shows the relative rates of diffusion for the three primary gases in the floats of *Physalia*. The data suggest that oxygen moves through the float more readily than either CO or N_2, and that the CO permeability is slightly higher than that of nitrogen. It is felt that the data are due largely to the tissue solubility and molecular weights of the gases. The remaining data of table 5 include a calculation of the rate of CO loss from the animals based on the diffusion rate for CO for each animal (column 7) and a knowledge of the original CO found in the animals when they were collected (column 9). Column 12, table 5 shows that the CO may be lost from these particular animals at a rate varying from 178.6 to 226.6 µl/hr. The data suggest that a CO secretion rate of approximately 200 µl/hr. may be required to maintain the CO level in the normal animal. This is a higher value than we were able to obtain in our secretion experiments (table 4). It is assumed,

TABLE 5

Relative diffusion rates of gases and calculated rate of CO loss from floats

1 Gas	2 Total volume	3 Each gas after repl.	4 Each gas 4 hr. later	5 Initial press. diff.	6 Gas permeating float/4 hr.	7 Crossing float wall	8 Relative diff. rate $O_2 = 1.00$
	ml	*ml*	*ml*	*mm Hg*	*ml*	*µl/mm/hr.*	
O_2		0.30	2.29	155.19	1.99	3.2	1.00
N_2	53	2.39	7.37	558.75	4.98	2.2	0.69
CO		50.31	43.34	721.39	6.97	2.4	0.75
O_2		0.43	1.84	156.03	1.41	2.3	1.00
N_2	94	3.79	7.94	562.40	4.15	1.8	0.78
CO		89.78	84.13	725.88	5.65	1.9	0.83
O_2		0.98	3.96	148.05	2.98	5.0	1.00
N_2	65	4.86	12.81	536.26	7.95	3.7	0.74
CO		59.16	48.22	691.75	10.94	3.9	0.78

9 CO of orig. gas	10 Press. differential for CO across wall	11 CO crossing float wall	12 Rate of CO loss
%	*mm Hg*	*µl/mm/hr.*	*µl/hr.*
12.37	94.01	2.4	226.6
15.54	118.10	1.9	178.6
7.34	55.78	3.9	217.6

therefore, that there is a continuous loss of the secreted carbon monoxide and a continuous replacement of the gas by oxygen and nitrogen in the normal animals.

Tissue respiration in the presence of inhibitors

Carbon monoxide, at tensions normally found in the float gases, apparently does not inhibit tissue respiration. When the carbon monoxide is as high as 80%, normal oxygen uptake rates are obtained if the oxygen is maintained near atmospheric concentrations (table 6). Inhibition is obtained, however, if the oxygen tension of the tissue is lowered by dilution of the oxygen with nitrogen (Harvey and Williams, '58). The level of inhibition resulting from a gas mixture of 4% O_2, 14% N_2 and 82% CO is significant. It should be noted that in the *in vivo* experiments where the normal float gases were replaced with almost pure carbon monoxide, the animals did not appear to experience ill effects. In these experiments, the oxygen was less than 1% of the float gases, but the diffusion of oxygen from the environment through the walls of the floats probably raised the available O_2 to an adequate level.

The use of other cytochrome inhibitors, such as CN^-, N_3^- and $S^=$ produced marked inhibition of oxygen uptake in the excised float tissues (table 7). Strong inhibition was also observed following treatment of the tissues with malonate. If one assumes that a succinoxidase system is the site of action of the malonate, one would conclude that this system probably constitutes a major part of the oxidative pathway in the tissues of the animal. Finally, the stimulation of O_2 uptake resulting from 2,4 dinitrophenol treatment is assumed to represent the uncoupling of an oxidative phosphorylation process.

DISCUSSION

The composition of the float gases reported previously by Wittenberg ('60) and

TABLE 6

Carbon monoxide inhibition of physalia float tissue respiration

Gas	Control flask gas	Exper. flask gas	O_2 Uptake control	O_2 Uptake exper.	Inhibition
	%	%	µl/g/hr. wet wt.	µl/g/hr. wet wt.	%
O_2	20.9	20.0	94.54	96.91	none
N_2	78.0	0.0			
Co	0.0	80.0			
O_2	3.5	2.2	75.94	47.66	37.24
N_2	96.5	7.8			
CO	0.0	90.0			
O_2	4.0	4.0	80.54	49.32	38.76
N_2	96.0	13.7			
CO	0.0	82.2			
O_2	4.3	2.9	75.64	48.66	35.67
N_2	95.7	10.5			
CO	0.0	86.5			
O_2	4.3	2.6	75.64	41.94	44.55
N_2	95.7	9.4			
CO	0.0	88.9			
O_2	2.1	4.4	105.58	63.62	39.74
N_2	97.9	16.8			
CO	0.0	78.8			
O_2	5.2	5.7	84.80	72.00	15.09
N_2	94.8	21.3			
CO	0.0	73.0			
O_2	5.4	5.4	87.12	63.62	26.97
N_2	94.6	23.9			
CO	0.0	70.7			

TABLE 7

Inhibition of physalia float tissue respiration

Inhibitor	Concentration (molar)	Control rate	Exper. rate	Inhibition or stimulation
		$\mu l\ O_2/g./hr.$ wet wt.	$\mu l\ O_2/g/hr.$ wet wt.	%
Cyanide	1.5×10^{-3}	96.9	25.5	73.7
Azide	1.5×10^{-3}	96.9	39.9	58.8
	1.7×10^{-3}	140.0	66.0	52.9
	5.0×10^{-3}	158.0	40.0	74.7
Sulfide	3.3×10^{-3}	146.0	26.0	82.2
Malonate	1.7×10^{-3}	150.0	80.0	46.7
	6.6×10^{-3}	134.0	68.0	49.3
	8.3×10^{-3}	158.0	40.0	74.7
	8.3×10^{-3}	170.0	54.0	68.2
Dinitrophenol	2.1×10^{-4}	127.0	149.0	+17.3
	2.1×10^{-4}	146.0	192.0	+31.5

Clark and Lane ('61) are in general agreement with those presented here, but the carbon monoxide values of the present series are considerably higher. The highest value reported by Wittenberg was 12.7%, while the maximum value given by Clark and Lane was 6.07%. Seven of the 46 specimens examined in the present series exhibited carbon monoxide concentrations exceeding 15%, with the highest being 20.8%. It is apparent, therefore, that animals with extremely high secretion rates can accumulate about one-fifth of an atmosphere of CO in their floats, but ordinarily the value is lower (table 1, 2). The wide variation in the observed levels of CO probably reflects the nutritional status of the animals. This also appears to be the explanation for the wide range of CO secretion rates observed in different animals.

Analyses of the float gases of dead specimens revealed low carbon monoxide and relatively high oxygen and nitrogen contents in contrast to normal living animals. This implies that the carbon monoxide originates from the metabolism of the animal rather than from bacteria or algae. This is further supported by the observation that, although bacteria can be isolated from the interior of both living and dead specimens, these microorganisms were not observed to secrete carbon monoxide when cultured separately.

As previously suggested by Wittenberg ('60), the maintenance of the float gases appears to be attained by a secretion of carbon monoxide with the simultaneous influx of oxygen, nitrogen and argon from the surrounding atmosphere and sea water. Perhaps the major factor in determining the composition of the float gases is the carbon monoxide secretion rate. It establishes the carbon monoxide content of the float gases by influencing the inward diffusion gradient for oxygen, nitrogen and argon, as well as the outward diffusion gradient for carbon monoxide itself. In the normal feeding animal in its natural environment, the secretion rate of carbon monoxide may well exceed 120 µl./hr., which was the maximum rate observed in our experiments.

There are several conditions, in addition to the carbon monoxide secretion rate, which will determine the final composition of the float gases. Among these the differential diffusion rate of the various gases is of primary importance. It has been suggested by Clark and Lane ('61) that the complex structure of the float membranes would have a tendency to minimize the influence of diffusion on determining the composition of the float gases. The data here, however, indicate an appreciable diffusion rate for all the gases, including the carbon monoxide. A barrier to diffusion may exist, as suggested by Clark and Lane, but we are unable to determine the nature or extent of it from the present data. The internal pressure of the float may be of importance, but the ability of

the animal to change the pressure by activity of the float wall musculature makes it difficult to interpret the magnitude of this influence. Other factors which help establish the final composition include the relative amount of the float surface area exposed to air and to sea water, the diffusion coefficients of the gases, and probably the rate of oxygen consumption from the float gas itself. It is apparent that most of these factors are so variable that a complete description of the maintenance of the float gases cannot be obtained from the present data.

Although the respiratory enzymes have not been assayed directly, inhibitors which act on oxidized form of cytochrome oxidase such as CN^-, N_3^-, and $S^=$ prove very effective, indicating the presence of a cytochrome pathway of primary importance in electron transport in the animals. Inhibition of tissue respiration by carbon monoxide could only be obtained when the oxygen was diminished to about 5%. The affinity of carbon monoxide for cytochrome oxidase, or its equivalent, apparently becomes competitive with oxygen when the latter is in a sufficiently low concentration to allow considerable amounts of the enzyme to exist in the reduced state. In the normal *Physalia*, the interior float gases are known to reach 20% carbon monoxide, but the oxygen is apparently adequate to prevent appreciable carbon monoxide inhibition. This is particularly true for the outer layers of the float and the tentacles. The animal, by virtue of its floating habit, is always assured of a near atmospheric oxygen tension in its ocean surface environment.

It should be noted that the local tension of carbon monoxide in the gas producing gland itself actually may reach inhibiting levels. In this regard, the secreting mechanism as well as the electron transport system of the gland tissue is worthy of much further examination.

SUMMARY

1. Analyses of the float gases of 32 Portuguese man-of-war by gas chormatography showed their average content to be 17.4% oxygen and argon, 74.1% nitrogen and 8.5% carbon monoxide. More complete analyses of the gases of 14 specimens by mass spectrometry revealed the following contents: 14.4% oxygen, 74.4% nitrogen, 8.9% carbon monoxide, 1.1% argon, and 0.4% carbon dioxide.

2. Living animals were found to contain more carbon monoxide and less of the atmospheric gases than dead animals.

3. The rate of secretion of carbon monoxide was measured in several animals by a technique of replacing the original float gases by equal volumes of pure nitrogen or oxygen. The rates were found to range from 7.5 to 120 μl/hr. per animal. It is pointed out that this may not be the highest secretion rate the animals can achieve.

4. It is suggested that the floats are probably filled and maintained by the the metabolic production of carbon monoxide and the subsequent diffusion of atmospheric gases into the floats.

5. The float tissue respiration was found to exhibit normal sensitivity to CO, CN^-, N_3^-, $S^=$ and malonate, indicating an electron transport system of the classical type. A special condition may exist, however, in the gas secreting gland tissue.

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