

THE SOURCE OF CARBON MONOXIDE IN THE FLOAT OF THE PORTUGUESE MAN-OF-WAR, *PHYSALIA PHYSALIS* L.

By JONATHAN B. WITTENBERG

*The Marine Biological Laboratory, Woods Hole, Massachusetts and Department of
Physiology, Albert Einstein College of Medicine, New York 61, New York*

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INTRODUCTION

The Portuguese man-of-war is one of the most exotic creatures of the ocean surface. The gas-filled float which serves to buoy up some 30 ft. of tentacles stands well above the surface to catch the wind and attracts attention by its vivid blues, purples and greens. The float of the large Caribbean form is 12 to 18 in. long and contains a full litre of gas. The smaller animals which drift north in the Gulf Stream and on which the bulk of the present work was done contain some 300–500 ml. of gas in the float. Scientists have long been aware (Blainville, 1826, 1830, 1834; Lane, 1960) that the animal can replenish this gas. Haeckel (1888, p. 324), who had travelled extensively in southern seas to study the siphonophores, writes 'By compressing the float voluntarily, the animal can extrude the included air through the apical stigma and sink down. After a short time has elapsed it can rise again, secreting a great mass of gas by the pneumadema, and filling the float. I often observed this process repeated, in December 1866, off the Canary Islands.'

The gases found accumulated in the float are clearly derived in large part from air. Schloesing & Richard (1896) record for two pooled samples of gas 0.0 and 1.7 % CO₂, 12.2 and 15.1 % oxygen, the balance being nitrogen and argon. Argon and nitrogen were present in the same proportion as in air, indicating that these gases entered the float from the air. In addition to the components derived from air, carbon monoxide has been found to comprise a significant proportion of the total gas volume in the floats of animals carried north in the Gulf Stream and captured off Martha's Vineyard Island in Massachusetts (Wittenberg, 1958).

The present experiments establish that the isolated gas gland or pneumadema forms carbon monoxide *in vitro* when supplied with L-serine. Carbon monoxide, it is suggested, probably serves to inflate the float and is later slowly replaced by air through diffusion and exchange.

METHODS AND RESULTS

The gases accumulated in the float

Carbon dioxide, oxygen and non-absorbable gases were determined in the float gases by the gasometric method of Scholander, van Dam, Claff & Kanwisher

(1955). Carbon monoxide was determined gasometrically by absorption with a solution of cuprous chloride in ammonium chloride (Wittenberg, 1960); or, when present in low concentration, estimated colorimetrically (Shepherd, 1947).

Carbon monoxide present in the float gases was identified by the concordance of the volumetric and colorimetric analyses and by the characteristic carboxy-haemoglobin colour and spectrum exhibited by haemoglobin solutions equilibrated with the float gases.

Table 1. *Float gases of Physalia*

Float		Gas composition	
Length (in.)	Volume (ml.)	O ₂ (%)	CO (%)
2	9	19.6	1.4
3	15	15.1	12.7
4	23	14.9	12.6
4	28	18.9	3.5
5	32	17.0	6.7
3	35	19.5	0.4*
5	39	16.5	12.3
4	44	19.4	1*
5	49	16.8	10.3
6	52	14.9	11.8
5	63	16.4	12.6
4	69	18.9	4.5
6	96	17.4	9.1
6	108	19.3	3.7
7	116	18.7	2.9
6	127	18.4	6.1
6	132	18.9	3.3
7	157	17.5	0.5*
6	159	17.8	7.4
6	167	19.4	0.4*
7	168	17.8	6.2
7	170	18.9	5.0
7	185	18.2	2.6
7	186	19.0	2.0
7	187	18.2	4.9
7	190	19.2	3.2
5	251	21.2	2.0
7	254	19.0	4.5
5	281	19.6	1*

* Colorimetric analysis (Shepherd, 1947).

The float gases of fifty individuals captured during the late summers of 1957, 1958 and 1959 at Woods Hole, Massachusetts, contained an average of 16% oxygen (range 13–20%), and an average of 2% carbon monoxide (range 0–11%). An approximate measurement of total combustible gas showed no combustible gas other than carbon monoxide. These specimens had of course come far from their native subtropical seas and were in relatively cool waters when captured. It was felt that a truer picture of the normal float gases would be obtained from animals captured in their native environment. In Table 1 are presented the compositions of the float gases of animals obtained near Miami, Florida, in February and March 1960. I am grateful to Mrs Peter Wangersky who obtained these specimens. It will be seen that the carbon monoxide content is higher than in the

specimens from Massachusetts and is often near 10%. The oxygen content of the float gases of animals from both areas is less than in air, suggesting a metabolic consumption. The carbon dioxide content of all specimens was less than 1%.

The site of carbon monoxide production

Tissues dissected from recently captured *Physalia* were incubated for 20 hr. at 23° C. in 30 ml. portions of sea water contained in 50 ml. syringes. The syringes were agitated frequently during the early hours of incubation. At the end of the incubation period, about 25 ml. of air was drawn into the syringe and any carbon monoxide present was extracted into the gas phase by vigorous shaking. The gas phase was transferred to a second syringe, the extraction with air was repeated, and the air extracts were combined. Because of the limited solubility of carbon monoxide in water, this procedure should extract essentially all of the carbon monoxide present. Carbon monoxide in the air extracts was estimated colorimetrically by the method of Shepherd (1947). The air is passed through a glass tube containing silica gel as a desiccant and a portion of silica gel is treated with palladium sulphate and ammonium molybdate.* The palladium-catalysed reduction of the molybdate leads to the production of a green colour which is estimated by comparison with standards.

The zoöxanthellae, symbiotic algae living in the outer layer of the float, the pneumatocodon, were considered to be a possible source of carbon monoxide. On this assumption, numerous attempts were made to demonstrate carbon monoxide formation by the green and non-green portions of the pneumatocodon, both in the light and in the dark, in the presence and in the absence of serine. No carbon monoxide production was detected in the pneumatocodon. Tentacles were not tested. Carbon monoxide was formed, however, by the inner layer of the float, the pneumatococcus. The data presented in Table 2 establish that in the presence of D,L-serine as substrate carbon monoxide production is sharply localized in the specialized area of the pneumatococcus known as the gas gland or pneumadema. Large bubbles of gas could be seen to form around the gland fragments during the early hours of the incubation.

The gas gland of these large, 7-8 in. long, animals weighed about 0.2 g. The pneumatococcus, of course, is very much larger than the gland and this fact should

Table 2. *The site of carbon monoxide production*

(The tissues were incubated in 30 ml. of 0.02 M D,L-serine in sea water for 20 hr. at 23° C.)

Animal no.	Carbon monoxide formed (ml.)	
	Gas gland	Pneumatococcus
A	0.2	0.000
B	0.4	0.000
C	0.4	0.005
D	0.2	0.000
E, F	0.1	0.000

* Indicating tubes for M-S-A Carbon Monoxide Tester, Catalogue No. DS 47134, Mine Safety Appliances Company, Pittsburgh 8, Pa.

be borne in mind in interpreting the data of Table 2, since the entire non-glandular part of the pneumatosaccus was incubated.

The substrate for carbon monoxide production

Dissected gas glands were divided in quarters and the fragments from several animals were distributed at random among a number of 50 ml. syringes so that each syringe received four fragments. 30 ml. portions of sea water containing dissolved substrates were added and the syringes were incubated 20 hr. at 23° C. Carbon monoxide formed was estimated as before. No carbon monoxide formation was detected with the following solutions of substrates prepared by adding to sea water concentrated solutions adjusted to the pH of sea water: 0.01 M D-lactic acid plus 0.01 M pyruvic acid; 0.02 M 2-ketoglutaric acid; 0.01 M L-aspartic acid plus 0.01 M L-glutamic acid; 0.02 M glycine; 0.02 M D,L-alanine; 0.02 M D,L-threonine; 0.02 M D,L-methionine; 0.05 M methanol; 0.02 M-NaCN. 0.02 M formic acid supported carbon monoxide formation to a limited extent.

Serine was found to be the substrate for carbon monoxide production. The data presented in Table 3 demonstrate the support of carbon monoxide production by L-serine. D,L-Serine was also effective. D-Serine was not tested.

Table 3. *L-Serine the substrate for carbon monoxide production*

(Gas glands were divided into halves and the halves incubated separately in either 30 ml. sea water or 30 ml. 0.01 M L-serine in sea water for 20 hr. at 23° C.)

Animal no.	Carbon monoxide formed (ml. per gas gland)	
	Sea water	0.01 M serine in sea water
G	0.02	0.2
H, I	0.02	0.2
J	0.15	0.4
K	0.00	0.1
L	0.04	0.2
M	0.1	0.4
N	0.1	0.4
O	0.04	0.4
P	0.02	0.2

In the intact animal the gas gland probably receives a continuous supply of nutriment from the main bulk of the stem on which it rests. In the experiments the gland is, of course, deprived of its source of food. In an attempt to replace this supply, gas gland fragments were incubated in 0.02 M D,L-serine solution containing either 0.01 M L-aspartic acid plus 0.01 M L-glutamic acid or 0.5 % casein hydrolysate* or fragments of pneumatocodon. In no case was there any increment in carbon-monoxide production over that found with serine alone. Oxygen may quite possibly be required for the formation of carbon monoxide, and it should be noted that the total supply of dissolved oxygen in the syringe was only 0.16 ml. and that the access of the tissue to this supply was limited by inadequate stirring.

* Tryptic hydrolysate of casein: N-Z-Case, Sheffield Chemical Co., Norwich, New York.

Some components of the gas gland

The gas gland appears faintly coloured to the eye and stands out in contrast to the surrounding pneumatosaccus which is water-white. The colour of the gland is not well defined but extracts are faintly pink. Water extracts of the gland exhibit intense absorption maxima at 260 and 305 $m\mu$ and a very much weaker maximum at 510 $m\mu$ to which the visible colour of the gland probably is referable. The absorbency, calculated for a layer of glandular tissue 1 cm. thick and relative to water, is 80 at 305 $m\mu$ and is 2.1 at 510 $m\mu$.

The reported rapid secretion of gas by *Physalia* implies a very high concentration in the gas gland of the chemical system responsible. It might be anticipated that co-factors utilized by the enzyme system would also be present in high concentration. *A priori* vitamin B₆ (pyridoxine, pyridoxamine, pyridoxal and the corresponding phosphates) might be expected to be required for the activation of serine, and folic acid might similarly be required for the manipulation of the one-carbon fragments which are ultimately transformed to carbon monoxide. Analyses of the gas gland and of the pneumatosaccus for these vitamins and for two vitamins which are probably not involved in carbon monoxide formation are presented in Table 4. Vitamin analyses were carried out by Dr Maria Burger of the Wisconsin Alumni

Table 4. *Vitamin contents of the gas gland*

	(μg. per g. wet weight.)			
	Tissues			
	Gas gland		Pneumato- saccus†	
Vitamin	1*	2†		Method
Folic acid	23.8	23.7	0.10	A.O.A.C. 1955, p. 830
Folinic acid	2.45	2.45	0.07	Sauberlich & Baumann, 1948
B ₆	2.74	2.78	0.15	Atkins <i>et al.</i> 1943
Thiamine	—	3.4	0.47	A.O.A.C. 1955, p. 819
Riboflavin	—	0.50	0.18	A.O.A.C. 1955, p. 823

* Pool of four glands.

† Pool of six glands.

‡ Pool of twelve animals, gas gland not included.

Table 5. *Vitamin contents of some mammalian tissues*

Tissue	(μg. per g. wet weight.)			
	Vitamin			
	Folic acid	B ₆ *	Thiamine†	Riboflavin‡
Beef liver	2.9 †	6.5	2.6	33
Beef kidney	0.58 †	6.7	3.7	26
Beef heart	0.03 †	2.4	5.8	8.9
Beef muscle	0.1 †	2.8	0.7	1.5
Rat liver	1.83 §	—	—	—
Rat kidney	2.50 §	—	—	—
Chicken liver	3.77 †	—	—	—

* Snell & Keevil (1954).

† Stokstad (1954).

|| Snell (1950).

‡ Spector (1956).

§ Mitchell & Isbell (1942).

Research Foundation. For comparison, Table 5 lists the vitamin contents of several animal tissues selected for their high content of folic acid. It will be seen that the vitamin B₉, folinic acid, thiamine and riboflavin contents of the gas gland are in no way remarkable. The folic-acid concentration on the other hand is very high, and is roughly ten times as great as that found in the mammalian tissues. One may infer that folic acid forms a part of the carbon monoxide-producing system and that this system is present in the gland in very high concentration.

DISCUSSION

The present work has established that the carbon monoxide found in the float gases originates in the structure known as the pneumadema or gas gland. This structure has been described (Haeckel, 1888, p. 342; Chun, 1887, p. 559) as a thin layer of ectodermal glandular cylindrical cells. It is necessary to inquire whether the gas gland is indeed responsible for the elaboration of the float gases. The question cannot be answered directly because it has not as yet been studied in *Physalia*. However, Jacobs (1937) has observed the rapid replacement of gas in the floats of the siphonophore *Stephanomia bijuga*. The gas was not analysed. Jacobs noted that the gas unquestionably arose from a structure described as a gas gland and located in the 'funnel' at the base of the simple float of this animal. Chun (1887, p. 559) has traced the embryological development of the gas gland of *Physalia* from a simple structure very similar to that of *Stephanomia* to the highly modified, asymmetric, flattened and expanded structure found in the adult. It is reasonable to conclude that the structure in *Physalia* which is called the gas gland is homologous with the gas gland of siphonophores of simpler structure and is therefore, in all probability, actually responsible for the secretion of gas.

Of great interest in this regard is the extraordinary development of the gas gland described by Haeckel (1888) in siphonophores captured aboard H.M.S. *Challenger* from the depths of the ocean. In these forms the gas gland is massively developed and is housed in a new structure, the aurophore, which provides a greatly increased area of contact between the gland cells and the gas phase of the float. Although the depth of capture cannot be known with certainty, the recorded depths, 640 and 516, 275, 600, fathoms for *Stephalia*, *Stephonalia* and *Rhodalia*, respectively, correspond to hydrostatic pressures of 50–120 atm., and the animals must be capable of secreting gases into the float in the face of these pressures.

The nature of the gases secreted by *Physalia* into the float can only be known by direct analysis of the secretion product of the gas gland, since the accumulated gases may be assumed to have exchanged material with the atmosphere. Unfortunately, the author has not had an opportunity to observe the re-inflation of the float or to analyse the newly secreted gas.

Carbon monoxide clearly is a component, although possibly not the sole component, of the secreted gas. Although the rates of carbon monoxide formation observed *in vitro* were small, they were certainly minimal since the tissue was deprived of a source of nutriment and had a very limited oxygen supply. On the other hand, the very large concentration of folic acid encountered in the gas gland

would imply that the gland is capable of converting L-serine to carbon monoxide very rapidly. Secretion of carbon monoxide, followed by exchange of gases with the atmosphere and metabolic consumption of oxygen, can account for the observed composition of the accumulated gas.

Carbon monoxide accumulation occurs but seldom in nature. The only other example known to the author is the accumulation which has been noted in the hollow stems of marine algae (reviewed in Blinks, 1951) where it may comprise 5% of the total gas. The source of this carbon monoxide is not known.

SUMMARY

1. Carbon monoxide, 0.5–13%, is found in the float gases of *Physalia*. Oxygen comprises 15–20% of the total gas. Carbon dioxide is present in negligible amounts.
2. The gas gland, pneumadema, is the site of carbon monoxide formation.
3. L-Serine is the substrate for carbon monoxide formation.
4. A strikingly large concentration of folic acid is found in the gas gland.
5. It is suggested that carbon monoxide secretion serves to inflate the float of *Physalia* and that carbon monoxide is later slowly replaced by air through diffusion and exchange.

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