THE CAUSE OF MUSSEL POISONING IN SOUTH AFRICA*

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During the past 130 years there have been several human deaths from mussel poisoning around the coast of South Africa. This paralytic type of shellfish poisoning is known to be caused by certain toxic species of planktonic dinoflagellates which are ingested by filter-feeding molluscs such as sand mussels, black mussels and oysters. The ingested toxin accumulates in the body of the molluscs, concentrating particularly in the digestive gland or liver, usually without harming the molluscs. The pharmacological action of the poison extracted from local mussels has been shown to be similar to that isolated from shellfish in other parts of the world.[23,25] However, until now the source of the poison involved in South Africa was not known. Many species of dinoflagellates become so abundant at times that they cause a reddish discoloration of the sea, known as red water.[1,7-9] Most of the species causing red water are probably harmless, but a few are known to produce mussel poisoning.

Both red water and mussel poisoning have been known for many centuries, although their association was not recognized by scientists or medical men until Sommer and his colleagues[27] established their relationship. They demonstrated a direct relationship between the number of *Gonyaulax catenella* in the sea and the toxicity of the mussel *Mytilus californianus*. The methods established by Sommer and Meyer[26] for extracting and assaying the poison form the basis of most studies today.

In the 30 years since Sommer and his associates demonstrated that *Gonyaulax catenella* was the source of the poison on the coast of California, several other dinoflagellates have been found to be sources of toxin. Koch[13] found that a dinoflagellate, *Pyrodinium phoneus,* was the source of the toxin involved in mussel poisoning in Belgium. Needler[17] produced evidence that the dinoflagellate *Gonyaulax tamarensis* is the primary cause of shellfish poisoning on the Atlantic coast of Canada. Prakash and Taylor[20] have found that *Gonyaulax acatenella* is a source of the toxin on the Pacific coast of Canada. In Portugal it has been suggested that *Prorocentrum micans* is the source of the toxin,[19] and in Japan a species of Prorocentrum has been implicated.[16]

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In May 1968, *Gonyaulax tamarensis* was found to be the cause of mussel poisoning off the Northumbrian coast of Britain, causing the illness of more than 8 people and the death of large numbers of sea birds and other marine fauna.[15,29] The primary source of the poison is not known in other parts of the world, although mussel poisoning has been recorded from many different countries. Halstead, in his review,[10] lists many early examples of mussel poisoning, some occurring as early as 1689. Woodcock[30] showed that the toxic products of *Gymnodinium brevis,* which causes red tides along the Florida coast, act as an upper respiratory irritant and do not produce mussel poisoning of the paralytic type.

SOUTH AFRICAN CASES

There have been cases of mussel poisoning around the coast of South Africa resulting in human deaths in 1837, 1888, 1948, 1957 and 1958.

The 1837 case has been referred to in reviews as mussel poisoning,[30] but the circumstances of the case as quoted by Gilchrist[4] suggest that it might have been gastroenteritis from eating decaying fish which washed up in masses: '. . . some of the poor blacks ate of the dead fish and many died with excruciating agonies therefrom'.

About 1888, many people became ill and some are believed to have died after eating shellfish when the waters of Table Bay at night 'appeared like a sea of phosphorus'. At about this time several baboons were seen lying dead on the beach near Simonstown with clam shells in their paws. Gilchrist[4] suggested that they 'must have been poisoned by eating the white mussel (*Donax serra*) which at certain seasons appears to be poisonous'. There would thus appear to have been red water causing mussel poisoning in both Table Bay and False Bay at that time.

In April 1948 several people became ill after eating white mussels (*Donax serra*) and black mussels (*Chloromytilus meridionalis*) from the Blouberg area near Cape Town. One man died as a result of eating the latter type.[22] The sea at Blouberg had been luminescent and an unusual red colour during the previous fortnight. Von Bonde[28] suggested that the source of the toxin was the planktonic flagellate *Gonyaulax catenella,* presumably on

the basis of the findings in California, as no examination of the plankton was made and *G. catenella* had not been recorded from South African waters at that time.

In December 1957, 5 people became ill and one died[24,24] after eating black mussels (*Perna perna*) collected from the rocks at Tongaat on the Natal coast. (They were referred to as *Mytilus meridionalis* at the time, but this is the west coast species and is not found on the Natal coast.) No red discoloration of the sea was noticed at the time. However, minute dinoflagellates were discovered in the rejection currents from the gills of all the mussels by W. Pople of Natal University. He suggested that they were *Gonyaulax catenella*, but admitted that with the equipment available it was impossible to identify them exactly. Their minute size (3 - 4 μ) appears to preclude the above identity; the permanent slides that were prepared were subsequently sent to the USA for study, but appear to have been lost.

In May 1958 a death occurred in Cape Town as a result of poisoning by black mussels (*Chloromytilus meridionalis*) collected at Paternoster on the west coast.[24] Other cases also occurred but no other fatalities were reported. It was suggested by Marchand (personal communication)[24] that Gymnodinium, which is one cause of red water on the west coast of Southern Africa, was the source of the poison. This organism was reported to have been identified in local mussels of the kind that have proved to be poisonous. However, enquiries have revealed that, as in previous cases, no critical examination of the causative organisms had been made.

Observations During 1966/67

During December 1966 red water caused by a high concentration of the dinoflagellate *Gonyaulax grindleyi* Reineke appeared off the west coast of South Africa. The red water resulted in the death of large numbers of white mussels (*Donax serra*), black mussels (*Chloromytilus meridionalis*) and many other marine invertebrates. Toxicity tests were carried out on mussels from the affected area. These tests revealed that both white and black mussels, including live specimens, were highly toxic. Warnings were issued over the radio and in the press, and no cases of human mussel-poisoning occurred. Tests in succeeding months revealed that mussels at Elands Bay remained toxic for 4 months, and mussels further south were toxic 6 months later. Tests of extracts from the cells of *Gonyaulax grindleyi* indicated that it did not contain toxins of the mussel-poison type. Therefore it appeared that *Gonyaulax grindleyi* had not caused the mussel poisoning, although it was apparently responsible for the mortality of marine invertebrates. Small numbers of *Gonyaulax catenella* (Whedon and Kofoid) were observed in offshore samples at that time. This was the first time the species had been recorded off South Africa. As *Gonyaulax catenella* is known to be a source of mussel poison off California, it was suggested that it was probably the cause of the mussel poisoning.[5,6] The distribution of toxicity in 1966/67 and the locations from which shellfish causing fatalities were collected are shown in Fig. 1.

Observations During 1967/68

In December 1967 *Gonyaulax catenella* appeared in large numbers off the west coast,* further supporting the suggestion that it was the cause of poisoning.

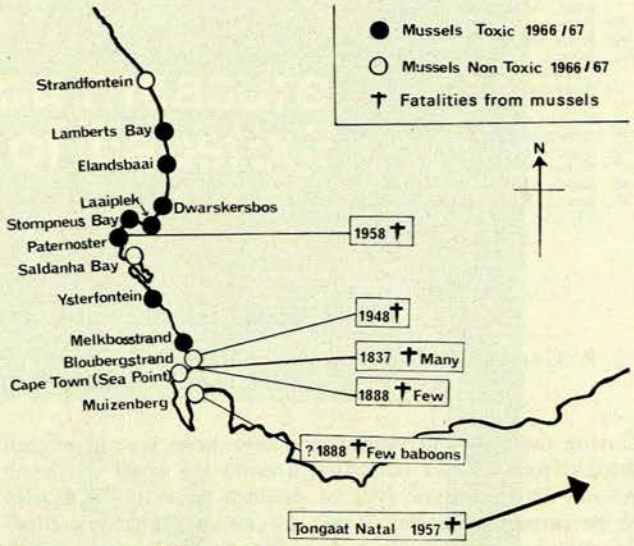

Fig. 1. Distribution of toxicity in summer and autumn 1966/67, and localities from which shellfish causing fatalities were collected.

Observations During 1968/69

On 8 December 1968 Elands Bay was again visited with all the necessary equipment for sampling. It was considered that the time and conditions were right for red water to be developing, and this proved to be correct. Local fishermen reported that the sea had become discoloured on 4 December and that there had been red water since 6 December. Light offshore winds had tended to concentrate the red water inshore, while warm weather and sunshine would have accelerated growth. On 8 December the weather was still sunny, the air temperature 27°C (in the shade) and the wind light south-westerly (Force 2 freshening to Force 3). As the wind freshened, upwelling of cold subsurface water occurred in Elands Bay, so that cool greenish water was present off the beach while red water was present off the point (Cape Deseada). Samples of phytoplankton and mussels were taken for testing. The material was refrigerated and flown back to Port Elizabeth for analysis. Warnings were again issued over the radio and in the press when the mussels were found to be dangerously toxic.

METHODS

Details of the hydrological and planktological methods employed in this work are given in a separate report on the earlier part of this investigation.[6] In the work described here, water samples for analysis and studies of phytoplankton and toxicity were taken at 3 points at Elands Bay on 8 December 1968: surface samples off the point (Cape Deseada), surface samples off the beach, and samples from a depth of 3 metres, at the fishing jetty. The number of phytoplankton cells per litre of the dominant species and the percentage composition of the samples were determined. Large volumes of water were filtered through

*Personal communication, E. A. Nel.

material with a 28-μ aperture size to obtain a sufficient weight of Gonyaulax cells for toxicity studies. The phytoplankton samples were centrifuged and the sedimented cells removed and weighed wet, and then weighed portions were dried to constant weight at 100°C. A sample of black mussels (*Chloromytilus meridionalis*) was collected to determine their toxicity.

Extracts of toxin from mussels and phytoplankton were prepared using the method of Sommer and Meyer.[10,26] Toxicity was determined by intraperitoneal injection into mice. Highly toxic extracts were diluted serially with distilled water to produce death in 4 - 10 minutes. Corrections were applied for the death time of the mice and the weight of the mice, employing Sommer's tables of time-dosage and time-weight relations.[10]

RESULTS

The results of the analysis of the phytoplankton are presented in Table I. The results of studies in 1966/67 have been presented separately.[5,6] *Gonyaulax catenella* was the only dinoflagellate occurring in large numbers at Elands Bay in December 1968. They comprised up to 40% of the cells in the phytoplankton in red water off Cape Deseada with a maximum concentration of $1{\cdot}14 \times 10^6$ cells/litre. Almost all the remainder of the phytoplankton was composed of diatoms, and chains of *Skeletonema costatum* predominated. They comprised 48% - 85% of the cells in the different samples, but their cell size is far smaller than that of *G. catenella*.

TABLE I. PHYTOPLANKTON

	Point (0 metres)	*Beach (0 metres)*	*Jetty (3 metres)*
Temperature	15·6°C	12·1°C	14·5°C
Salinity	35·053%	35·066%	34·677%
Sea colour	Reddish-brown	Greenish-brown	(sub-surface)
Gonyaulax catenella (Whedon and Kofoid)			
Concentration	$1{\cdot}14 \times 10^6$/litre	$0{\cdot}12 \times 10^6$/litre	Nil
% of cells	45 - 49%	5 - 9%	0%
Cell pigmentation	Orange-brown	Greenish-yellow	—
Other phytoplankton			
Concentration	$1{\cdot}24 \times 10^6$/litre	$1{\cdot}32 \times 10^6$/litre	$1{\cdot}56 \times 10^6$/litre
% of cells	51 - 55%	91 - 95%	100%
Composition (% of total cells)			
Skeletonema costatum (Greville) Cleve	48%	71%	85%
Nitzschia seriata Cleve	—	4%	3%
Chaetoceros spp.	1%	3%	3%
Asterionella japonica Cleve and Möller	—	3%	—
Pleurosigma capense Karsten	—	2%	—
Stephanopyxis turris (Greville and Arnot) Ralfs	—	2%	3%
Thalassionema nitzschioides Hustedt	—	—	2%
Biddulphia sp.	—	—	2%
Gyrosigma sp.	—	2%	—
Diploneis sp.	—	2%	—
Nitzschia closterium (Ehrenberg) W. Smith	—	—	1%
Acnanthes sp.	—	1%	—
Peridinium depressum Bailey	1%	—	—
Unidentified resting stages	1%	—	1%
Colourless flagellates	—	2%	—

The results of the toxicity determinations are presented in Table II. The high toxicity of the phytoplankton containing *Gonyaulax catenella* leaves little doubt that this dinoflagellate is the source of the mussel poison here. Further confirmation of this is provided by the correlation between the percentage of *Gonyaulax catenella* and the toxicity of the samples. The toxicity of one milligram of Californian *Gonyaulax catenella* is given as 66 mouse units.[10] The toxicities determined here for 45% and 8% *G. catenella* correspond closely to the Californian figure. Owing to the smaller size of most of the other phytoplankton cells, the correlation is not quite as close as implied by these figures, but it is still similar. The toxicity of the *Gonyaulax* in the beach sample is relatively lower; this may be related to their greenish-yellow pigmentation.

It has been suggested[20,27] that the development of the reddish colour is indicative of physiological changes which might be related to the occurrence of toxin. The greatly reduced toxicity of the dried phytoplankton results from the toxin being denatured.[10] The toxicity of the mussels at 42,000 units/100 G is dangerous to man, as the estimated lethal dose for man is 36,500 mouse units.[10] The toxicity of mussels from Holy Island in the recent British outbreak reached 20,800 mouse units/100 G,[3] while toxicities in excess of 100,000 mouse units/100 G have been recorded from the Bay of Fundy, Canada.[10]

As black mussels form an important part of the diet of the Cape rock lobster, *Jasus lalandii*, which is widely eaten by man, it was considered advisable to examine rock lobsters for toxicity. Rock lobsters from Lamberts Bay and Elands Bay were examined while the mussels were toxic in 1967. The results are summarized in Table II. It would appear that mussel poison is not taken up by the bodies of the lobsters although it may appear in the proventriculus, presumably in the form of undigested portions of mussel. The tail of the lobster, which is the part normally eaten by man, was quite unaffected.

TABLE II. TOXICITY

Material examined	*Toxin found*		
Shellfish:			
Black mussels (*Chloromytilus meridionalis*)	42,000 mouse units/100 G		
Phytoplankton:			
From point (45% *G. catenella*)	30 mouse units/mg. dry weight		
From point (after drying)	2 mouse units/mg. dry weight		
From beach (5 - 9% *G. catenella*)	0·5 mouse units/mg. dry weight		
From beach (after drying)	< 0·1 mouse units/mg. dry weight		
Rock lobsters:	*Locality*		
	Proventriculus	*Digestive gland*	*Tail*
Lamberts Bay	+	—	—
Elands Bay	+	—	—
Sea Point (mussels non-toxic)	—	—	—

The Causative Organism

The form of *Gonyaulax catenella* found here is essentially similar to that described from California by Whedon and Kofoid. Specimens are illustrated in Fig. 2. A few chains of 2, 3 and 4 cells were observed, but most cells occurred singly. The size and proportions of the cells varied considerably (30 - 43 μ × 37 - 50 μ; mean 35 × 42 μ). The squat proportions of the cells, the presence of a curtain fin on the girdle and the arrangement of the thecal plates characterize the species. The form of the plates in the ventral region was found to be somewhat variable. Prakash and Taylor[20] have noted the resemblance between *Gonyaulax catenella* and *G. tamarensis*, and they note that *G. catenella* shares some common features. Pieterse and Van der Post[18] have recorded *Gonyaulax tamarensis* in Walvis Bay in concentrations up to 4×10^6 cells/litre and

noted cells linked together in chains of 2 to 3 cells in the manner of *G. catenella*. (They found no indication of toxicity in mussels in that area despite regular testing.) The resemblances between the various toxic species emphasize their close relationship.

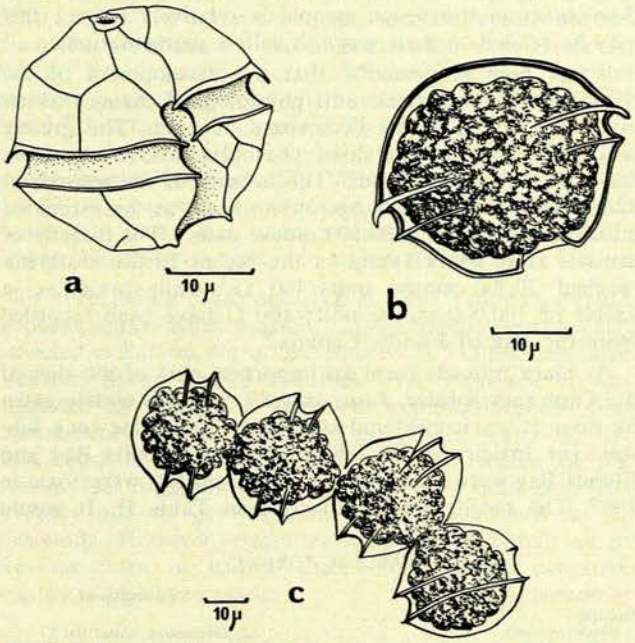

Fig. 2. Gonyaulax catenella from Elands Bay. a: Ventral view of theca showing plates. b: Typical appearance of living cells. c: Chain of 4 cells. Scale lines represent 10 μ.

MUSSEL POISON

Mussel poison is known by various names, including paralytic shellfish-poison, mytilotoxin and saxitoxin. The toxin from mussels and clams as well as *Gonyaulax catenella* and *G. tamarensis* has been found to have identical physical, chemical and pharmacological properties.[20,21] The toxin from local mussels also has similar pharmacological properties.[23,25] The toxin is basic in nature, forming salts with mineral acids. The molecular formula is $C_{10}H_{15}N_7O_4 . 2HCl$ and the molecular weight is 370.[12] It has been suggested that ring structures are present and that several of the nitrogen atoms are involved in a heterocyclic structure.[21]

The toxin is one of the most lethal biological toxins known. The principal action is on the central nervous system (respiratory and vasomotor centres) and the peripheral nervous system (neuromuscular junctions, cutaneous tactile endings and muscle spindles). The toxin is absorbed slowly from the gastro-intestinal tract and excreted rapidly by the kidneys. It depresses respiration, the cardio-inhibitory and vasomotor centres and conduction in the myocardium.[12,21]

In the recent British outbreak of mussel poisoning it was found that the level of serum creatine kinase was raised in 2 severely affected patients and it was suggested that this might be used as an indication of the severity of intoxication.[15]

It has been suggested that the toxin might be formed by bacteria with the dinoflagellates, but several American workers have shown that bacteria-free cultures are still capable of producing the poison.[21]

Clinical Characteristics of Paralytic Shellfish-Poisoning

Paralytic shellfish-poisoning may be diagnosed readily by the presence of pathognomonic symptoms which usually appear within 30 minutes. A tingling sensation of the lips, gums, tongue and face is felt initially and gradually spreads to the neck, arms, fingertips and toes. The paraesthesia later changes to numbness, so that voluntary movements are made with difficulty. In severe cases ataxia is accompanied by a 'floating' sensation. Constrictive sensations of the throat, incoherent speech and aphonia occur in severe cases. In the terminal stages of the disease, motor weakness and paralysis become progressively more severe, and death occurs as a result of respiratory paralysis usually within a period of 12 hours. The prognosis is good if the patient survives the first 12 hours.[10]

Paralytic shellfish-poisoning must be distinguished from gastro-intestinal shellfish-poisoning caused by bacterial pathogens, with a relatively long incubation period of 10 - 12 hours and symptoms of nausea, vomiting, diarrhoea and abdominal pain. Erythematous shellfish-poisoning is caused by allergy, has an incubation period of a few hours, and symptoms characteristic of an allergic reaction occur, including diffuse erythema, swelling and urticaria that particularly affect the face and neck.

No specific antidote is known. Treatment of paralytic shellfish-poisoning is therefore largely symptomatic. Alkaline fluids, such as sodium bicarbonate solution for stomach wash-out, are of value since the toxin is unstable in alkaline media. Adsorbents may be tried. Diuresis may be instituted. Assisted ventilation is important if there is respiratory embarrassment. Drug therapy has varying degrees of success. Anticurare drugs such as neostigmine are useful in aiding artificial respiration. Amphetamine, noradrenaline and ephedrine have also been recommended, while oximes such as pralidoxime have been advised to reactivate acetylcholine esterase.[10,22]

DISCUSSION

Mussel poisoning appears to be very rare on the south and east coasts of South Africa.

Although *Gonyaulax catenella* has been shown to be the cause of mussel poisoning on the west coast of South Africa, different organisms may be involved on the eastern seaboard. Indeed, because of the completely different hydrological conditions, it is unlikely that the same organism would be involved.

The only recorded death from mussel poisoning on the east coast occurred at Tongaat on the Natal coast in 1958. The causative organism is not known. No red water was present.

In October 1968 low levels of toxicity of mussels and oysters were recorded in Algoa Bay near Port Elizabeth following red water caused by the dinoflagellate *Prorocentrum micans*. This species has been associated with mussel poisoning in Portugal[19] and Japan.[16] Mussels and oysters are widely eaten on the south and east coasts of South Africa, particularly by the indigenous population. The

rarity of cases of mussel poisoning on the south and east coasts of South Africa suggests that it is of relatively slight importance on these warmer coasts.

It is interesting that although the Strandloper middens round the South African coast provide evidence that the diet of these people was composed largely of shellfish, they were not decimated by mussel poisoning even on the west coast. Archaeologists have not found a single example of human mass mortality attributable to mussel poisoning, although Hoffman[11] suggested that a diet of 90% of Mytilus played a role in the pygmyation of 'pre-bushman' peoples.

There does not appear to have been any local folklore or taboos among the indigenous people of South Africa regarding mussel poisoning. However, in North America, before Europeans reached the Pacific coast, the Red Indians are reputed to have watched the sea for streaks of red water and luminescence at night (caused by the presence of toxic dinoflagellates). The chiefs forbade the taking of mussels and posted guards to warn those not acquainted with the dangers.[2] Interviews with residents on the west coast of South Africa suggest that there are prejudices among the residents against eating mussels today, but visitors are likely to be unaware of the danger. The incidence of mussel poisoning on the west coast suggests that there is a need for regular testing of shellfish for toxicity.

SUMMARY

The occurrence of mussel poisoning in South Africa and the causes of mussel poisoning elsewhere are reviewed. Recent investigations of mussel poisoning on the west coast of South Africa are described. It is shown that the cause of mussel poisoning in this area is the planktonic dinoflagellate *Gonyaulax catenella*. This is confirmed by extraction of the toxin directly from the phytoplankton.

It is demonstrated that the toxicity of *Gonyaulax catenella* cells off the west coast of South Africa is similar to that determined off California (30 mouse units/mg., 45% Gonyaulax). The toxicity of black mussels at Elands Bay on 8 December 1968 was 42,000 mouse units/100 G (estimated lethal dose for man 36,500 mouse units). In 1967 mussels at Elands Bay remained toxic for 4 months. The nature and action of the toxin and the significance of mussel poisoning in South Africa are discussed.

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REFERENCES

1. Braarud, T. (1957): Galathea Deep Sea Expedition, **1**, 137.
2. Carson, R. L. (1951): *The Sea Around Us*, p. 33. New York: Oxford University Press.
3. Clarke, R. B. (1968): Lancet, **2**, 770.
4. Gilchrist, J. D. F. (1914): Marine Biological Report, Cape Town, **2**, 8.
5. Grindley, J. R. and Nel, E. A. (1968): S. Afr. J. Sci., **64**, 420.
6. *Idem* (1969): Fisheries Bulletin, South Africa (in the press).
7. Grindley, J. R. and Taylor, F. J. R. (1962): Nature (Lond.), **195**, 1324.
8. *Idem* (1964): Trans. Roy. Soc. S. Afr., **37**, 111.
9. *Idem* (1969): *Ibid.* (in the press).
10. Halstead, B. W. (1965): *Poisonous and Venomous Marine Animals of the World*, vol. I, p. 157. Washington, DC: US Government Printing Office.
11. Hoffman, A. C. (1952): Navorsinge van die Nasionale Museum, Bloemfontein, **1**, 281.
12. Kao, C. Y. (1966): Pharmacol. Rev., **18**, 997.
13. Koch, H. J. (1938): Arch. méd. Soc. Hyg. (Brux.), **9**, 796.
14. Mann, N. M. and Winship, W. S. (1958): S. Afr. Med. J., **32**, 548.
15. McCollum, J. P. K., Pearson, R. C. M., Ingham, H. R., Wood, P. C. and Dewar, H. A. (1968): Lancet, **2**, 767.
16. Nakazima, M. (1965): Bulletin of the Japanese Society of Scientific Fisheries, **31**, 198.
17. Needler, A. B. (1949): Journal of the Fisheries Research Board of Canada, **7**, 490.
18. Pieterse, F. and Van der Post, D. C. (1968): Investigational Reports of the Marine Research Laboratory, South West Africa, **14**, 1.
19. Pinto, J. S. and Silva, E. S. (1956): Notas e Estudos do Instituto de Biologia Maritima, Lisboa, **12**, 1.
20. Prakash, A. and Taylor, F. J. R. (1966): Journal of the Fisheries Research Board of Canada, **23**, 1265.
21. Russell, F. E. (1965): Advances in Marine Biology, **3**, 255.
22. Sapeika, N. (1948): S. Afr. Med. J., **22**, 337.
23. *Idem* (1953): Arch. int. Pharmacodyn., **93**, 135.
24. *Idem* (1958): S. Afr. Med. J., **32**, 527.
25. *Idem* (1968): *Ibid.*, **42**, 1213.
26. Sommer, H. and Meyer, K. F. (1937): Arch. Path., **24**, 560.
27. Sommer, H., Whedon, W. F., Kofoid, C. A. and Stohler, R. (1937): *Ibid.*, **24**, 537.
28. Von Bonde, C. (1948): S. Afr. Med. J., **22**, 760.
29. Wood, P. C. (1968): Nature (Lond.), **220**, 21.
30. Woodcock, A. H. (1948): Journal of Marine Research, **7**, 56.