

# Biological Effect of Asbestos Dust on the Peritoneal Viscera of Rats\*

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## SUMMARY

Crocidolite and chrysotile asbestos suspensions were injected intraperitoneally into rats. Peritoneal mesotheliomas were induced in 90% of the crocidolite group and in 30% of the chrysotile group.

The pathology and possible pathogenesis are described and discussed. It is postulated that the asbestos fibres are encapsulated by fibrous tissue and that a soluble carcinogen then diffuses from these fibres to the mesothelial cells, causing anaplastic changes.

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Reports from many countries<sup>1-4</sup> indicate the association of asbestos with malignancy. After a comprehensive review Wagner *et al.*<sup>5</sup> concluded that exposure to chrysotile alone, is a rare cause. Exposure to crocidolite or amosite or both substances, with or without chrysotile, is to blame in the majority of cases, whereas the position of amosite is intermediate. From a recent survey<sup>6</sup> of 232 cases with diffuse pleural mesothelioma diagnosed by the National Research Institute for Occupational Diseases, Johannesburg, it appeared that no direct relationship exists between exposure to crocidolite and the development of pleural mesothelioma. It is further suggested that crocidolite and amosite must be associated with another factor, possibly a mineral, before malignant changes can be induced.

The experimental induction of asbestosis was commenced by Gloyne<sup>7</sup> and extended by Vorwald *et al.*<sup>8</sup> The tissue response to asbestos consisted of a foreign body reaction with giant cells, granulation tissue and the appearance of ferruginous bodies. After exposure of rabbits, monkeys, and guinea pigs<sup>9</sup> to chrysotile, these species showed an increasing response in the order stated, whereas amosite induced an equally severe response. Asbestosis<sup>9</sup> produced by chrysotile in rats appeared not to be progressive, but was progressive and lethal in hamsters.

Experimentally, lung tumours were successfully induced in mice<sup>10,11</sup> and rats<sup>9</sup> after inhalation of chrysotile. Better results were obtained by the injection of dusts into the pleural cavity of rats<sup>12,13</sup> and hamsters.<sup>14</sup> Peritoneal tumours were also produced by the injection method.<sup>15-17</sup>

From clinical evidence it would appear that exposure to crocidolite asbestos seems to be associated with a higher incidence of malignant pleural mesothelioma, than from exposure to chrysotile. Owing to species differences, no conclusive evidence has been gained from animal ex-

periments to confirm the clinical observations. A long-term experiment was planned, therefore, to investigate the biological effect of crocidolite and chrysotile asbestos on the abdominal viscera of the rat after intraperitoneal injection of dust suspensions.

## MATERIALS AND METHODS

### Asbestos Samples

Two UICC standard reference asbestos samples, i.e. crocidolite and chrysotile, were used to prepare suspensions in normal saline (50 mg/ml). The suspensions were sterilized by autoclaving before use.

### Animals

Female albino rats of the same age and weight (155 ± 10 g) were used. The animals were divided at random into 2 groups of 10 each. Group 1 received crocidolite, and group 2 chrysotile suspension.

### Injection of Dusts

The animals were lightly anaesthetized with ether, and tied to a dissecting board. A sterile needle was carefully inserted in the left inguinal region through the anterior abdominal wall, and 1 ml of suspension followed by 1 ml of air, were forced into the abdominal cavity.

### Duration of Experiment and Examination Technique

A survival experiment was planned. The animals were inspected daily for signs and symptoms of abdominal tumours. An animal was killed as soon as ascites developed, the abdomen exposed, and the organs carefully inspected. Blocks of tissue for histological examination were selected from areas with pathology. The preparations were stained with haematoxylin and eosin and Perl's iron stain for evaluation of the histopathology. Smear preparations of ascitic fluid were made and stained with Leishmann's stain.

## RESULTS

In the crocidolite group, 9 animals developed peritoneal tumours, whereas only 3 rats from the chrysotile group showed a similar pathology. Macroscopically the tumours

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appeared to be spherical nodules and plaque-like growths (Fig. 1). The spherical nodules on the viscera varied from nodules with a diameter of 1-2 mm to big ones with a diameter of more than 1 cm. The plaque-like growths were localized, especially on the viscera in the vicinity of the stomach, spleen, pancreas, and liver. In some areas these plaques joined the organs into a tight mass.

In 4 rats injected with chrysotile, peritoneal abscesses developed. Two others survived 18 months without signs of pathology. One rat from each group died soon after the intraperitoneal injection. Apart from an acute inflammatory response, no pathology could be detected in these animals.

The volume of ascitic fluid varied from 10 ml to more than 100 ml. It was mostly haemorrhagic and yellowish brown.

All the tumours developed 10-17 months after the start of the experiment.

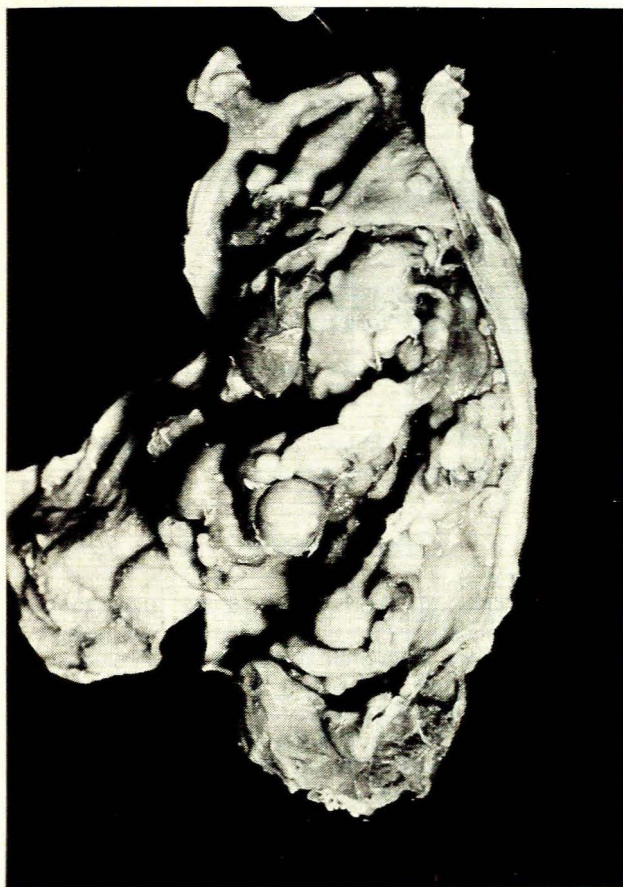


Fig 1. Exposed abdomen of a rat illustrating nodular and plaque-like growths.

## Histology

The peritoneal nodules consisted of a fibrous centre encapsulating large numbers of asbestos fibres, either lying free in the interstitial tissue or partly phagocytosed by

macrophages or foreign-body giant cells. Most of the fibres stained iron-negative, but some gave a positive reaction and showed up ferruginous bodies. These bodies were always closely associated with the fibrous nodules, but could not be detected in the H. and E.-stained preparations. Malignant cells were found proliferating from the mesothelial layer of these nodules (Fig. 2). They formed secondary nodules of malignant cells only (Fig. 3).

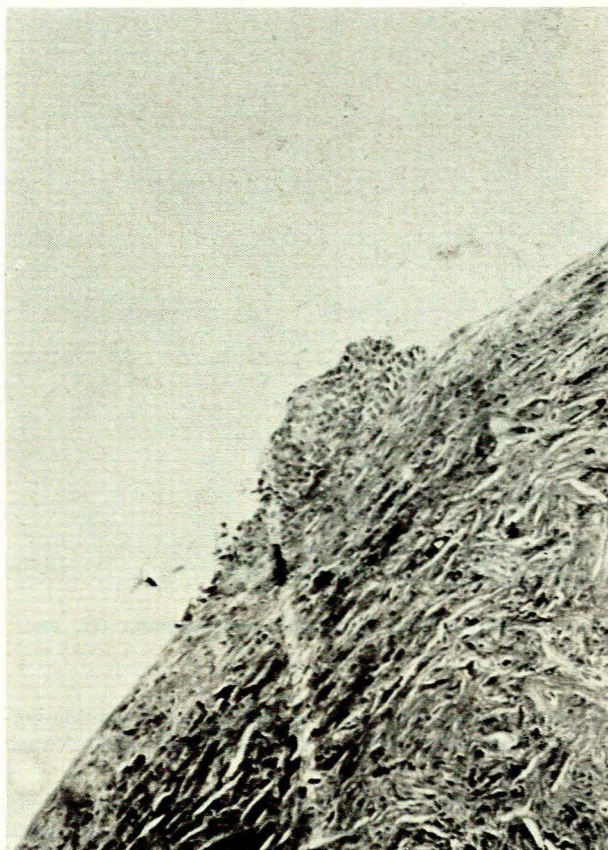


Fig. 2. Malignant mesothelial cells proliferating at the edge of fibrous nodule (H. and E.  $\times 100$ )

The plaque-like growths consisted mainly of tumour cells either surrounding or infiltrating the various organs. Areas of fibrous tissue containing many asbestos particles were seen in the tumours, which might indicate that these plaques were nodular in origin.

The cyto-architecture of these tumours varied greatly from area to area within one tumour and also between different tumours. The following patterns with transitional forms were observed.

1. Loosely arranged polyhedral cells with little intercellular substance. The polygonal cells contained eccentric nuclei, the appearance of which ranged from vesicular to hyperchromatic, with an eosinophilic cytoplasm (Fig. 4).

2. Solid sarcoma-like tissue consisting of spindle-shaped cells and varying amounts of intercellular tissue. The

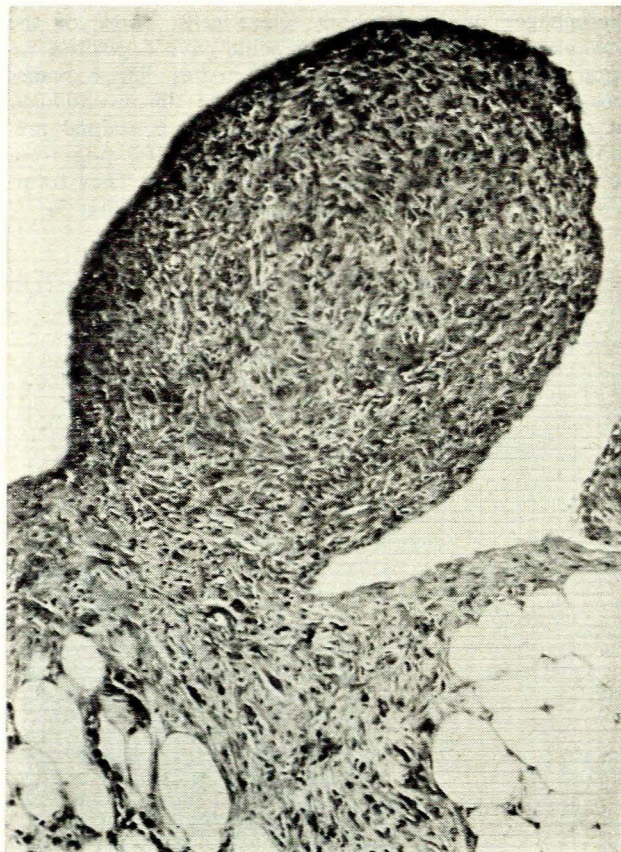


Fig. 3. Small nodular malignant mesothelioma (H. and E.  $\times 100$ ).

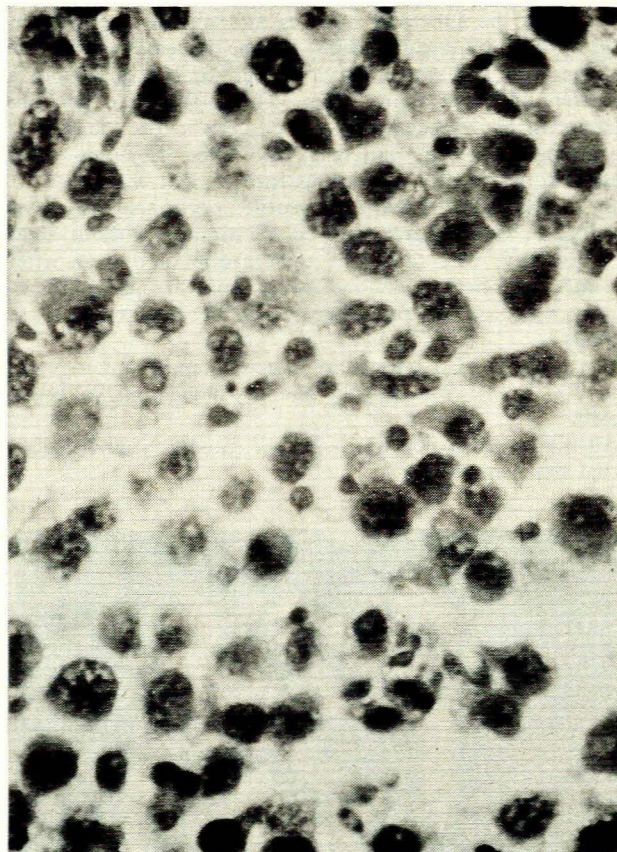


Fig. 4. Polyhedral tumour cells (H. and E.  $\times 250$ ).

nuclei were pleomorphic with elongated forms predominating. The cytoplasm was eosinophilic with well-defined margins (Fig. 5).

3. Gland-like, partly papillary tissue with polygonal and spindle-shaped stromal cells. The tissue clefts were lined by big polygonal and cuboidal cells with much eosinophilic cytoplasm and very large round and oval vesicular nuclei (Fig. 6).

4. Tissue consisting of spindle-shaped stroma cells with clefts which were lined by polygonal cells (Fig. 7). Mitotic figures were abundant in all cell types.

One rat from the crocidolite group developed a tumour of the uterus. Structurally, this tumour was entirely different from the others described and consisted of a hyalinized stroma with large numbers of spindle-shaped cells. Included in the stroma were islets of concentrically arranged polygonal cells with an acidophilic cytoplasm and moderately pleomorphic nuclei. On the periphery, spindle-shaped cells continuous with the hyalinized stroma were found.

Tumour cell infiltration of the liver, pancreas, spleen, abdominal wall and gut wall occurred in almost every case. In 1 case metastases were found in the liver.

The cell population of the ascitic fluid consisted of erythrocytes and malignant cells with phagocytic charac-

teristics. Many of the latter cells had fragments of, or whole, erythrocytes in their cytoplasm.

## DISCUSSION

Previous experimental work indicated that the initial reaction induced by asbestos intrapleurally or intraperitoneally, consisted of a granulomatous inflammation and subsequent fibrosis.<sup>15,17</sup> According to Jagatic *et al.*<sup>15</sup> small nodules and plaque-like structures appeared at a later stage on the serosal surface of the abdominal viscera. This finding correlates well with the histopathology found in the present investigation.

Malignant cells were first observed on the periphery of the fibrotic areas in close approximation to the mesothelial layer. These cells proliferated to form secondary nodules which eventually changed into plaque-like tumours. The fibrotic areas seen in these plaques might be indicative of their nodular origin.

From our observations it would appear that the pathogenesis of these tumours might proceed through different stages:

1. The granulomatous stage during which the asbestos fibres were actively phagocytosed by macrophages, foreign body giant cells, and, possibly, by mesothelial cells,

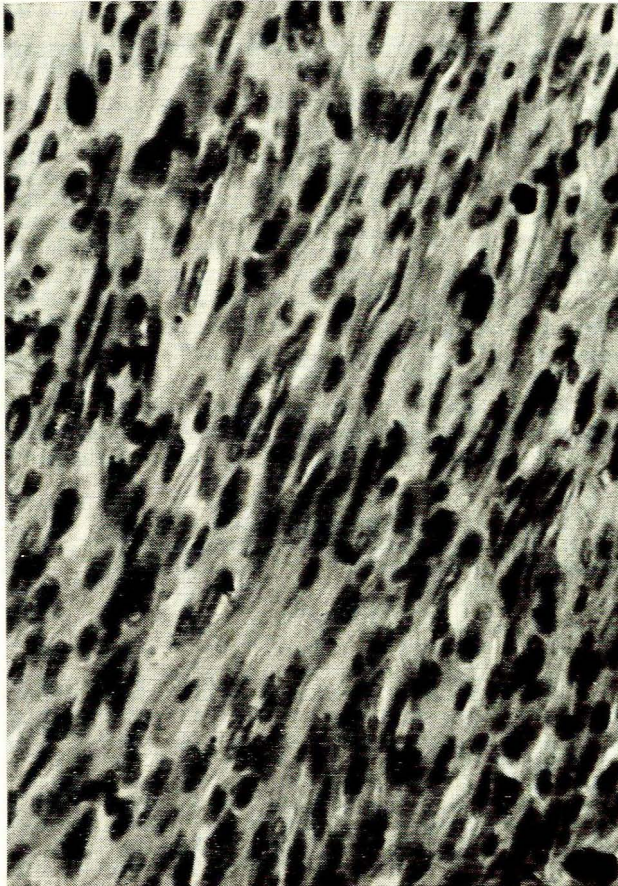


Fig. 5. Spindle-shaped tumour cells (H. and E.  $\times$  400).



Fig. 6. Gland-like, partial papillary arrangement of tumour cells (H. and E.  $\times$  400).

2. The stage of fibrosis characterized by fibroblast proliferation to form a fibrous granuloma covered by a layer of mesothelial cells.

3. The stage of anaplasia in which the mesothelial cells were transformed into malignant cells, possibly owing to one or more soluble carcinogens diffusing slowly from the asbestos particles.

4. The stage of neoplastic proliferation of malignant cells to form secondary nodules some of which are subsequently transformed into plaque-like tumours.

5. The stage of differentiation of the malignant cells into any of the tumour cells described.

In the present investigation malignant mesothelioma was found in 30% of the rats in the chrysotile group and in 90% of the crocidolite group. This significant difference might indicate that crocidolite is much more carcinogenic than chrysotile. This finding also correlates well with the results of some clinical surveys which showed a higher incidence of mesothelioma. Differences in chemical composition and physical structure between the 2 types of asbestos might be responsible for the difference in their biological effect:

1. Their crystal structures are entirely different. Chrysotile belongs to the serpentine series, whereas crocidolite is an amphibole asbestos.

2. Chrysotile is flexible and silky, with curly fibres and a microtubular structure. Crocidolite has a high elasticity and consists of needle-like fibres.

3. Chrysotile contains higher concentrations of Mg, Co, Cr, Pb and Sc and crocidolite, whereas the latter has higher concentrations of Fe, Na, SiO<sub>2</sub> and Mn.

From the physical nature of these dusts it would appear that crocidolite could cause greater mechanical irritation than chrysotile fibres. If this factor is involved in carcinogenesis, it might explain the higher incidence of malignant mesothelioma associated with the more irritating crocidolite. Smith *et al.*<sup>18</sup> could demonstrate that brittle varieties of chrysotile induced mesothelioma in hamsters, whereas the soft variety was non-carcinogenic. If mechanical irritation is an important factor in carcinogenesis, it seems unlikely from our results that fibres encapsulated in the fibrous nodules could be involved. The free-lying fibres, therefore, should be the initiating factor. This observation is in accord with clinical observations that no direct dose-response relationship exists, but rather a time-dependent response. However, the present histopathological findings do not support the mechanical theory.

From the possible chemical mode of action it would appear that labile cations, attached to the silicon-oxygen

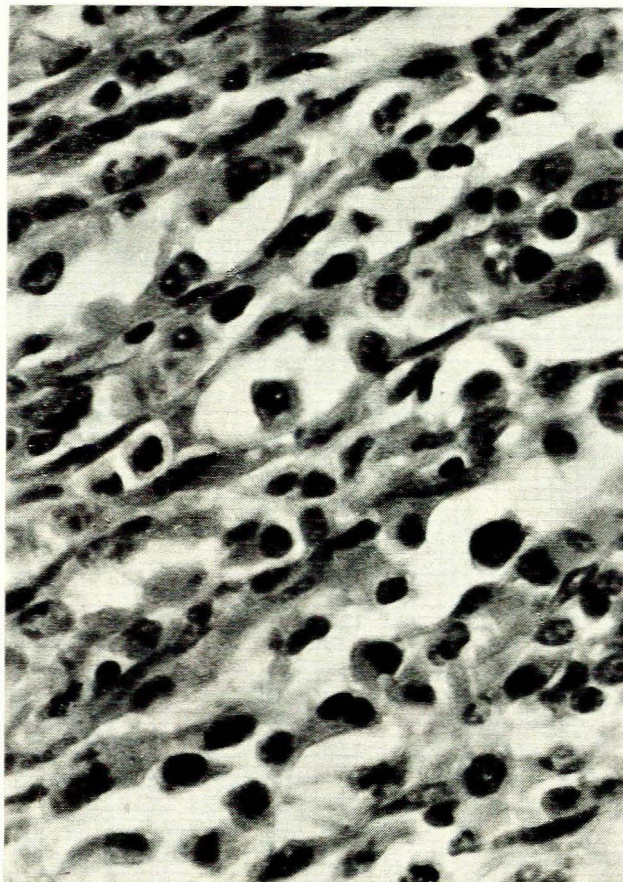


Fig. 7. Stromal spindle-shaped cells forming clefts lined by polygonal cells (H. and E.  $\times$  400).

lattice, metallic, or organic compounds which may become absorbed on the fibres during their geological genesis or during commercial handling, may be the key factor in the carcinogenic potential of asbestos.<sup>17,19</sup> Benz(a)pyrene is a known carcinogenic contaminant of crocidolite. Although

this compound, together with asbestos, might be involved in the induction of mesothelioma, the significance of these hydrocarbons in asbestos carcinogenesis, is still unproved.

It is also known that the UICC reference samples contained small amounts of oils, and they may also be contaminated by tetratertiary butyl diphenquinone. The biological significance of these organic compounds requires further investigation.

Some metals are directly carcinogenic, while others attain carcinogenic properties only when bound into macromolecular complexes. Iron belongs to the latter group and in the complex form may become carcinogenic. Harington and Roe<sup>20</sup> postulated that the  $Fe^{2+} : Fe^{3+}$  ratio might be important in carcinogenesis. Even silica, implanted in the peritoneum, induced mesothelial tumours.<sup>12</sup> Since the iron and silica content of crocidolite is relatively high, it is suggested that these aspects be further investigated.

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#### REFERENCES

1. Wagner, J. C., Gilson, J. C., Berry, G. and Timbrell, V. (1971): *Brit. Med. Bull.*, **27**, 71.
2. Selikoff, I. J., Hammond, E. C. and Churg, J. in Shapiro, H. A., ed. (1970): *Pneumoconiosis: Proceedings of the International Conference on Pneumoconioses, Johannesburg, 1969*, p. 180. Cape Town: Oxford University Press.
3. Stumphius, J. and Meyer, P. B. (1968): *Ann. Occup. Hyg.*, **11**, 283.
4. McDonald, A. D., Harper, A., El Attar, O. A. and McDonald, J. C. (1970): *Cancer*, **26**, 914.
5. Webster, I. (1973): *S. Afr. Med. J.*, **47**, 165.
6. Gloyne, S. R. (1930): *Tubercle (Edinb.)*, **12**, 54.
7. Vorwald, A. J., Durkan, T. M. and Pratt, P. C. (1951): *Arch. Ind. Hyg. Occup. Med.*, **3**, 1.
8. Wagner, J. C. (1963): *Brit. J. Ind. Med.*, **20**, 1.
9. Gross, P. and De Treuille, R. T. P. (1967): *Arch. Environm. Hlth*, **15**, 638.
10. Nordmann, M. and Sorge, A. (1941): *Z. Krebsforsch.*, **51**, 168.
11. Lynch, K. M., McIlver, F. A. and Cain, J. R. (1957): *Arch. Ind. Health*, **15**, 207.
12. Wagner, J. C. (1962): *Nature (Lond.)*, **196**, 180.
13. Wagner, J. C. and Berry, G. (1969): *Brit. J. Cancer*, **23**, 567.
14. Donna, A. (1970): *Med. Lavoro*, **61**, 1.
15. Jagatic, J., Rubnitz, M. E., Godwin, M. C. and Weiskopf, R. W. (1967): *Environm. Res.*, **1**, 217.
16. Graham, J. and Graham, R. (1967): *Ibid.*, **1**, 115.
17. Reeves, A. L., Puro, H. E., Smith, R. G. and Vorwald, A. J. (1971): *Ibid.*, **4**, 496.
18. Smith, Wm. E., Miller, L., Churg, J. and Selikoff, I. J. (1965): *J. Mt Sinai Hosp.*, **10**, 93.
19. Harington, J. S. and Roe, F. J. C. (1965): *Ann. N. Y. Acad. Sci.*, **132**, 439.