

X-RAY DIFFRACTION AND ELECTRONMICROSCOPIC STUDIES ON THE FINE STRUCTURE OF THE TRACHEAE OF A CENTIPEDE *SCUTIGERA LONGICORNIS*, TOGETHER WITH OBSERVATIONS ON THEIR CHEMICAL COMPOSITION

It is well known that in Onychophora, Myriapoda and Insecta, respiration takes place by means of internal air-tubes, the tracheae.¹ The wall of the tracheae has spirally coiled chitinous intima which keeps the tracheae distended and prevents it from collapsing, to ensure free passage of air.² There is no diffusion of gases across the wall of tracheae, as blood in these arthropods does not take part in respiration.³ An exception to this is found in scutigeromorph centipedes where the tracheae do not have chitinous intima, but are still distended and non-collapsible⁴; there is diffusion of gases across the wall of tracheae as blood in these myriapods contains haemocyanin and takes part in respiration.⁵ But no attempt has so far been made to study the fine structure and chemical composition of the tracheae in Scutigeromorpha in relation to their exceptional properties; hence, the present investigation.

The material used was scutigeromorph centipedes of the species *Scutigera longicornis*, which is available in plenty in the humid regions of the Alagarkoil forest near Madurai. They were collected, brought to the laboratory and maintained as detailed elsewhere.⁶

For X-ray diffraction studies, tracheae isolated from freshly decapitated animals were washed thoroughly in distilled water, stretched, dried and stacked to give a final thickness of about 0.1 mm. CuK α radiation was used at a specimen to film distance of 4 cm. For electron-microscopic studies, freshly isolated and thoroughly washed tracheae were taken onto formvar-coated glass screens and dried in air at 20-25° C. Such preparations were shadow-cast with palladium and examined with RCA EMU-2 electronmicroscope.

The procedure adopted for the extraction of protein was based on the work of Hackman⁷ and the method of quantitative estimations of individual amino-acids has been given elsewhere.⁸ Details of histochemical techniques employed are given in appropriate contexts.

The X-ray diffraction diagram obtained for the tracheae of *S. longicornis* (Fig. 1) reveals a diffuse scattering in the central region and

2.9 Å meridional spacing. This would suggest that chitin is absent in the tracheae. If chitin is present, one would expect rings at 10.3 Å, 4.6 Å and 3.4 Å.⁹ On the other hand, the 2.9 Å meridional spacing would indicate the



FIGS. 1-2. Fig. 1. X-ray diffraction diagram of stretched trachea of *Scutigera longicornis*, recorded with CuK α radiation. Fig. 2. Tracheal wall of *Scutigera longicornis*. Shadow cast with palladium, $\times 45,000$.

presence of collagen.¹⁰ Diffuse scattering in the central region of the diagram obscures other features of X-ray wide angle diagram. Possibly, in the native state the fibrils of collagen are in perpendicular layers and only those fibrils which are parallel to the stretch become oriented, which results in the appearance of the meridional arc.

Electronmicroscopic examinations of the tracheae lend support for this suggestion. Figure 2 presents an electronmicrograph of tracheae of *S. longicornis*; it disclosed that the wall of

the tracheae has a network consisting of two systems of transverse and longitudinal fibrils, disposed in regular order at right angles delimiting square cells. The fibrils are all of uniform diameter measuring 4 to 6 millimicrons. The square regions in between the fibrils seem to be thin homogeneous membranes.

TABLE I

Amino-acid composition of the protein extracted from the tracheae of Scutigera longicornis in residues amino-acid/1000 total residues (amide value omitted)

No.	Amino-acids		Amonnt
1	Alanine	..	112.0
2	Arginine	..	39.8
3	Aspartic acid	..	55.6
4	Glutamic acid	..	71.0
5	Glycine	..	335.7
6	Hydroxylysine
7	Hydroxyproline	..	68.4
8	Leucine	..	29.0
9	Lysine	..	28.6
10	Histidine	..	12.9
11	Isoleucine	..	15.0
12	Methionine	..	15.2
13	Phenylalanine	..	0.9
14	Proline	..	86.4
15	Serine	..	68.9
16	Threonine	..	45.7
17	Tyrosine	..	1.9
18	Valine	..	23.3

Collagen from different sources differs markedly in its amino-acid composition, although all give the characteristic collagen X-ray diagram. However, the presence of glycine, hydroxyproline and proline in large quantities is considered characteristic of collagen.¹¹ The protein extracted from the tracheae was hydrolysed and subjected to quantitative estimations of individual amino-acids. The results are given in Table I which may show that glycine forms about one-third of the total amount of amino-acids; hydroxyproline and proline are also found in larger amounts. A notable feature is the absence of hydroxylysine which recalls the condition noted in annelid collagen.¹²

In addition to collagen, the tracheae of *S. longicornis* react positively to tests for acid mucopolysaccharides. Sections of tracheae stained deep blue with alcian blue and red with toluidine blue. The metachromasia with toluidine blue may indicate the presence of highly acidic mucopolysaccharide. The metachromasia was not removed by washing with 95% ethanol indicating presence of esters of sulphuric

acid.¹³ Methylene blue stained the tracheae at pH 2.6 and the extinction point lies between pH 1.0 and pH 1.4 and the capacity to bind methylene blue below pH 4.0 is considered indicative of presence of sulphate groups. These observations may suggest that the trachea of *S. longicornis* contains sulphated acid mucopolysaccharides.

The roles of collagen and acid mucopolysaccharides have been discussed by several workers.¹⁴ An important function of collagen is its role as a skeletal structure especially in instances where chitin is absent.¹⁵ Acid mucopolysaccharides present in the respiratory tubes of vertebrates are said to make them permeable to respiratory gases.¹⁴ In the light of these observations, it is suggested that in the absence of chitinous intima, collagen fibrils may act as skeletal structure in the tracheae and acid mucopolysaccharides may render the wall of the tracheae permeable to respiratory gases in *Scutigera longicornis*.

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