im Meso- und Metatergum befindlichen „akzessorischen Herzen" am weitesten entfernt gelegene Geäder sich als besonders intensiv durchblutet erweist, während gerade umgekehrt in diesen proximalen Flügelabschnitten eine schwächere und langsamere Durchspülung mit Hämolymphe hätte vermutet werden können. Die tatsächlich reziprok ausgebildeten Verhältnisse lassen nur den Schluß zu, den Pterostigmen im Libellenflügel eine pulsierende Funktion zuzuerkennen.

Den Herren Dr. HERIBERT KIEBITZ-Bielefeld und Dr. GERHARD JURZITZA vom Botanischen Institut der Technischen Hochschule in Karlsruhe sei an dieser Stelle für das mir nach Soest zugestellte lebende Libellenmaterial gedankt. Besonders haben sich die aus dem regen Schriftwechsel mit dem Kollegen in Karlsruhe eröffnenden Perspektiven sehr günstig auf meine autoradiographischen Studien am Libellenflügel ausgewirkt. Schließlich bleibe ich noch Herrn Professor Dr. MANFRED LÜDICKE vom Zoologischen Institut der Universität Heidelberg für briefliche Informationen verbunden.

Studies on the cuticle of an annectant symphylid *Polyxenella krishnani* together with observations on its phylogenetic significance

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The structure, staining and chemical properties of the cuticle of the annectant symphylid *Polyxenella krishnani* are compared with those of *Scutigerella* sp. The peculiarities in the cuticle of *Polyxenella krishnani* refer to the mode of stabilization of the cuticularprotein by sulphur linkages and to the possession of an outer epicuticle not found in *Scutigerella*. These differences appear to be correlated with morphological characters like the position of the gonopore. The significance of such features of the cuticle of *Polyxenella krishnani* is discussed. It is suggested that *Polyxenella krishnani* may be representative of an ancestral type from which the extant Symphyla and possibly the insects may have been derived.

Polyxenella krishnani, whose occurrence in South India has recently been reported (SUNDARA RAJULU [1b]), shows a unique combination of typical symphylid characters with features like posterior gonopore and presence of eyes. Such a combination appears significant in the light of the observations of TIEGS and MANTON [2] that the symphylan theory of ancestry of insects, notwithstanding the many points in its favour, may not be acceptable on account of the anamoly of the gonopore and absence of eyes. The above authors suggested that the insects may have been derived from a group, the so-called Protosymphyla which probably had a posterior gonopore and possessed eyes. *Polyxenella krishnani* shows all the essential features envisaged by TIEGS and MANTON in the progenitors of insects. It is therefore of interest to study the nature of its cuticle in the light of its morphological peculiarities already reported (SUNDARA RAJULU [1b]). The cuticle is considered one of the most fundamental attributes of arthropods and of supreme functional importance in the success that attended the arthropod evolution (TIEGS and MANTON). Therefore a study of the cuticle may be expected to throw light on the interrelationships of arthropod groups. Although the importance of Symphyla in the context of the origin of insects has been discussed by a number of workers, no attempt has so far been made to examine the cuticle of this group to see if it would yield evidence supporting the observations based on a study of the morphological characters. It is with this object the present study has been undertaken. Since no information is available regarding the cuticle of any symphylid, a study of a typical representative of the group *Scutigerella* sp. has been made for purposes of comparison with that of the annectant type *Polyxenella* to see if the cuticular organization would reflect the morphological characteristics.

[1a] G. SUNDARA RAJULU, Curr. Sci. **32**, 412 [1963 a].
[1b] G. SUNDARA RAJULU, Ann. Mag. Nat. Hist. [London] **13**, 6, 136 [1963 b].
[2] O. W. TIEGS and S. M. MANTON, Biol. Rev. Cambridge philos. Soc. **33**, 255 [1958].

Material and method

Scutigerella sp., used in the following study occurs in the vicinity of Madurai, South India. These are easily collected from soft mud and humus soon after the commencement of the rainy season from September to December. In the summer months they are scarce probably due to their habit of burying themselves. Average sized specimens measure between 1 to 2 mm. long and about 0.25 mm. wide. *Polyxenella krishnani* occurs in the same region as *Scutigerella* sp. These are about 2 to 2.5 mm. long and about 0.2 mm. wide, dorsoventrally compressed, pale brown to creamy white in colour. Like *Scutigerella* sp. they have been found in soft mud soon after the onset of rainy season, but are much less abundant.

For a study of the cuticle, sections of the entire animals were prepared by double embedding with celloidin and paraffin as well as by gelatin impregnation. The histochemical tests applied are mentioned in appropriate context. For the visualization of disulphide and sulphydril groups application of the Blue-Tetrazolium test of BARNETT and SELIGMAN [3] gave consistently good results. Location of chitin by chitosan test in the cuticular layers was rendered difficult on account of the extreme thinness of the layers concerned which lost their integrity by the violent treatment involved. This difficulty was overcome by applying a modified procedure of S c h u l t z test for identification of chitin using a mixture of L u g o l's iodine solution and dilute sulphuric acid after a preliminary treatment with sodium hypochlorite and sodium hydroxide.

Previous work

The cuticle of symphylids has not so far been studied. It is thought that in general it may be similar to the cuticle of diplopods and chilopods (FUHRMAN [4]; LANGNER [5]; CLOUDSLEY-THOMPSON [6]; BLOWER [7]; KRISHNAN [8]). In these groups the main point arising out of the previous work is with reference to the presence or absence of an epicuticle. BLOWER contended that the chilopods and diplopods studied by him lack an epicuticle comparable to that of insects and what resembles the epicuticle of insects is the tanned exocuticle which may be amber coloured. In a later study (KRISHNAN [9]) it has been shown that the tanned outermost layer in the chilopod cuticle is exocuticular in nature and there is present external to it a very thin hyaline membrane corresponding to the epicuticle of insects, which is stabilized by –S–S– bonds. This supports an earlier study by CLOUDSLEY-THOMPSON [6] in which an epicuticle has been differentiated from the underlying layers by treatment with chlorated nitric acid. A difficulty in reconciling the apparently contradictory views of previous workers on the occurrence of an epicuticle is obviated by the work of SUNDARA RAJULU [1a]. He noted that in the diplopod *Cingalobolus bugnioni* an epicuticle is present in summer months, wenn they lie buried under the soil and on emerging out with the onset of rains it undergoes a moult and develops a new cuticle which lacks an epicuticle. Such periodical shedding of the epicuticle may be a common feature in diplopods and probably account for the differing views of previous workers referred to above. Barring the Symphyla and Pauropoda about which no information is available, in the chilopods and diplopods the cuticle may be said to comprise an epicuticle overlying a tanned exocuticle and an untanned endocuticle. The epicuticle in diplopods is tanned while in chilopods it is stabilized by sulphur linkages. In the former a lipid epicuticle is absent while in the latter there is a very thin aniline blue-staining layer forming the boundary of the epicuticle and conforms in staining and chemical reactions to the outer epicuticle of insects.

Cuticle of *Scutigerella* Sp.

Transverse sections passing through the anterior part of the body show a very thin colourless cuticular layer measuring 3 μ thick which is distinguishable into an outer very thin homogenous layer less than a μ thick. This layer is produced into a number of outwardly projecting spines. Internal to the homogenous layer is a comparatively wider part marked by transverse striations (Fig. 1). With MALLORY's stain the outer homogenous layer and the spines take a red colour, the outer half of the striated region also stains red though less intensely than the homogenous layer while the inner half of this region

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[6] J. L. CLOUDSLEY-THOMPSON, Nature [London] **165**, 692 [1950].
[7] J. G. BLOWER, Quart. J. microscop. Sci. **92**, 141 [1951].
[8] G. KRISHNAN, Quart. J. microscop. Sci. **24**, 11 [1953].
[9] G. KRISHNAN, Physiol. Zoöl. **29**, 324 [1956]; Quart. J. microscop. Sci. **99**, 359 [1958]; Zool. Anz., in Press.
A. KRISHNAKUMARAN, Zool. Jb. Anat. **80**, 49 [1962].

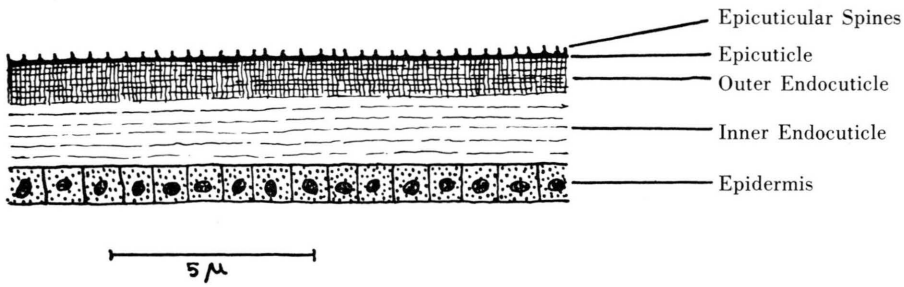

Fig. 1. Transverse section through the cuticle of *Scutigerella Immaculata* stained by Mallory's method.

is blue. With Heidenhain's haematoxylin the outer membranous layer and the spines stain deep blue or black, the outer half of the striated region blueblack and the inner half is very light blue or grey. The structural and staining characteristics suggest that the outer homogenous layer may correspond to the epicuticle of other arthropods, the striated region to the procuticle which is differentiated into an outer layer showing chemical characters differing from the inner, the differences reflected in the staining reactions noted above.

The results of histochemical tests are shown in Table I. The layers of the cuticle which are fuchsinophil are positive to Hg/nitrite, Millon's and xanthoproteic tests indicative of the presence of a protein rich in phenolic groups (BAKER[10]; PEARSE[11]). The absence of amber coloration in the epicuticle or in any part of the procuticle shows that the protein is not sclerotized, but it seems to have undergone partial hardening probably by tanning involving the oxidation of tyrosine residues resulting in the fuchsinophily of these regions as has been suggested to occur in the presumptive exocuticle of *Periplaneta* (DENNELL[12]). Such a suggestion is supported by the observation that the cuticle after pretreatment with alkaline stannite solution, the

No.	Tests	References	Epicuticle	Outer Endocuticle	Inner Endocuticle
A)	*Staining Reactions*				
1.	Mallory	MALLORY and WRIGHT[17]	Intensley red	Red	Blue
2.	Mallory after alkaline stannite	TRIM[13]	Blue	Blue	Blue
3.	Mallory after Sodium sulphide	BROWN[18]	Intensley red	Red	Blue
4.	Heidenhain's haematoxylin	LILLIE[19]	Black	Blueblack	Grey
5.	— do — after alkaline stannite	TRIM[13]	Paleblue	—	—
6.	— do — after Sodium sulphide	BROWN[18]	Black	Blueblack	Grey
B)	*Chitin*				
7.	Chitosan test	LISON[20]	—	+	+
8.	Schultz modified	Present Authors	—	+	+
C)	*Lipids*				
9.	Sudan Black-B	BAKER[21]	++	—	+
10.	Nile Blue	CAIN[22]	++	+	+
11.	Lieberman-Burchardt test	LISON[20]	+	—	—
D)	*Protein*				
12.	Biuret test	FEARON[23]	—	—	+
13.	Xanthoproteic test	LILLIE[19]	++	++	+
14.	Millon's test	PEARSE[11]	++	++	—
15.	Hg/nitrite	Baker[10]	+++	++	+
16.	Blue tetrazolium	BARNETT and SELIGMAN[3]	—	—	—
17.	Lead acetate test	LILLIE[19]	—	—	—

Table 1. Results of histochemical tests on the sclerite cuticle of *Scutigerella Sp.*

[10] J. R. BAKER, Quart. J. microspoc. Sci. **97**, 161 [1956 a].
[11] A. G. E. PEARSE, Histochemistry theoretical and applied. Churchill, London 1961; R. J. POCOCK, Zool. Anz. **16**, 271 [1893 a].
[12] R. DENNELL, Biol. Rev. **33**, 178 [1958].

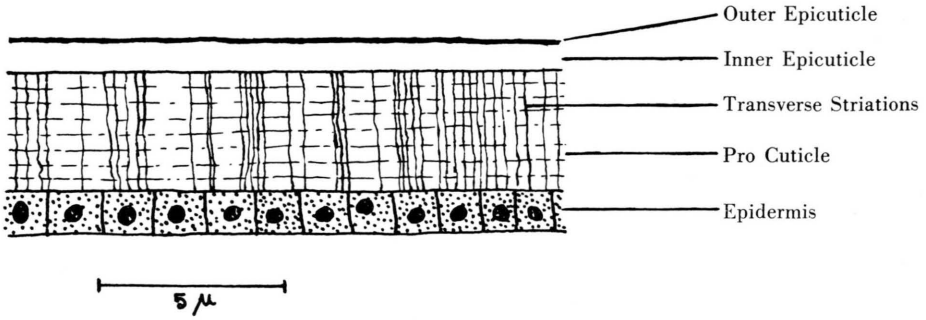

Fig. 2. Transverse section through the partially hardened region of the cuticle of *Polyxenella Krishnani* stained by Mallory's method.

staining of the fuchsinophil region of the procuticle changes from red to blue with Mallory. Since alkaline stannite solution is known to detan, tanned proteins (TRIM [13]), the reversal of staining noted above may be attributed to the transformation of the partially tanned protein to its original condition (DENNELL [12]). The sequence of changes in staining reactions of the cuticular layers following detanning parallel those reported in the cuticle of *Periplaneta* by DENNELL and MALEK [14].

The general cuticular organization and the mode of hardening conform to the pattern reported in diplopods (BLOWER [7]; SUNDARA RAJULU [1a]). A feature of the epicuticle is the absence of lipid outer epicuticle staining blue in Mallory preparations. The inner epicuticle is devoid of chitin and is moderately hardened by phenolic tanning. The procuticle is not modified to form an amber exocuticle, but the outer regions show differentiation into a fuchsinophil part comparable to the region staining red with Mallory in the cuticle of millipedes (BLOWER [7]). A comparison with the cuticle of chilopods shows marked differences. In *Scolopendra subspinipes* KRISHNAN [9] described an epicuticle containing a protein rich in cystine residues, stabilized by $-S-S-$ bonds, and with an outer lipid epicuticle staining blue with Mallory.

Cuticle of *Polyxenella krishnani*

The cuticle of *Polyxenella krishnani* comprises two optically discernible regions, an outer homogenous hyaline layer measuring about a μ thick and a comparatively wider region, 3 μ thick, marked by horizontal lamellations and with transverse striations (Fig. 2). These two regions are distinguishable by staining with Mallory. The outer hyaline layer is non-reactive to stains, but a very thin membrane overlying it takes up a blue colour in Mallory preparations, and the inner lamellated region stains

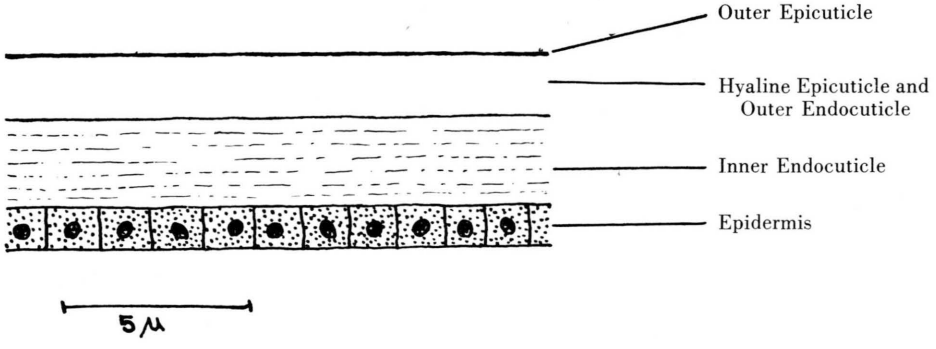

Fig. 3. Transverse section through the fully hardened region of the cuticle *Polyxenella Krishnani* stained by Mallory's method.

[13] A. R. H. TRIM, Biochem. J. **35**, 1088 [1941]. V. B. WIGGLESWORTH, J. exp. Biol. **21**, 97 [1945].

[14] R. DENNELL and S. R. A. MALEK, Proc. Roy. Soc. [London], Ser. B, **144**, 545 [1956].

red in the outer half and blue in the inner half. The hyaline layer with the blue staining membrane may correspond to the epicuticle, the inner lamellated region to the procuticle, the fuchsinophil part of it corresponding to the mesocuticle (SCHATZ [15]). The cuticle shows varying degrees of hardening which are reflected in the staining reactions. In the tergites in the anterior part of the body which shows pronounced hardening, not only the epicuticle but also the outer part of the procuticle are hyaline and the rest of the cuticle is fuchsinophil (Fig. 3). In those regions where the cuticle is least hardened the inner epicuticle stains red and the entire procuticle blue with Mallory's stain. Similar results were obtained by staining with Heidenhain's haematoxylin with which the regions staining red with Mallory take a blue-black colour and the aniline blue-staning layers like the inner endocuticle take a light grey colour. The outer epicuticle stains black.

The fuchsinophily of the epicuticle and the mesocuticle seems to be due not to the presence of tyrosine-rich proteins as in insects, in which it has been suggested that the protein may undergo a process of self-tanning responsible for its staining properties. In *Polyxenella krishnani* a preliminary treatment of the cuticle with a detanning agent like alkaline stannite solution followed by staining with Mallory did not produce any change in staining as has been noted in *Scutigerella* sp. The xanthproteic and Millon's tests in the fuchsinophil regions were also negative. However, the Hg/nitrite test of BAKER [10] gave a very feeble reaction. It is suggestive that the fuchsinophily of the cuticle protein of *Polyxenella krishnani* is attributable to cause other than those reported in the insect cuticles (DENNELL [12]).

Suggestive evidence regarding the nature of protein present in the hyaline regions of the cuticle is afforded by the results of staining after treating the

No.	Tests	References	Outer Epi-cuticle	Inner Epi-cuticle	Outer Endo-cuticle	Inner Endo-cuticle
A)	*Staining Reactions*					
1.	Mallory	MALLORY and WRIGHT [17]	Blue	Hyaline	Hyaline	Red
2.	Mallory after alkaline stannite	TRIM [13]	Blue	Hyaline	Hyaline	Red
3.	Mallory after sodium sulphide	BROWN [18]	Blue	Red	Red	Red
4.	Heidenhain's haematoxylin	LILLIE [19]	Black	Hyaline	Hyaline	Grey
5.	— do — after alkaline stannite	TRIM [13]	Black	Hyaline	Hyaline	Grey
6.	— do — after sodium sluphide	BROWN [18]	Black	Blueblack	Blueblack	Grey
B)	*Chitin*					
7.	Chitosan test	LISON [20]	—	—	+	+
8.	Schultz modified	Present Authors	—	—	+	+
C)	*Lipid*					
9.	Sudan Black-B	BAKER [21]	++	—	—	—
10.	Nile blue	CAIN [22]	++	—	—	+
11.	Lieberman-Burchardt test	LISON [20]	+	—	—	—
D)	*Protein*					
12.	Biuret test	FEARON [23]	—	—	—	—
13.	Xanthoproteic test	LILLIE [19]	—	—	—	—
14.	Millon's test	PEARSE [11]	—	—	—	—
15.	Hg/nitrite test	BAKER [21]	—	—	—	—
16.	Lead acetate test	LILLIE [19]	—	+	+	—
17.	Ferricyanide test	PEARSE [11]	—	+	+	+
18.	Blue tetrazolium	BARNETT and SELIGMAN [3]	—	++	+	+
19.	Sodium Nitroprusside	PEARSE [11]	—	++	+	+
20.	Thioglycollate	BROWN [18]	—	Swells	Swells	—

Table II. Results of histochemical tests on the sclerite cuticle of *Polyxenella Krishnani.*

[15] L. SCHATZ, Ann. Ent. Soc. Amer **45**, 678 [1952]
[16] J. A. RYDER, Amer. Naturalist **14**, 375 [1880].
[17] F. B. MALLORY and J. H. WRIGHT, Pathological technique. Saunders, Philadelphia 1924; A. S. PACKARD, Proc. Boston Soc. Nat. Hist. **16**, 111 [1873].
[18] C. H. BROWN, Quart. J. microscop. Sci. **9**, 331 [1950 a].
[19] R. D. LILLIE, Histopathologic technic and practical histochemistry. Blackistan, New York 1954.
[20] L. LISON, Histochimie et cytochemie animale. Gauthir Villars, Paris 1953.
[21] J. R. BAKER, Quart. J. microscop. Sci. **87**, 441 [1946].
[22] A. J. CAIN, Quart. J. microscop. Sci. **88**, 383 [1947].
[23] W. R. FEARON, William Heinman, London 1946.

cuticle with alkaline sodium sulphide. The hyaline epicuticle which is non-reactive to stains after the above treatment stained red with Mallory. A similar effect has been reported in the epicuticle of scorpion *Palamneus swammerdami* (KRISHNAN [8]) in which the hyaline epicuticle has been shown to be hardened by sulphur linkages which are broken by alkaline sodium sulphide treatment, resulting in a resumption of staining properties. Histochemical tests carried out on the cuticle provide suggestive evidence of the protein constituent of the cuticle (Table II). The nitroprusside test for SH groups was positive in the fuchsinophil regions of the cuticle. Thioglycollate treatment causes swelling of the hyaline epicuticle indicative of the presence of sulphur linkages in those regions. The lead acetate test is positive in the epicuticle and the outer layers of the procuticle. With blue-tetrazolium method (BARNETT and SELIGMAN [3]) the epicuticle showed an intense blue coloration. The above mentioned tests indicate that the protein substrate in these regions is rich in sulphur-containing amino-acids. The resumption of the staining properties of the hyaline epicuticle after a treatment with sodium sulphide may suggest that the mode of hardening is by sulphur linkages as in keratinous protein. In this respect *Polyxenella krishnani* recalls the condition noted in the epicuticle of the chilopod *Scolopendra subspinipes* (KRISHNAN [9]).

Discussion

The cuticular organization of *Polyxenella krishnani* differs from that of *Scutigerella* sp. in two important respects, namely in the mode of stabilization of the cuticle-proteins involving $-S-S-$ bonds and in the presence of a lipid outer epicuticle. The general pattern recalls the type met with in chilopods like *Scolopendra subspinipes* (KRISHNAN [9]). On the other hand in structure and composition the cuticle of *Scutigerella* is similar to those of a diplopod for in both, an outer lipid epicuticle is absent and the mode of hardening is solely by phenolic tanning (BLOWER [7]; SUNDARA RAJULU [1a]). In these observations suggestive evidence may be discerned that in myriapods the mode of hardening of the cuticle may be correlated with the position of the gonopore. But the information in regard to the cuticle of this group, is still incomplete, for among the progoneates only the diplopods have so far been investigated in some detail while with the exception of the present study, very little is known of Symphyla and the Pauropoda have not received any attention. In the opisthogoneates only in *Scolopendra* the epicuticle has been shown to be hardened by $-S-S-$ bonds and in the chilopods studied by BLOWER an epicuticle is said to be absent.

It has been suggested that the progoneates and opisthogoneates represent two main trends in the evolution of Myriapoda. In this connection it is of interest to note that *Polyxenella* shows a combination of characters reminiscent of both progoneates and opisthogoneates, in the presence of short 8-segmented antennae, a labium recalling gnathochilarium of diplopods, and a posterior gonopore. To these peculiarities may be added the characteristics of the cuticle, like the mode of hardening by $-S-S-$ bonds and the presence of a lipid epicuticle not found in typical symphylids. In view of the suggestion of TIEGS and MANTON [2] that progoneate myriapods may have been derived from ancestors which were opistogoneate, it may be suggested that a type like *Polyxenella* may have been such an ancestral form. If such an assumption is valid, it is of interest to note that sulphur bonding as a means of hardening of the cuticle may have preceded phenolic tanning. In this context a significant feature is the remarkable agreement seen in the mode of hardening with that found in insects like the thysanuran *Machilis* in which studies being carried out in this laboratory show that the cuticle contains organic sulphur which is probably involved in the hardening of the cuticular proteins by sulphur linkages. In addition, the absence in thysanuran cuticle of a tyrosine-containing protein corresponding to the so-called arthropodin which is a necessary precursor of the tanned sclerotin of the cuticle, lends support to the view that in this cuticle hardening is not by phenolic tanning but by sulphur bonds. The resemblence noted above between a symphylid like *Polyxenella* and primitive insects belonging to the group Thysanura may be considered significant in the context of the theory of derivation of insects from symphylid forebears (RYDER [16]).