Some Aspects of Cuticular Organization of the Branchiopod, *Streptocephalus dichotomus*

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SUMMARY

The structural features of the cuticle of *Streptocephalus* are described. The epicuticle and endocuticle, unlike those of other Crustacea, stain alike and show similar chemical characters (apart from the absence of chitin in the epicuticle). This feature appears to be related to the absence of tanning of the cuticle, which remains soft and colourless in all stages of growth. In the epicuticle the outermost very thin layer, whose presence can be inferred from chemical tests, seems to correspond to the outer epicuticle of Crustacea, but this layer lacks histological distinctness. The protein constituent of the cuticle is unlike that of other arthropod cuticles so far studied. It differs markedly from arthropodin or sclerotin and recalls collagen. The significance of the peculiarities of the cuticle is discussed. It is suggested that the structure and chemical characteristics of the cuticle may indicate that it represents a generalized and unspecialized condition of the arthropod cuticle.

INTRODUCTION

RECENT studies indicate a non-uniformity in the chemical composition of the cuticle of arthropods. In *Palamneus*, *Scolopendra*, and *Propallene* it has been noted that the amino-acid composition of the protein constituent of the epicuticle differs from that of its counterpart in insects in containing cystine residues, which are absent in the latter (Krishnan, 1953, 1954, 1955, 1956). The presence of such sulphur-containing amino-acids appears to be related to the mode of hardening of the cuticle, which involves the formation of —S—S-bonds as in the keratin of vertebrates, as opposed to the condition reported in the cuticle of insects, which is hardened by phenolic tanning. It is becoming increasingly clear that the cuticle of arthropods, far from conforming to any one chemical pattern, may show a range of variation. The significance, if any, of such variations merits further study. In this connexion it is of interest to recall the work of Reed and Rudall (1948), who called attention to the problem of the relationship of the arthropod cuticle to that of annelids. The two cuticles are very different, that of annelids being composed of a protein resembling collagen, whereas the so-called arthropodin is of keratinous β-protein type (Astbury, 1945). In the light of the above observations the finding, in the cuticle of a primitive branchiopod crustacean, of a protein hitherto undescribed, seemed to warrant a more intensive study of its structural and chemical constitution.

Although the cuticle of decapod Crustacea has been studied in considerable

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detail (Drach, 1939; Dennell, 1947; Krishnan, 1951; Richards, 1951), it is not known whether the characteristics of the cuticle found in them are of general validity for the entire group. From what little is known of the cuticle of entomostracan Crustacea (Lafon, 1941, 1943; Thomas, 1944; Richards and Cutkomp, 1946), it would appear that chitin is present in the cuticle of representative species of Branchiopoda, Cladocera, Copepoda, and Cirrepedia. Lafon (1943), from a biochemical analysis of the cuticle of a number of types, reported the relative percentages of chitin and protein. Thomas (1944), in the course of his studies on the tegumental glands of the cirripedes such as *Lepas*, *Balanus*, and *Lithotrya* found that the cuticular organization in these species recalls that of decapods like *Homarus* in consisting of a very thin, non-chitinous, protein layer overlying a broad endocuticle formed of a chitin-protein complex. The information regarding the other classes of Entomostraca is more scanty. Dennell (1947), from his observations on the cuticle of *Apus cancriformis*, a branchiopod, suggested that it may be similar to that of the other Crustacea, but he noted that it was too thin and delicate to preserve even the body shape. It may be inferred that the cuticle in this species is unhardened, but no information is available of its structural characteristics and chemical composition beyond the fact that both chitin and protein are present. Lafon (1943) has not indicated the protein value for the branchiopod *Triops*, although he recorded that chitin amounts to about 61·4%. From the above brief review of our knowledge of the cuticle of Entomostraca, especially Branchiopoda, it would be clear that there is need for information regarding the structure of the cuticle, the mode of hardening, if any, and the nature of the chemical components of the cuticle. It is realized that protein is by far the most important constituent of the cuticle, which forms as it were the central unit around which the other cuticular components are built and which largely accounts for the properties of the cuticle. In the following study an attempt has therefore been made to investigate the structure and chemical composition with special reference to the protein constituents of the cuticle of *Streptocephalus* by histological, histochemical, and microchemical procedures. The object has been to arrive at a more complete understanding of the cuticular organization of a hitherto less known group of Crustacea.

Structure and Staining Reactions

Streptocephalus dichotomus is an anostracan branchiopod, characterized by the absence of a shell-fold. It is of common occurrence in fresh-water ponds in Madras and its environs. The body, composed of 19 segments behind the head region, presents an almost vermiform appearance. The average length of an adult varies from about 5 cm to about 8 cm, the females being smaller than the males. The general body surface is covered by a very thin cuticle, closely adhering to the epidermis. By careful manipulation the cuticle can be separated from the underlying soft parts. It is colourless, soft, and flexible, and remains so at all stages of growth. Sheets of cuticle taken from the thorax

or abdomen show in a surface view minute polygonal patterns, marked by well-defined lines. This has been noted in a number of Crustacea (Drach, 1939). In *Cancer pagurus* Dennell (1947) distinguished two distinct patterns. In one of these the boundary lines are wavy or corrugated; this is found in the newly-formed cuticle of an animal that has just moulted. In the other type, seen in cuticles some time after the moult, the boundaries are marked by vertical lines. The significance of the corrugated lines is said to be that they provide for expansion during the rapid growth of the cuticle after the moult. It has been pointed out that these patterns may indicate the mode of formation of the cuticle by the activity of the underlying cells (see Dennell, 1947).

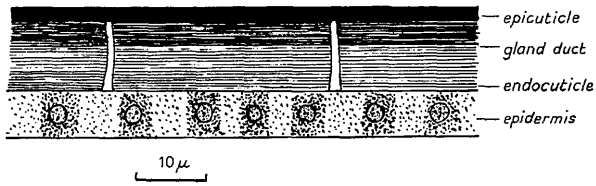

FIG. 1. Transverse section through the tergite cuticle of *Streptocephalus*, stained by Mallory's method.

Sections passing transversely through the thorax and abdomen show the cuticle as a very thin, colourless layer about 4 μ thick, overlying the epidermis (fig. 1). The cuticle is of more or less uniform thickness over the tergites and sternites. Laterally the cuticle is thinner. In unstained sections of the cuticle, an outer membrane less than 1 μ thick is distinguishable from the inner region of the cuticle. This layer shows staining reactions and chemical characteristics different from the rest of the cuticle, and, as will be shown in the sequel, corresponds to the epicuticle of other arthropods, while the inner, broader, colourless region is the procuticle or endocuticle, which shows faint horizontal lamellations extending up to the epicuticle. A division is discernible in the endocuticle, marking an outer and inner endocuticle. In the former the lamellations are closer and less prominently seen than in the latter, in which they are wider apart and conspicuous. Gland ducts traversing the cuticle from the epidermis to the outer surface are sparsely distributed. Pore-canals could not be made out in unstained preparations.

The cuticle presents more or less similar features in all regions of the body surface. It is not amber-coloured. In insects and most other arthropods the appearance of an exocuticle brings about a modification of the basic structure of the cuticle, resulting in a differentiation of the sclerite cuticle from the arthrodial membrane. The transition between the two being gradual, a region representing an intermediate condition designated the 'intermediate sclerite cuticle' has been distinguished. In such forms, the arthrodial membrane consists only of the epicuticle and endocuticle. In *Streptocephalus* the cuticle throughout the body surface, apart from minor modifications, is soft and

consists only of the epicuticle and endocuticle, so that there can be no distinction into the arthrodial membrane condition and the sclerite condition noted in other arthropods (see Blower, 1951; Dennell and Malek, 1955*a*).

The above-mentioned differences between the cuticle of *Streptocephalus* and that of other arthropods are further emphasized by a study of the staining reactions. With Mallory's triple stain, the epicuticle takes on a deep blue colour and the endocuticle a lighter blue. In the latter, the inner region stains more faintly than the outer. With Masson's trichrome stain the epicuticle stains a dark green colour, while the endocuticle is only lightly stained green, the innermost region being more feebly stained. The endocuticle is not stained with Heidenhain's haematoxylin but the epicuticle becomes deep brown or blue. In the lateral regions connecting the tergite and sternite the cuticle is markedly attenuated, the endocuticle being reduced; the epicuticle is indicated by its affinity for haematoxylin. These staining reactions differ from those reported for the cuticles of decapod Crustacea. In the latter, even in the freshly-moulted condition, the epicuticle stains characteristically red and the endocuticle blue with Mallory. With the onset of phenolic tanning after the moult, the epicuticle gradually loses its ability to stain and turns amber-coloured, while the presumptive exocuticle tends to stain red like the unhardened epicuticle; this is followed by the formation of an amber-coloured exocuticle and loss of ability to stain (Dennell, 1947; Krishnan, 1951, 1956). It would appear, therefore, that the cuticle of *Streptocephalus* is not comparable to that of a new-moulted decapod crustacean, although in being unhardened and soft the two resemble one another. Dennell and Malek (1955*a*) in the course of their studies of the cuticle of the cockroach observed that although the cuticle that has just moulted bears a strong resemblance to the arthrodial membrane, the unhardened cuticle of the tergites differs in some respects from this. The differences lie in the staining reactions, which indicate the presence of lipoprotein in the epicuticle of the tergites. This lipoprotein stains red with Mallory. In the soft arthrodial membrane, on the contrary, the epicuticle and endocuticle stain alike blue with Mallory and green with Masson's trichrome stain. The staining reactions of the cuticle of the arthrodial membrane and of the tergite of *Streptocephalus* may suggest the absence from both of a protein capable of being tanned. It is found, however, that the epicuticle—unlike that of the arthrodial membrane—stains with haematoxylin. The observations of Dennell and Malek (1955*b*) may indicate that the substance that stains with haematoxylin in the insect cuticle is a protein rich in tyrosine, which is later involved in tanning. In a cuticle such as that of *Streptocephalus* the substance that stains with haematoxylin cannot be identical with that in cockroach cuticle. It would be reasonable to assume that in arthropod cuticles, haematoxylin staining may be due to different substances. For example, in the arachnid *Palamneus*, the epicuticle of the arthrodial membrane stains with haematoxylin although it does not undergo tanning, and the protein constituent is very different from the protein rich in tyrosine that is characteristic of the insect epicuticle (Krishnan, 1954).

Chemical Composition

Histochemical tests performed on both fresh and frozen sections lend support to the suggestion made above that there is a marked similarity in the composition of the tergite cuticle of *Streptocephalus* and that of the arthrodial membrane of insects (table 1).

Table 1

The responses of the tergite cuticle of Streptocephalus *to chemical tests*

Test	*Epicuticle*	*Endocuticle*	
		Outer endocuticle	*Inner endocuticle*
Millon's	−	−	−
xanthoproteic	−	−	−
biuret	+	+	+
argentaffin	++	+	−
Sudan black B	++	+	−
Morner's	−	−	−
ferric chloride	−	−	−
chitosan	−	+	+
concentrated HNO_3 (cold)	dissolves slowly	dissolves rapidly	dissolves rapidly
concentrated HCl (cold)	,, ,,	,, ,,	,, ,,
concentrated KOH	dissolves	no apparent effect	no apparent effect

The negative Millon and xanthoproteic tests indicate the absence from both the epicuticle and the endocuticle of a protein containing phenolic substances. That the protein constituent of both the epicuticle and endocuticle is similar may be inferred from the above-mentioned reactions and the positive biuret reactions in both the regions. These reactions, together with the negative Morner's test, strongly recall those reported for the arthrodial membrane cuticle of *Periplaneta*; but here, though not in *Periplaneta*, the Sudan black test is positive in the epicuticle and in the outer regions of the endocuticle. A feature of interest is that those regions giving a positive reaction with Sudan black are also positive to the argentaffin test. In both these reactions the epicuticle is more intensely positive than the endocuticular regions. Dennell and Malek (1955*a*) have discussed at length the significance of a positive argentaffin reaction in the cuticles of insects and pointed out that by itself a positive reaction is indicative of no more than the presence of reducing substances. In the cockroach cuticle the positive argentaffin reaction taken with other histochemical tests has been shown to indicate a protein rich in tyrosine; this protein is present in those regions of the cuticle which undergo tanning. In contrast it has been shown that the cuticle of the arthrodial membrane shows a negative reaction to the argentaffin test. In the absence of tanning at any stage in the cuticle of *Streptocephalus*, it is obvious that the positive argentaffin reaction noted here is indicative of a reducing substance other than tyrosine or a protein containing tyrosine. The negative ferric chloride test rules out the possibility of phenols being present in the cuticle.

The close correspondence between the sudanophil regions of the cuticle and those giving a positive argentaffin reaction may suggest that the argentaffin-positive substance in the cuticle of *Streptocephalus* may be a lipid, for it is known that lipids may react positively to ammoniacal silver nitrate.

Notwithstanding the differences noted above, the chemical composition of the cuticle suggests a similarity to the cuticle of the arthrodial membrane of an insect like *Periplaneta*. The histochemical tests for proteins give similar reactions in both the cuticles, which suggests that a similar type of protein forms a basal matrix that is not impregnated at any stage by another protein. The absence of an amber coloration is common to both. Susceptibility to the action of mineral acids and in general a lack of chemical stability are other features common to the cuticle of the arthrodial membrane of insects and that of *Streptocephalus*. Although a lipid is said to be wanting in the epicuticle of the arthrodial membrane of the cockroach, its reported occurrence in the cuticulin layer from the very inception of its development in insects like *Calliphora* suggest the possibility of the presence of a lipid constituent in the arthrodial membrane of insects, as in the cuticle of *Streptocephalus*.

Nature of the Epicuticle

From the results of the histochemical tests, it may be inferred that the epicuticle of *Streptocephalus* corresponds to the epicuticular layer of the cuticle of the arthrodial membrane of insects. Histological examination did not reveal the presence of an outer epicuticle as in the decapod Crustacea. Sections stained with Mallory's and Masson's trichrome stains show the epicuticle as an apparently single layer. Although with Sudan black the outermost portion of the epicuticle becomes darker, the colouring is diffuse, so that it is not possible to distinguish a separate outer layer on the basis of the sudanophil reaction. It has been observed that concentrated hydrochloric acid rapidly dissolves the endocuticle, but the epicuticle is only slowly attacked. On prolonged treatment, however, the greater part of the epicuticle is disrupted, leaving a very thin outermost membrane which resists for some time the action of cold hydrochloric acid. Pieces of entire cuticle were kept in concentrated hydrochloric acid for periods varying from 1 hour to 2 days, and later embedded in paraffin and sectioned. A comparative study of the cuticle after having been kept in hydrochloric acid for varying periods, shows that the most resistant part of the cuticle is the outermost very thin layer of the epicuticle. Material kept in concentrated hydrochloric acid for about a day and later sectioned and stained with Heidenhain's haematoxylin shows in the epicuticle a marked distinction into an outermost very thin membrane staining more intensely with haematoxylin and a wider inner part which is very lightly stained and shows signs of disruption (fig. 2) when sections of the same material are treated with Sudan black. The outermost layer referred to above takes up a black coloration while the inner part is not or only feebly reactive. The presence of a membrane answering to the description given above may be made out in some preparations of the cuticle subjected to a less violent

treatment than is involved in the chitosan test, as for example when the material is treated with concentrated potash for about half an hour at 100° C and later sectioned. The membrane in question is clearly seen in relation to the endocuticle and the surviving parts of the epicuticle, of which it forms the outer rim. When stained with Heidenhain's haematoxylin, it was coloured black, whereas the endocuticle hardly took up the colouring agent. Similarly, when sections of the cuticle are treated with cold chlorated nitric acid, the dissolution of the cuticle is rapid, starting with the endocuticle and extending

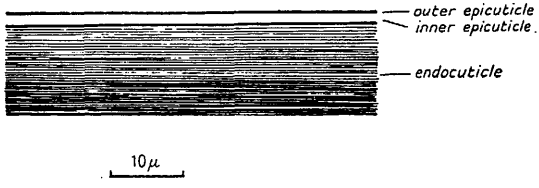

FIG. 2. Transverse section of the tergite cuticle of *Streptocephalus* after treatment with concentrated hydrochloric acid, stained with iron haematoxylin.

outwards. After some time the entire section of the cuticle breaks down, but the portion which survives to the last is the very thin outermost membrane of the epicuticle, corresponding to that noted after maceration with concentrated hydrochloric acid. The sequence of disruptive changes correspond with those reported for the epicuticle of the cockroach (Dennell and Malek, 1955*a*), in which a thin membrane that survives to the last has been thought to be homologous with the outer epicuticle or the outer resistant part of the cuticulin layer of insects.

In the adult epicuticle of *Palamneus* an outermost, thin, resistant layer is distinguished as the outer epicuticle. Histochemical and X-ray diffraction studies show that it contains long-chain paraffins (Krishnan, 1954). The chemical features of the outermost part of the epicuticle of *Streptocephalus* recall in some respects the outer epicuticle of *Palamneus*.

It will be seen from the foregoing account that the cuticle conforms to what may be called the basic structure of the arthropod cuticle in comprising two fundamental layers, the epicuticle and endocuticle, of which the epicuticle gives evidence of being constituted of two parts, an outermost, very thin, layer containing lipid, and an inner lipoprotein part. In the absence of hardening by tanning or by —S—S-bonding it differs from that of other arthropods but recalls strongly the arthrodial membrane condition of the adult cuticle of such an insect as *Periplaneta*.

CUTICULAR PROTEIN

The peculiarities of the cuticle of *Streptocephalus*, especially its chemical composition, are attributable to the nature of the cuticular protein. Although in some respects there is a similarity to the protein of the arthrodial membrane

cuticle of *Periplaneta*, a direct comparison with it is precluded by a paucity of information on the nature of this protein in the insect. It is, however, clear from the results reported by Dennell and Malek (1955*a*) that the protein constituent of the arthrodial membrane is different from arthropodin, which is characterized by a high tyrosine content and presumably stains red with Mallory. A similar inference may be made regarding the protein of the tergite cuticle of *Streptocephalus*. In table 2 are given some of the results of the tests performed on the cuticle which support the above suggestions.

TABLE 2

The responses of the tergite cuticle of Streptocephalus *to further chemical tests*

Test	*Result*
pepsin + HCl	swells and disrupts
saturated ammonium sulphate	slowly disintegrates
trichloracetic acid 10% (cold)	swells and disrupts
boiling water	swells
H_2SO_4 (dilute)	swells and disrupts
lead acetate test	negative
sodium nitroprusside test	negative

The above results suggest that the cuticular protein of *Streptocephalus* has none of the characteristics described for the sulphur-containing protein of the cuticle of *Palamneus* (Krishnan, 1953, 1954), which gives positive colour reactions with the sodium nitroprusside and lead acetate tests, and shows marked swelling effect on treatment with alkaline sodium sulphide. Nor does this protein accord with the arthropodin of the soft cuticle of insects, which is characterized by its insolubility in cold 10% trichloracetic acid and its failure to coagulate in hot water. On the other hand the reactions to boiling water, its dissolution in pepsin, and saturated ammonium sulphate, as well as in cold 10% trichloracetic acid, while distinguishing it from arthropodin or sclerotin, suggest a collagenous type of protein.

To test the validity of the suggestion made above a microchemical analysis of the cuticle was made by a modification of the method of Spencer, Morgulis, and Wilder (1937). These authors applied the above method successfully for a microdetermination of the collagen content of the muscles of the rabbit.

The cuticle was separated by cutting open the body longitudinally; the contents were removed. The cuticle was cleaned by scraping with a blunt scalpel in a washing medium of 60% alcohol, so as to remove as completely as possible all adhering tissue. The cuticular material was cut into small bits and a 100 mg sample was homogenized with 1 ml of distilled water in a Potter–Elvehjam homogenizer. Drying the material with acetone was omitted (see Harkness, 1952). The homogenized cuticle was placed in a boiling water bath for 10 to 15 min with 5 to 10 times its weight of water and later stored in a refrigerator. Next day it was autoclaved for 3 h at 20 lb pressure. By auto-

claving, the collagen in the cuticle (if any) would be converted into gelatin. The material was then centrifuged at 4,000 rev/min for 1 h and the supernatant fluid drawn off and treated with 5% tannic acid, as in the original method. By this treatment a precipitate was obtained which indicates the presence of gelatin derived from the cuticle (see Spencer and others, 1937).

To test further the nature of the precipitate, it was subjected to a chromatographic analysis. The material was treated with 10 times its weight of 6 NHCl in a sealed tube and hydrolysed at 105° C for 24 h. After hydrolysis the solution was transferred to a small beaker and dried in a vacuum desiccator containing potassium hydroxide. When drying was complete the material was used for an analysis of its amino-acid constituents by paper partition chromatography. The technique followed was the capillary ascent method of Williams and Kirby (1948). The hydrolysate was dissolved in a small quantity of distilled water and a 20 μl sample was applied to a sheet of Whatman no. 1 filter paper. The chromatogram was run with butanol acetic acid water as the solvent. Simultaneously a number of chromatograms were run under identical conditions with solutions of pure amino-acids for purposes of comparison. The amino-acids were each prepared by dissolving 5 mg in 5 ml of distilled water. The chromatograms were examined after spraying 0·1% ninhydrin in butanol. For one set of chromatograms the spraying agent was prepared as above and to this a few drops of 2:4:6 collidine were added, which is said to improve the sensitiveness of the reagent. It was found that by this means, the amino-acid spots on the chromatograms were more sharply defined. An estimation of the amino-acids present in the hydrolysate was made by the application of Rf values of amino-acids as well as by comparison with chromatograms obtained with pure amino-acids.

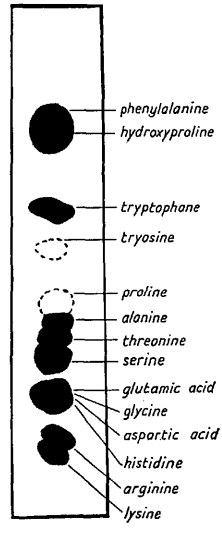

FIG. 3. Diagram of a paper chromatogram of the amino-acids in an acid hydrolysate of cuticular material of *Streptocephalus*, estimated by the method of Spencer and others (1937). Dotted lines represent spots which are visible in the original but did not appear in the photographic reproduction.

The amino-acids in an acid hydrolysate of the protein of the cuticle of *Streptocephalus* include most of those obtained from the collagen of ox-hide (Bowes and Kenten, 1949), that is, alanine, arginine, asparatic acid, glutamic acid, glycine, histidine, hydroxyproline, lysine, phenylalanine, proline, serine, threonine, and tyrosine (see fig. 3). However, the cuticular protein is lacking in cysteic acid, hydroxylysine, leucine, and valine, present in the collagen from ox-hide, while it includes tryptophane, which is absent from the latter. It has been observed that the same type of protein drawn from different sources

may vary in some respects in regard to amino-acid composition (Hackman, 1953). In view of the above observations, and considering the number of amino-acids common to the cuticle protein and collagen, it would appear reasonable to suggest the essential identity of the cuticular protein and collagen.

As a further test, the chromatogram obtained from cuticle protein was compared with that of pure gelatin, prepared for chromatographic analysis in a manner identical with that applied to the material of the cuticle that is precipitable by tannic acid. There was a marked resemblance between the amino-acids of the gelatin and those of the cuticle.

Discussion

The results reported above show that the cuticle of *Streptocephalus* presents certain peculiar features in regard to its structure and chemical composition. The epicuticle, though conforming in general to the type found in other Crustacea, lacks the differentiation into sub-layers corresponding to the outer and inner epicuticle of decapods and cirripedes. The staining reactions suggest an apparent homogeneity, for the outermost resistant part, distinguished by chemical tests, was not evident on histological examination. Another noteworthy feature is the marked similarity in the chemical characteristics of the epicuticle and endocuticle, apart from the distinction based on the absence of chitin in the former. These two layers stain alike with Mallory's and Masson's trichrome stain and show similar reactions to histochemical tests for proteins and lipids, although quantitative differences exist. It is known that in decapod Crustacea as well as in cirripedes the two fundamental layers of the cuticle are distinguished by their different chemical composition. Apart from the absence of chitin, the epicuticle in all Crustacea so far studied is characterized by the presence of a protein rich in phenolic substances, which is either absent in the endocuticle or appears at a later stage in the growth of the cuticle and then distinguishes the presumptive exocuticle. The absence of such a distinction cannot be explained as due solely to the non-occurrence of tanning. This does not by itself provide an explanation, for there are a number of instances among the decapod Crustacea in which, even where tanning does not occur, the protein in question is still present in the epicuticle, as for example in the soft cuticle lining the gut of *Homarus* and the tergite cuticle of *Penaeus* (Yonge, 1932; Krishnan, 1956). It would appear that the protein constitution of the cuticle in *Streptocephalus* is such that it lacks the mechanism necessary for undergoing tanning and is destined to remain permanently in a soft and flexible state, comparable in some respects to the arthrodial membrane of the insect cuticle. The significance of such a feature may be that the cuticle in this type is in an unspecialized condition.

The above suggestion receives further support from a consideration of the nature of the cuticular protein. Until recently few attempts have been made to characterize the proteins of the arthropod cuticle. The observations of Trim (1941) on the cuticular protein of two insects, *Sarcophaga falculata* and

Sphinx ligustri, show that it is different from collagen and in some respects resembles sericin. Fraenkel and Rudall (1947), while confirming in general the observations of Trim, emphasized the unique features of this protein, which made it difficult to relate it to any known protein. They felt the necessity of designating it by a new name, arthropodin. Among the features distinguishing arthropodin may be mentioned its occurrence always in a β or extended pattern, its high tyrosine and low glycine content, solubility in hot but not cold 10% trichloracetic acid, and its failure to coagulate in hot water. When hardened by tanning, arthropodin is said to give rise to sclerotin, which shows a similar amino-acid composition. Later, Blower (1951) in his studies on the cuticle of myriopods noted at least two different protein components during the growth-phase of the cuticle before tanning took place, the one staining blue with Mallory and found in the inner regions of the endocuticle that never undergo tanning, the other appearing shortly before tanning and connected with that process. On account of this feature Blower designated it prosclerotin. A protein identical in staining and chemical characteristics with the so-called prosclerotin is found in insects; it has been described in detail by Dennell and Malek in *Periplaneta*, where it is shown as impregnating a basal protein matrix of the epicuticle and the presumptive exocuticle. In staining reactions, amino-acid composition, and histochemical reactions this protein is indistinguishable from arthropodin. The identity of the tyrosine-rich protein of the cockroach cuticle with arthropodin has also been indicated by Dennell and Malek (1955*b*). It may be suggested that the tyrosine-rich, fuschinophil protein, reported from the cuticles of various Crustacea, is identical with the above-mentioned protein in cockroach cuticle. From the wide occurrence of this type of protein in arthropods it was assumed that it is characteristic of the arthropod cuticles. However, the observations on the epicuticular protein of some arachnids and myriopods (Krishnan, 1954, 1956) have not confirmed such a view. In them the principal protein component of the epicuticle is different from its counterpart in insects in containing organic sulphur and in being hardened by a process similar to keratinization. Very little is known of its chemical nature except that it is rich in cystine residues and that in spite of a superficial resemblance, it is not identical with vertebrate keratin as revealed by its X-ray diffraction pattern. As in the arachnids referred to above, the cuticular protein of *Streptocephalus* is unlike arthropodin or its derivative sclerotin. An analysis of the protein suggests a marked likeness to collagen. The nature of the protein forming the basal matrix of the cuticle of the arthrodial membrane of insects or that forming the protein constituent of the unhardened regions of the endocuticle in insects and decapod Crustacea is not known. Hitherto the homogeneity of the cuticular protein has been assumed, and recently Hackman (1953) called attention to the possibility of the occurrence of a number of protein components constituting the total protein content of the cuticle. The above author found from an electrophoretic examination of the cuticular protein of the insect *Diaphonia dorsalis* that it is formed of a number of components. The fastest anodic component showed an

amino-acid composition different from that of the entire protein preparation of the cuticle in not containing serine, threonine, phenylalanine, proline, or hydroxyproline. It seems clear that in the cuticle of arthropods we have to deal with several protein fractions showing different amino-acid composition. This feature may account for the apparently anomalous results obtained from an analysis of the total protein content of the cuticle.

In the foregoing study of the cuticle of *Streptocephalus* a type of protein has been isolated which is shown by chemical tests to be allied to collagen. Its amino-acid analysis also indicates a pattern closely resembling that of collagen. The occurrence of a collagenous protein has not till now been reported from the cuticles of arthropods. Till very recently it has been assumed that there is only a single protein component in the cuticle and that this is a keratinous β protein, so that the other protein components have been ignored. Even in the insects which have received much attention in this respect, very little is known of the protein fractions of the cuticle other than arthropodin or its derivative sclerotin. For example, no information is available regarding the nature of the protein component of the cuticle of the arthrodial membrane of insects, which is known to differ in staining and histochemical reactions from those of arthropodin. It is of interest to point out that in the above-mentioned features, it resembles to some extent the cuticular protein of *Streptocephalus*. But till more extended observations are made regarding the nature of the protein of the arthrodial membrane cuticle, a valid comparison with it is not possible. An interesting point for consideration is the possible significance of the occurrence of collagen in *Streptocephalus*, for this is a primitive crustacean type, showing many features in its anatomy reminiscent of annelid organization. In the light of these facts, the presence in its cuticle of a protein identical with that found in the annelid cuticle may be considered significant.

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