Nature of the Cuticle Protein of the Branchiopod Crustacean *Streptocephalus dichotomus*

It has been reported that the cuticle of *Streptocephalus dichotomus* remains in a permanently untanned condition and its protein component conforms in staining and histochemical reactions with the basal protein of the tanned cuticles of insects like *Periplaneta*[1-3]. But very little is known of the basal protein referred to above, except that it is different from the protein precursor of tanning. In view of the absence of tanning, the cuticle of *Streptocephalus* provides suitable material for a study of the protein in question. In an earlier study, the collagenous nature of the cuticular protein of *Streptocephalus* has been suggested, based on the presence of amino acids like proline and hydroxyproline. But it is realized that such an evidence may not by itself be indicative of collagen as hydroxyproline has been reported to occur in proteins other than collagen[4,5].

The criteria suggested for characterizing collagenous protein are based on the molecular organization, by which collagen may be distinguished from the other large class of proteins keratin, myosin, epidermin and fibrin[6]. The molecular characteristics of collagen are indicated by a typical high angle X-ray diffraction pattern showing the 2.9 Å meridional reflection and a characteristic axial period of 600–700 Å in low angle X-ray diffraction as well as a banded pattern in electron micrographs. However, it has been reported that certain invertebrate collagens may not show the axial periodicity of 600–700 Å as in the cuticular collagen of the earthworm[7,8]. But the chemical characteristics of collagen are said to provide means for distinguishing collagenous proteins from others. A valid criterion is the unusually high content of glycine which in all collagens so far studied forms about $^1/_3$ of the total amino acid residues[9].

In the light of the observations reported above, a quantitative estimation of amino acids of the cuticle protein of *Streptocephalus* was made as a sequel to the earlier study of the amino acid analysis already reported[1]. The procedure adopted for the extraction of the water-soluble protein of the cuticle was based on the work of HACKMAN[10]. Aliquots containing 1–2 mg of protein were used for analysis by means of an automatic amino acid analyser similar to the one described by SPACKMAN et al.[11]. Quantitative estimation of the amino acids was based on the ninhydrin colouring intensity of the effluent from ion exchange columns. The colour was developed by passing the mixture of the reagent with the effluent through a spiral capillary tubing kept in a boiling water bath. The absorbancy of the coloured solution was estimated at 570 μ and 440 μ in a spectrophotometer. The peaks on the recorded curves were integrated for loads varying from 0.1–3.0 μmoles of each amino acid. The ninhydrin positive constituents of the effluent from the chromatographic columns were analyzed. The results were compared with those obtained with pure gelatin treated in an identical manner. The Table shows the relative quantities of amino acids present in the cuticle protein and those in a pure sample of gelatin. The values were compared with those obtained by an analysis of the rat skin collagen[12] and of the earthworm cuticle[13]. Although the relative proportions of amino acids of the protein of *Streptocephalus* cuticle show some differences in comparison with gelatin, collagen of earthworm cuticle and rat skin, the amino acid pattern conforms to what has been considered characteristic of collagenous protein which includes appreciable quantities of hydroxyproline, proline, hydroxylysine together with a comparatively large proportion of glycine forming nearly one-third of the total residues, and the absence or occurrence in traces of sulphur-containing and aromatic amino acids[9].

To verify whether the amino acids analyzed above are derived from a single homogeneous protein fraction, an analysis of the water-soluble protein extracted from the cuticle of *Streptocephalus* was made using an electrophoretic set up under following conditions: Phosphate buffer pH 5.8, ionic strength 0.2 (0.16 *M* KH_2PO_4 – 0.0167 *M* KOH) voltage 15.0 v/cm, duration of the run 3 h, and cooling temperature for the apparatus 10–15 °C. The electropherograms were scanned using Carl-Zeiss extinction recording instrument model ERI-10. It was found that electrophoretically the protein of the cuticle of *Streptocephalus* behaves like gelatin.

It has been reported that the protein component of the cuticle of *Streptocephalus* shows staining properties and histochemical reactions which suggest a similarity to collagen. The results reported in the present study

Amino acid composition of protein component of the cuticle of *Streptocephalus dichotomus*

Amino acids	Rat skin collagen (PIEZ and GROSS[12])	*Lumbricus* (Annelida) cuticle gelatin (WATSON[13])	Gelatin	*Streptocephalus* cuticle
Alanine	106	103	110	110
Arginine	49	21	53	48
Aspartic acid	47	56	44	43
Glutamic acid	74	81	69	75
Glycine	327	324	339	328
Hydroxyproline	100	165	59	59
Hydroxylysine	5.7	0	6.3	6.3
Lysine	29	15	25.7	22
Histidine	5.1	0	11.7	12.9
Methionine	6.3	0	15.7	16.1
Phenylalanine	13	11	8.4	0.2
Proline	117	13	99	97
Serine	41	105	59	59
Threonine	20	52	24	26
Tyrosine	3.2	0	2.7	1.9
Valine	22	17	27.6	29.1

Residue amino acid/1000 total residues.

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provide evidence of the collagenous nature of the protein in question, based on quantitative estimation of glycine. In this context it is of interest to recall the observations of RUDALL[14] that the protein of the soft cuticles of lobsters and insects show chemical features reminiscent of collagenous proteins.

Zusammenfassung. Der einzige Proteinbestandteil des Branchiopodenkrebses *Streptocephalus dichotomus* zeigt ähnliche chemische Eigenschaften wie kollagene Eiweissverbindungen. Merkmal der Aminosäurezusammensetzung ist eine relativ grosse Menge von Glyzin (etwa $^1/_3$ des Gesamtresiduums), zusätzlich zu Hydroxyprolin und Prolin. Dies wird als Kriterium zur Unterscheidung von kollagenem Protein von den übrigen Eiweissen (K-mef-Gruppe) benützt.

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Division of *Entamoeba invadens* Rodhain, 1934, in Cultures[1]

The nuclear division of *Entamoeba* has been studied by several authors. The Table summarizes these observations. So far, very little attention has been paid to the cytokinesis of *Entamoeba*. The term 'cytokinesis' has been used here as defined by MAZIA[2] which includes all the schemes of cytoplasmic division during the process of mitosis.

From the survey of the literature on the cell division of *Entamoeba*, it appears that HARRIS[3] first saw the cytoplasmic division of a trophozoite of *E. histolytica* (Amoeba dysenteriae) in the faecal smears. He observed the trophozoite elongated to many times its width, and one end moved more rapidly, creating a constriction near the middle. The daughter amoebae remained connected for a while by a long but less that 1 μ wide 'band, composed entirely of ectosarc'. Usually, division into 2 was observed by HARRIS but once he saw a division into 3 (his Figure 1d, p. 568). DOBELL[4] considered that HARRIS observed a pathological process, because it was studied at room temperature. DOBELL[4] has argued that the sudden change of temperature might have caused such division referred to above in *E. histolytica* trophozoites.

SHAFFER et al.[27] have studied the cytokinesis of *E. histolytica* strain K-9, from cultures with the help of time lapse cinemicrophotography. In their experimental conditions the nucleus of the amoeba could not be seen. Their description of the cytoplasmic division supports the observation of HARRIS[3] and adds further details to the process. Usually the trophozoites of *E. histolytica* divided into 2 equal halves, following a parallel procedure described above[3]. Mostly the division was completed within 3–8 min. Sometimes, 1 of the 2 sister amoebae divided into a 'normal' and a 'very small' sized amoeba (Figure 3, p. 170 of SHAFFER et al.[27]). The latter also behaved as a typical *E. histolytica*. Occasionally the 3 daughter amoebae, thus produced, were of the same size (see Figure 1, p. 568 of HARRIS[3]). SHAFFER et al.[27] have also frequently observed a process suggestive of conjugation of 2 *E. histolytica* trophozoites. 2 amoebae became apposed to each other and the membranes at their point of contact appeared to break; a few seconds after, the 2 amoebae separated. In the following, attempts have been made to follow the cytokinesis of *E. invadens* in cultures.

Materials and methods. Monoxenic cultures of *E. invadens* strain BC was grown in biphasic 'HSre + S' and 'HShsm + S' media at 24 °C. Further details of culturing and the history of the strain of amoeba used are given elsewhere[28]. During the log phase of growth[28], the amoebae were examined in paraffin sealed smears under a Wild M20 phase contrast microscope at room temperature (22 °C).

Results. Figure 1 is a photomicrograph of *E. invadens* BC taken from a 10-day-old culture. When the amoeba

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