Résumé

Le nombre diploïde des chromosomes du *Polididus armatissimus* est de 14. L'X et l'Y sont de taille presque égale. L'ensemble des chromosomes de cette espèce représente un karyotype primitif pour la famille des Reduviidae.

Concerning the Free Amino Acids in the Hydroid Tubularia[1]

Recently FAULHABER and TARDENT[2] demonstrated the presence of fourteen free amino acids in alcohol extracts of two to three hydranths of *Tubularia larynx*. Only six of these amino acids were detected in extracts of 40–60 regenereted hydranths and 3 mm long segments of hydrocauli. These results suggest that relatively few free amino acids are present in tubularian tissues and that marked qualitative differences in the distribution of free amino acids may exist between the morphologically distinct hydranth and hydrocaulus. The results also suggest that the free amino acid composition of hydranths recently regenarated from hydrocaulus tissues is more like that of the hydrocaulus than that of mature hydranths.

The present report is also concerned with the qualitative distribution of free amino acids and related substances in the mature hydranth, regenerated hydranth, and hydrocaulus of *Tubularia*. The results, contrary to those of FAULHABER and TARDENT, suggest that there are few if any qualitative differences in the free amino acid composition of the hydrocaulus and the hydranth.

Materials and Methods. Hydranth and hydrocaulus tissues of *Tubularia crocea* and *T. spectabilis* were prepared for alcohol extraction of free amino acids by first removing the hydranth and then the distal 4 mm of the hydrocaulus. The latter was discarded. The remaining unencrusted portions of the hydrocauli and the hydranths were frozen, lyophilized, and stored at −20°C. The nitrogenous substances were extracted from the lyophilized tissues with 80% ethanol according to AWAPARA[3]. Each extract was centrifuged, filtered, and mixed in a separatory funnel with three volumes of chloroform. After standing, the aqueous layer was drawn off and evaporated to dryness. The residue was taken up in 10% isopropanol and the nitrogen content adjusted to 11–12 mg N/ml. In order to detect as many substances as possible 4, 8, 12, 16, and 20 µl quantities of the extracts were chromatogrammed.

Chromatograms also were prepared of live tissues which were rinsed first in 10^{-3} *M* ethylenediamine tetraacetic acid (EDTA) in sea water, then in 10^{-3} *M* EDTA in distilled water, and applied directly to the paper and crushed[4]. The following amounts of material were chromatogrammed in this way: 5–10 mature hydranths with gonophores, 30–180 regenerated hydranths which lacked gonophores and actinula larvae, and 30–50 hydrocaulus segments 1 cm long which were first homogenized and then chromatogrammed.

Amino acids were separated by one- and two-dimension descending paper partition chromatography, using Whatman number 1 filter paper. The solvent system used for one-dimensional chromatograms was 1-butanol:acetic acid:water (4:1:1). With two-dimensional chromatograms this solvent was employed in the first direction and phenol:water:8-hydroxyquinoline (80:20:04) in the second direction. The amino acid spots were revealed by dipping the chromatograms in a solution of 0.5% ninhydrin in acetone. The spots were identified by comparing their ninhydrin color and R_f's with those of known amino acids on chromatograms prepared simultaneously.

Certain amino acids were identified further by treating one-dimensional chromatograms of hydranths and hydrocauli with other reagents. The sulfur amino acids—cysteine, cystine, methionine—were detected with sodium nitroprusside reagents[5]; arginine with the Sakaguchi reaction and nitroprusside reagent[6]; proline with isatin[7]; tyrosine and histidine with modified Pauly's reagent[8]; the hydroxyamino acids—serine and threonine—with the Nessler-periodate test[6]; and hydroxyproline by first treating the chromatogram with 0.2% isatin in acetone and then spraying with Ehrlich's reagent[9]. Tryptophane was detected by its fluorescence under ultraviolet light.

Taurine was not identified, nor were α- and β-alanine distinguished as was done in the study of FAULHABER and TARDENT[2].

Results. The following sixteen free amino acids were detected on chromatograms of crushed hydranths of *T. crocea:* alanine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, hydroxyproline, leucines, lysine, methionine, proline, serine, threonine, tyrosine, and valine. Chromatograms of *T. crocea* crushed hydrocauli were similar but lacked hydroxyproline, proline, cystine, and methionine.

Chromatograms of alcohol extracts of *T. crocea* revealed five additional free amino acids in hydranth tissues — arginine, asparagine, histidine, phenylalanine, and tryptophane. Chromatograms of alcohol extracts of hydrocauli and of crushed regenerated hydranths were similar to each other as well as to those of extracts of mature hydranths, except that cystine, methionine, and phenylalanine were not detected. Thus of the twenty-one free amino acids present in mature hydranths, eighteen were detected in hydrocauli and regenerated hydranths.

The free amino acid composition of alcohol extracts of *T. spectabilis* hydranths was similar to that of hydranth extracts of *T. crocea* except that phenylalanine and histidine were not detected on *T. spectabilis* chromatograms. Although similar in free amino acid composition to alcohol extracts of *T. crocea* hydrocauli, the extracts of *T. spectabilis* hydrocauli lacked—in addition to cystine, methionine and phenylalanine—asparagine, histidine, hydroxyproline, and proline. The qualitative differences between the free amino acids of hydranths and hydrocauli of the two species may be significant since the alcohol extracts had similar nitrogen contents.

Seventeen unidentified spots which did not correspond to any simple amino acid spots were detected on chromatograms developed with ninhydrin and other reagents. Table I shows eleven unidentified ninhydrin positive substances detected on two-dimensional chromatograms of *T. crocea* tissues. The patterns of crushed tissues were

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Tab. I. Unidentified ninhydrin positive substances on two-dimensional chromatograms of *Tubularia crocea*

Spot	R_f		Crushed			Alcohol Extract	
	a	b	Hydrocaulus	Mature Hydranth	Regen. Hydranth	Hydrocaulus	Mature Hydranth
1	0.34	0.92	+	+	+	+	+
2	0.54	0.94	+	+	–	–	–
3	0.09	0.10	+	+	+	+	+
4	0.07	0.05	+	+	+	–	–
5	0.05	0.03	+	+	+	–	–
6	0.03	0.02	+	+	+	–	–
7	0.08	0.90	–	–	–	–	+
8	0.06	0.79	–	–	–	+	+
9	0.06	0.67	–	–	–	–	+
10	0.25	0.11	–	–	–	+	+
11	0.03	0.07	+	–	+	–	–

[a] R_f in butanol: acetic acid: water. [b] R_f in 80% phenol.

Discussion. The detection in the present study of more free amino acids in tubularian tissues and few if any qualitative differences in the distribution of these amino acids as compared to the number reported previously[2] is probably due to the larger amounts of material chromatogrammed in the present study. The present results, however, do not negate the possibility of morphogenetically significant quantitative differences in the free amino acid composition of mature and newly regenerated hydranths and hydrocauli as well as hydrocaulus tissues during hydranth regeneration.

The qualitative distribution patterns of several unidentified substances suggest that their identity and fate during hydranth regeneration should be examined.

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Tab. II. Non-amino acid substances of alcohol extracts of *T. crocea* and *T. spectabilis* with reagents for imidazoles (modified Pauly's tests), guanidines (Sakaguchi reaction), -SS- bonds (nitroprusside test), SH groups (nitroprusside test), the hydroxyamino acids, serene, and threonine (Nessler periodate test)

Spot	R_f a	Character of Spot						Presence or Absence of Spot			
								T. crocea		*T. spectabilis*	
		Ninhydrin	Imidazoles	Guanidine	-SS-	SH	Hydroxyamino acid	Hydranth	Hydrocaulus	Hydranth	Hydrocaulus
1	0.34	+	–	+	–	+	+	+	+	+	–
3	0.09	+	+	+	+	–	+	+	+	+	–
12	0.12	–	–	–	+	–	–	+	+	+	+
13	0.22	–	–	–	–	+	–	+	–	+	–
14	0.28–0.32	–	+	–	–	–	–	+	+	+	+
15	0.44	–	–	+	–	–	–	+	–	+	–
16	0.66	–	+	–	–	–	–	+	+	+	–
17	0.88	–	+	–	–	–	–	–	+	–	+

[a] R_f in butanol: acetic acid: water.

similar except that spot 11 was not present on mature hydranth chromatograms and spot 2 was not present on regenerated hydranth chromatograms. The absence of spots 2, 4, 5, 6, and 11 from chromatograms of hydrocaulus and hydranth alcohol extracts is probably due to alcohol precipitation of substances associated with these spots. Spots 7, 8, 9, and 10 appeared on chromatograms of hydranth extracts; but only spots 8 and 10 were detected on chromatograms of hydrocaulus extracts.

On treating one-dimensional chromatograms of alcohol extracts with reagents for functional groups on certain amino acids, spots 1 and 3 were developed with several reagents (Table II). As seen in Table II of the six additional spots which were ninhydrin negative, spots 13 and 15 were detected only in hydranth extracts of the two species while spot 17 was detected only in stem extracts of the two species. In addition spots 1 and 3 were not detected in extracts of *T. spectabilis* hydrocauli.

Résumé

La composition de l'amine acide libre dans les deux espèces de *Tubularia* a été examinée au moyen de la chromatographie. Les tissus d'hydranthe de *T. crocea* avaient 21 amines acides libres dont 18 ont été identifiées dans les hydrocauli et dans les hydranthes régénérés de *T. crocea*, 19 dans les hydranthes de *T. spectabilis* et 14 dans les hydrocauli de *T. spectabilis*. La différence principale entre les hydranthes et les hydrocauli réside dans la distribution de certaines taches non-identifiées qui apparurent dans les chromatogrammes developpés avec la ninhydrine et les réactifs pour les groupes des imidazoles, guanidines, ou sulfhydriles.

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