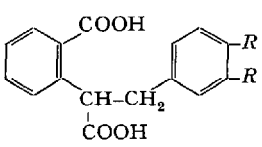
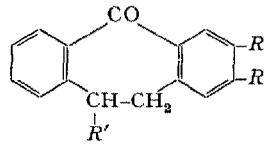
an alkali-insoluble compound, $C_{20}H_{18}O_4$ (m. p. 176–177°C) resulted. The latter is identified as the diethoxyindeno*iso*coumarin (VI). α-Benzylhomophthalic acid itself afforded only the *iso*coumarin (VII, m. p. 173°C) on cyclisation. This structure is confirmed by the sharp lactone carbonyl absorption at 5·78 μ (KBr pellett) and by conversion into 1:2-Benzo-4-azafluorene (m. p. 163–164°C; picrate, m. p. 234–235°C) by conventional methods. Further, reduction of the *iso*coumarin (VII) by lithium aluminium hydride[4] gave the related *iso*chromene (m. p. 103–104°C) which was readily converted into indeno-(3′:2′-3:4)-*iso*-benzopyrylium perchlorate (m. p. 197–199°C) by treatment with triphenylmethylperchlorate in acetic acid[5].

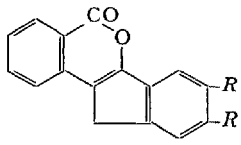
Other substituted benzylhomophthalic acids so far examined have afforded only the indeno*iso*coumarins.



I R = OMe
IV R = OEt



II R = OMe, R' = COOH
III R = OMe, R' = H
V R = OEt, R' = COOH



VI R = OEt
VII R = H

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Résumé

Les cyclisations des acides α-benzylhomophthaliques ont produit un keto-acide à 7 termes, un indenoisocoumarin ou un mélange des deux.

Chromosome Mechanism in *Polididus armatissimus* (Reduviidae-Heteroptera)

The genus *Polididus* has so far been known[1,2] cytologically by only a single species, *Polididus armatissimus*. The diploid number of chromosomes in this species is twelve, which includes a typical XY pair of sex chromosomes. The latter are of almost equal size and behave post-reductionally during meiosis. This chromosome number is the lowest among all the reduviids so far investigated which are otherwise characterised by the presence of a multiple X chromosome and a high number of chromosomes varying from twenty-three to thirty-two. Only in a few exceptional cases is the X simple. On account of the simple XY sex-determining mechanism and the lowest number of chromosomes, the *Polididus* constitutes an interesting material for cytological investigations.

During the studies on the chromosomes of Indian Heteroptera, the author[3–5] happened to examine *Polididus armatissimus* which was found to differ markedly in its karyotype from that already described[1,2].

At the spermatogonial metaphase (Fig. 1) there are fourteen chromosomes out of which two pairs can be distinguished as rod-shaped from the remaining ten more or less round ones. The eight elements at the metaphase of the first meiotic division (Fig. 2) form the typical reduviid pattern—the six autosomal bivalents forming a ring, slightly outside which are the two sex chromosomes, X and Y. The sex chromosomes reveal only a slight difference in size among themselves. The metaphase of the second meiotic division (Fig. 3) also presents six autosomal elements forming a ring sorrounding the centrally placed 'pseudo-bivalent' formed by X and Y. The two components of the 'pseudo-bivalent' differ slightly in their size (Fig. 4).

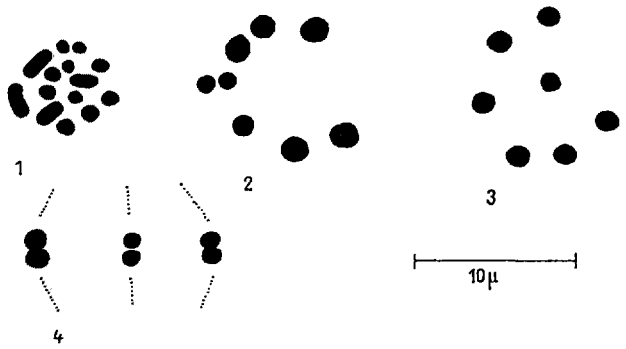

Fig. 1. Spermatogonial metaphase (polar view). Fig. 2. Metaphase I (polar view). Fig. 3. Metaphase II (polar view). Fig. 4. Metaphase II (side view), only three elements are shown.

Utilising the chromosome data to establish the phylogenetic relationship in the various families of Heteroptera, MANNA[6] and BANERJEE[2] conclude that the number '12 + XY' represents the primitive karyotype in Heteroptera and that the evolution of Reduviidae from Lygaeoidea has been accompanied by an increase in the basic number of chromosomes and a change from a simple X to multiple X condition. Such an assumption would point to the primitive nature of the karyotype in the genus *Polididus*, where the '12 + XY' condition appears to have survived as such. From the facts that, in the various species of the family Reduviidae with multiple X chromosome, the size of the Y is quite large as compared with those of the Xs, and that the sizes of the individual Xs decrease as their number increases, it may be concluded, as already suggested by TROEDSSON[7], that the increase in the number of Xs in the family has been accomplished by the fragmentation of the original X. The more or less equal size of X and Y in the genus *Polididus*, thus further supports the primitive nature of its karyotype amongst the reduviids.

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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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