Tabelle I. Zusammenstellung der Ergebnisse aus Versuch A (nähere Angaben im Text)

Tiergruppe		Normal	Normal + Öl	Normal + A-homo-Testosteronacetat	Normal + Testosteron
Organgewichte in mg	Hoden (Durchschnitt einseitig)	85,5 ± 1,8	76 ± 14	85 ± 3,7	63 ± 6,2
	Samenblasen (Durchschnitt einseitig)	71,3 ± 3,4	70 ± 20	58 ± 6,5	115 ± 7,5
	Präputialdrüsen	87,5 ± 5	78 ± 20	64 ± 8	109 ± 12
Kernvolumen der Leydigzellen des Hodens in μ^3/100 Kerne		14311	14854	14439	7151
Gewichtszunahme während des Versuches (Durchschnitt pro Tier) in g		3,6 ± 0,4	3,8 ± 0,5	1,9 ± 1	5,3 ± 0,4
Histologie	Hoden (Grösse der Zwischenzellen)	±	±	±	—
	Samenblasen (Epithelhöhe)	±	±	±	+
	Präputialdrüsen (Aktivität)	±	±	±	++

Tabelle II. Zusammenstellung der Ergebnisse aus Versuch B (nähere Angaben im Text)

Versuchsgruppen		Kastriert	Kastriert + Öl	Kastriert + A-homo-Testosteronacetat	Kastriert + Testosteron
Organgewichte in mg	Samenblasen (Durchschnitt einseitig)	5,0 ± 0,3	4,15 ± 0,4	6,1 ± 0,4	33,6 ± 2,1
	Präputialdrüsen	29 ± 2,2	26,8 ± 2,2	31,1 ± 2,1	58 ± 3,6
Histologie	Samenblasen (Epithelhöhe)	—	—	—	±
	Präputialdrüsen (Aktivität)	—	—	—	±

samkeit haben (Lit. siehe [5]). Über die Wirkung von Ringerweiterungen im Sterangerüst liegen noch keine sicheren Ergebnisse vor. Aus dem Ergebnis unserer Untersuchungen, die mit einer mittels Chromatographie gereinigten Substanz vorgenommen wurden, kann geschlossen werden, dass die Erweiterung des A-Ringes in der Position 3,4 bei Testosteronacetat zu einem Verlust der androgenen Wirksamkeit führt. Dies ist aus dem Ausbleiben der Substitutionswirkung beim kastrierten Tier erkenntlich. Ausserdem entfällt eine Hemmwirkung auf die gonadotrope Partialfunktion des HVL. Aus dem Verhalten des Gewichtszuwachses im Versuch A kann auf eine fehlende anabole Wirksamkeit geschlossen werden. Die von JOHNSON et al.[2] veröffentlichten vorläufigen Untersuchungsergebnisse gehen anscheinend von einer ungenügend von der Ausgangssubstanz gereinigten Verbindung aus. Bekanntlich genügen aber schon Spuren von Testosteron, um eine Wirkung zu entfalten. Eine solche Verunreinigung der Substanz scheint bei den genannten Untersuchungen vorzuliegen, worauf vor allem die Verzögerung des Wirkungseintritts hinweist.

Summary. The biological efficiency of a high purified A-homo-testosterone-acetate was tested on male mice. We found no inhibition of the gonadotropic partialfunction of the anterior pituitary by the substance; and furthermore no substitution of testosterone deficit after castration was possible.

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Anatomisches Institut der Universität Bonn (Deutschland), 11. Oktober 1962.

[5] H. LANGECKER, 9. Symp. Dtsch. Ges. Endokrinologie, Mainz, 3.–5. 5. 62 (Springer Verlag), im Druck.

Chromatographic Identification of Porphyrins in Several Species of Turbellaria

The occurrence and identification of uroporphyrin in the planarian, *Dugesia dorotocephala*, has been reported by the author[1]. Subsequently the uroporphyrin was localized in the intracellular rhabdites of epidermal and subepidermal cells of *Dugesia dorotocephala* and *Dugesia tigrina* (MACRAE[2]). The present report concerns the identification by paper chromatography of the porphyrins found in eight different species of fresh-water flatworms (Order Tricladida, Class Turbellaria, Phylum, Platyhelminthes). Only uroporphyrin, an octacarboxyl porphyrin, could be detected in some species. In other species a tetracarboxyl porphyrin, coproporphyrin, was detected. The distribution of these porphyrins among the species investigated seems to follow generic differences.

The species investigated included four North American species: *Dugesia dorotocephala* Woodworth, *Dugesia tigrina* Girard, *Cura foremanii* Girard (Genus *Cura* Family Planariidae) and *Phagocata gracilis gracilis* Haldeman (Genus

[1] E. J. KRUGELIS-MACRAE, Biol. Bull. *110*, 69 (1956).
[2] E. K. MACRAE, Science *134*, 331 (1961).

Phagocata, Family Planariidae). Specimens of four species found in Japan were also examined. These included: *Dugesia gonocephala* Duqès (Genus *Dugesia*, Family Planariidae), *Phagocata vivida* Ijima and Kaburaki, *Phagocata iwamai* Ichikawa and Kawakatsu (Genus *Phagocata*, Family Planariidae) and *Bdellocephala brunnea* Ijima and Kaburaki (Genus *Bdellocephala*, Family Dendrocoelidae)[3].

By means of fluorescence microscopy described by MACRAE[2], the red fluorescence due to porphyrins was consistently localized in the intracellular rhabdites of epidermal and subepidermal cells of these species.

Porphyrins were extracted from these species by HCl. As a preliminary, it was necessary to extract the worms either with ethyl alcohol or neutral formalin. This first extraction was found to facilitate the subsequent elution of porphyrin absorbed on talc during the purification procedure; it probably removed some interfering substance. 10 ml of 95% ethyl alcohol or of 10% neutral formalin were used per 10–20 worms. Extraction was allowed to proceed for at least 24 h. Extractions with ethyl alcohol and formalin were compared for the two North American species of *Dugesia*; no differences were noted as far as the porphyrin identification was concerned.

After the preliminary extraction the solvent was decanted and 2.5 *N* HCl was added to the worms (10 ml/10 to 20 worms). Extraction in HCl was allowed to proceed at 5°C for 3–4 days. The acidic solution was then neutralized with 0.2 *N* NaOH, and adjusted to pH 3.0–4.0 with acetic acid. This solution was concentrated by absorption on a thin disc of acid-washed talc using an aspirator filtering apparatus. The talc was washed with water and eluted with 20 drops of concentrated NH_4OH. Most of the porphyrin came off the talc in the first ten drops. The location of the porphyrin during this procedure was followed by observing its red fluorescence under the ultraviolet light (3660 Å).

The concentrated solution of porphyrin was then ready for paper chromatography; the method of NICHOLAS and RIMINGTON[4] was used. Spots of porphyrin were placed 2.5 cm from the edge of a Whatman No. 1 paper sheet (14 × 16 cm). The paper was rolled into a cylinder and placed standing on the bottom of a jar which contained the solvent system to a height of 1.5 cm. The solvent system was 2,4-lutidine-water (100 ml:70 ml). The jar was tightly covered and the paper chromatograph was allowed to develop in the dark at room temperature for $2^1/_2$ h. The location of the porphyrins were visualized by their fluorescence under the ultra-violet light, and the Rf values calculated. The results are shown in Table I.

Table I

Species	Genus	Rf	Number of carboxyl groups	Porphyrin
Of North America				
Dugesia dorotocephala	*Dugesia*	0.12	8	Uroporphyrin
Dugesia tigrina	*Dugesia*	0.12	8	Uroporphyrin
Cura foremanii	*Cura*	0.11	8	Uroporphyrin
Phagocata gracilis	*Phagocata*	0.68	4	Coproporphyrin
gracilis		0.38	—	Unidentified
Of Japan				
Dugesia gonocephala	*Dugesia*	0.12	8	Uroporphyrin
Phagocata iwanai	*Phagocata*	0.70	4	Coproporphyrin
		0.40	—	Unidentified
Phagocata vivida	*Phagocata*	0.68	4	Coproporphyrin
		0.40	—	Unidentified
Bdellocephala	*Bdello-*	0.68	4	Coproporphyrin
brunnea	*cephala*	0.40	—	Unidentified

Table II

Known sample	Rf	Number of carboxyl groups
Mesoporphyrin	0.91	2
Coproporphyrin	0.68	4
Uroporphyrin	0.12	8

The identification of the porphyrins is based on the Rf value. NICHOLAS and RIMINGTON[4] showed that the distance the porphyrins travel in the lutidine solvent system has a linear relationship to the number of carboxyl groups on the porphyrin molecule. Known samples of porphyrins were run along with the unknowns. The Rf values of the known samples are given in Table II.

In Table I, the first four species listed were collected in North America, while the second group of four species listed were collected in Japan. Uroporphyrin was the only porphyrin detected in the representatives of the genus *Dugesia*, regardless of their geographical source. It is known that *Dugesia tigrina* is similar to the Eurasian species *Dugesia gonocephala*[5], and the similarity of the porphyrin is evident. The three species of the genus *Phagocata* also represent different geographical sources, but contain a similar type of porphyrin which is distinct from that in the genus *Dugesia*. One species of the genus *Bdellocephala* has a porphyrin similar to that of the genus *Phagocata*. An unidentified red fluorescent spot with an Rf value of 0.38–0.40 was found in these species having coproporphyrin. Porphyrins with 2, 3, 4, 5, 6 and 7 carboxyl groups have been demonstrated by BOGORAD and GRANICK[6] as related to intermediates in the biosynthesis of protoporphyrin in *Chlorella*, and it is now generally accepted that these porphyrins may be intermediates or oxidized forms of intermediates in a stepwise enzymatic decarboxylation from uroporphyrinogen to protoporphyrin (BOGORAD[7]). Since reduced uroporphyrin is necessary for coproporphyrin formation, the occurrence of coproporphyrin in one genus and of uroporphyrin in another genus of Turbellaria may be reflecting a phylogenetic development.

On the other hand, these porphyrins may be biochemical phenotypes representing selected random mutations among the species. In any case, these data suggest that the identification of the porphyrins in Turbellaria may be biochemically characteristic of species or genera and might be added to the morphological characteristics by which they are generally classified[8].

Résumé. Chez les Turbellaires, l'auteur a trouvé de l'uroporphyrine dans les vers des genres *Dugesia* et *Cura*, et de la coproporphyrine dans ceux des genres *Phagocata* et *Bdellocephala*. Les porphyrines ont été identifiées par la chromatographie sur papier. Elles sont localisées dans les rhabdites des cellules de l'épiderme et du sous-épiderme de ces vers.

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[8] This investigation was supported by a Public Health Service Grant (No. A-3980-C1). A more detailed report of this investigation will be published elsewhere.