calculated by comparing tibia width responses to 1 dose of pituitary homogenates with the responses to 2 doses of GH standard (bracketed 3-point assay). Significance of differences in epiphyseal cartilage width was determined by Student's $t$ test. A sample of venous blood was withdrawn from experimental rats at the time of sacrifice for blood glucose determination (Glucostat, Worthington, Biochemicals).

*Results and discussion.* From the results reported in the Table it appears that of the 3 experimental conditions investigated, i.e. insulin-induced hypoglycemia, exposure to a cold environment or fasting for 60 h, no one was able to induce significant changes in pituitary GH as measured by RIA, with the exception of cold exposure, which in experiment 2 induced a clearcut increase of pituitary GH levels. In contrast with these negative results is the dramatic decrease in rat pituitary GH concentration observed in the aforementioned situations when pituitary GH levels were determined by BA in the same pituitary extracts. It would appear, in agreement with our previous results[5], that the more effective stimulus in releasing pituitary GH, as judged by bioassay, is cold exposure (experiment 3 from 70.0 µg/mg to 4.1 µg/mg), but also insulin hypoglycemia and severe fasting were highly active as stimuli.

The almost all negative results obtained till now in the rat by measuring plasma and pituitary GH levels by RIA, reflect the inability to modify in whichever direction plasma or pituitary GH levels and raise again the problem of the adequacy of this assay in the non-primate species. Even if the BA certainly suffers from lack of specificity[14], nevertheless results obtained in the rat by BA are compatible with the findings obtainable in primates by RIA. In addition numerous indications reached by BA in rat often served to further progress in the clarification of some aspects of the CNS involvement in the control of GH secretion in primates[15,16].

The possibility that what is currently measured as GH by RIA in plasma or pituitary of the manipulated rat may not be identical to the molecule responsible for the metabolic and growth effect of the hormone[8], is not completely divorced from the present-day reality[17].

Efforts aimed to study further the specificity of the antisera currently used, as well as the possible presence in the pituitary extracts or in plasma of substance(s) aspecifically interferring with the antigen-antibody binding, are urgently needed. On this line of investigation work is under way also in our laboratories[18].

*Zusammenfassung.* Zur Bestimmung des Gehalts an Wachstumshormon in der Hypophyse wurden die radioimmunologische und die biologische Methode verwendet. Die Resultate der immunologischen Bestimmung von Wachstumshormon nach Insulinstress, Kälte oder Hunger waren negativ, während im biologischen Versuch eine deutliche Abnahme des Gehalts an Wachstumshormon in der Hypophyse festzustellen war.

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[13] G. Falconi and G. Rossi, Endocrinology *75*, 301 (1964).
[14] I. I. Geschwind and C. H. Li, in *The Hypophyseal Growth Hormone, Nature and Actions* (Eds. R. W. Smith Jr., O. H. Gaebler and C. N. H. Long; McGraw Hill, New York 1955), p. 28.
[15] E. E. Müller, S. Sawano, A. Arimura and A. V. Schally, Endocrinology *80*, 471 (1967).
[16] W. G. Blackard and S. A. Heidingsfelder, J. clin. Invest. *47*, 1407 (1968).
[17] J. C. Beck, Int. Symp. Integration of Endocrine and Non-Endocrine Mechanisms in the Hypothalamus, Stresa 1969 (Abstract).
[18] Acknowledgments. We would like to thank Dr. S. Ellis, Nasa, Moffet Field, Calif., USA, for the generous supply of rat GH and the Endocrinology Study Section of the NIH for ovine GH. The skilful technical assistance of Miss Vannisa Albrici is gratefully acknowledged.
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## Iodine Accumulation by the Nephridia of *Sipunculus* (Sipuncula)

Previous studies related to the nephridia of Sipuncula have been concerned mostly with histological detail, especially those of Harms[1] and Kelley[2]; recent information on physiological and biochemical aspects of osmoregulation, nephridial contents or function of nephridia in osmoregulation can be found in the papers by Koller[3], Gross[4], Edmonds[5], Kamemoto and Larson[6] and Virkar[7]. Towle and Giese[8], while studying biochemical changes during reproduction and starvation, determined the water content and protein level of the nephridia of Phascolosoma.

The present report presents data on the iodine content in the nephridia of 2 species of Sipunculus, namely *S. multisulcatus* Fischer 1913[9] and *S. natans* Fischer 1954[10]. All specimens were collected at São Sebastião, on the littoral of the State of São Paulo, during the months of September and October 1968; no evidence was found of developing gonads, the animals therefore being considered immature.

Specimens of *S. multisulcatus* ranged in weight from 23–43 g, the size varying from 15–18 cm in length. In *S. natans* the weight varied from 80–120 g, the length being 20–25 cm; in both species the sac-like nephridia may attain 3–4 cm in length. The method used for the determination of iodine has already been reported[11].

[1] W. Harms, Arch. Entwmech. Org. *47*, 307 (1921).
[2] L. Kelley, *The Histology of the Nephridia of Golfingia gouldi*, Thesis (Library Fisk University, Nashville, Tenn., USA 1953).
[3] G. Koller, Verh. dt. zool. Ges. *41*, 440 (1939).
[4] W. J. Gross, J. exp. Biol. *31*, 402 (1954).
[5] S. J. Edmonds, Aus. J. mar. Freshwat. Res. *8*, 131 (1957).
[6] F. I. Kamemoto and E. J. Larson, Comp. Biochem. Physiol. *13*, 477 (1964).
[7] R. A. Virkar, Comp. Biochem. Physiol. *18*, 617 (1966).
[8] A. Towle and A. C. Giese, Comp. Biochem. Physiol. *19*, 667 (1966).
[9] W. Fischer, Mitt. naturh. Mus. Hamburg *30*, 93 (1913).
[10] W. K. Fischer, Ann. Mag. nat. Hist. *7*, 238 (1954).
[11] F. B. de Jorge and J. N. Kara, Nature *214*, 491 (1967).

Iodine content (μg/100 g fresh tissue)

| Region or structure | *Sipunculus natans* Average | Range | Factor[a] | *Sipunculus multisulcatus* Average | Range | Factor[a] |
|---|---|---|---|---|---|---|
| Muscles of anterior body wall | 55.7 | (47.3– 62.0) | 4.95 | 58.4 | (50.5– 72.6) | 4.82 |
| Muscles of median body wall | 27.5 | (22.3– 35.4) | 4.80 | 25.8 | (23.4– 28.0) | 4.55 |
| Muscles of posterior body wall | 16.7 | (10.6– 23.5) | 5.04 | 18.6 | (16.4– 20.5) | 4.51 |
| Proboscis | 136.4 | (120.5– 157.0) | 5.21 | 131.9 | (122.0– 141.0) | 4.63 |
| Oesophagus | 56.8 | (40.3– 75.5) | 4.92 | 46.9 | (44.2– 50.5) | 4.98 |
| Tentacles | 79.0 | (65.0– 90.2) | 5.55 | 76.5 | (70.5– 82.2) | 5.34 |
| Retractor muscles of introvert | 6.8 | (5.0– 9.1) | 5.44 | 6.3 | (5.0– 7.4) | 5.07 |
| Nerve cord | 6.3 | (4.3– 9.0) | 4.09 | 5.7 | (4.1– 8.2) | 3.98 |
| Digestive tract | 236.1 | (143.4– 343.5) | 6.79 | 372.4 | (296.5– 434.5) | 5.78 |
| Nephridia | 2123.2 | (2058.3–2200.3) | 3.25 | 2538.6 | (2460.2–2618.5) | 3.04 |
| Coelomic fluid | 11.3 | (9.8– 13.7) | 20.75 | 12.1 | (10.0– 13.2) | 14.24 |

[a] The factor presented in the Table allows the calculation of the results in relation to the dry weight.

The results are summarized in the Table. They show very low values of iodine for structures like the nerve cord and the retractor muscles of the introvert; higher values were obtained for the tentacles, proboscis and digestive tract. Exceptionally high values, however, were found in the nephridia of both species, such accumulation being so far unknown for this group of animals.

Accumulation of iodine in unexpected sites or structures in invertebrates has been reported, among others, for the hepatic region of *Balanoglossus* (Enteropneusta)[12], the pharynx of *Lineus* (Nemertina)[13] and the setae of polychaetes[14,15].

In relation to the possible function of iodine in the nephridia of *Sipunculus* it is of interest to mention that HARMS[1] had already ascribed, on morphological and physiological grounds, an important hormonal function to the nephridia, considering them as equivalent to the vertebrate adrenal glands. The present findings suggest that the nephridia may also be involved in the iodine metabolism.

*Résumé.* La détermination du contenu d'iode dans les différents organes des espèces du genre *Sipunculus* (Sipuncula) a montré que les néphridies présentent la plus haute concentration de cette substance, ensuite vient l'intestin et la trompe. La forte accumulation d'iode dans les néphridies laisse supposer que ces organes sont responsables du métabolisme des iodoprotéines dans ce groupe d'Invertébrés.

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[12] F. B. DE JORGE, P. SAWAYA, J. A. PETERSEN and A. S. F. DITADI, Science *150*, 1182 (1965).
[13] W. E. BALFOUR and E. N. WILLMER, J. expl. Biol. *46*, 551 (1967).
[14] A. GORBMAN, M. CLEMENTS and R. O'BRIEN, J. expl. Zool. *127*, 75 (1954).
[15] F. B. DE JORGE and J. A. PETERSEN, Comp. Biochem. Physiol. *27*, 51 (1968).

## Effect of Body Temperature on Intracellular pH

Though it is well known how temperature affects simple buffer systems and the acid-base properties of the blood, there are no data reported about the intracellular buffering and its relation to temperature. As a first approach to this problem we determined whole-body intracellular pH in nephrectomized dogs[1] with the DMO method[2]. The dogs were anaesthetized with Nembutal and paralysed with Succinyl choline. The body temperature was adjusted by means of an arterio-venous bypass through a heat exchanger, the arterial $pCO_2$ was kept at desired levels between 13 and 120 mm Hg by appropriate ventilation with a pump. The extracellular space was obtained from the distribution of inulin, total body water was assumed to be 60% of the body weight. The effect of temperature on the pK value of DMO was

[1] E. D. ROBIN, R. J. WILSON and P. A. BROMBERG, N.Y. Acad. Sci. *92*, 539 (1961).
[2] W. J. WADDELL and T. C. BUTLER, J. clin. Invest. *38*, 720 (1959).