Variations in Nitrogenous Compounds in the Urine of *Strophocheilus* (Pulmonata, Mollusca) with Different Diets

The snail *Strophocheilus oblongus musculus* Becquaert 1948 is an exclusively terrestrial pulmonate gastropod, found in Argentina, Paraguay and the south of Brazil[1]. The family Strophocheilidae, restricted to South America, belongs to the sub-order Mesurethra, which is constituted by animals having a very short ureter[2].

Problems of nitrogen excretion in Pulmonata have been studied in families other than Strophocheilidae, mostly on the Helicidae. Recent reviews on this subject are those of FLORKIN[3] and POTTS[4].

Previous studies by DE JORGE et al.[5] have shown that the blood of *Strophocheilus* contains 1.42 ± 0.18 g/100 ml of protein, 30.5 ± 1.3 mg/100 ml of urea and 0.34 ± 0.04 mg/100 ml of uric acid. Further biochemical investigations on this animal[6] have shown that the kidney presents the highest level of nitrogen in relation to any other tissues.

The specimens of the snail used in the present study were collected at São Leopoldo and Porto Alegre, Rio Grande do Sul (Brazil), transported to São Paulo and maintained at the vivarium of the Department of Zoology, University of São Paulo. The experimental animals were kept in covered crystallizing dishes about 20 cm in diameter, in which the relative humidity was always kept at saturation point.

Ten snails, weighing 64.12 g in average (range 40.51 to 87.10 g), were fed with lettuce (*Lactuca sativa*) which contained 206.4 mg/100 g of nitrogen and 95.65 g/100 g of water; another 10 snails weighing 73.95 g in average (range 61.15–91.00 g) were fed with cabbage (*Brassica oleracea* var. *acephala*) which contained 481.2 mg/100 g of nitrogen and 89.63 g/100 g of water. After an accommodation period of at least 10 days in each particular diet (lettuce or cabbage) the urine of 24 h of each animal was collected at the bottom of the glass container for 5 days.

An average of 2.565 g of lettuce, containing 5.294 mg of nitrogen and 2.45 ml of water, was ingested daily by each snail, which excreted 1.83 ml of urine per day. In the other group, an average of 1.776 g of cabbage, containing 8.546 mg of nitrogen and 1.75 ml of water, was ingested by each snail, 1.18 ml of urine being excreted per day. During the experiments the temperature of the room was maintained at 22–23 °C, the barometric pressure varying around 695 mm Hg.

The analytical methods used were: total nitrogen as KJELDAHL[7], urea as LEVINE et al.[8], creatinine as OWEN et al.[9], ammonia as CONNERTY et al.[10] and uric acid as HENRY et al.[11]. All analytical determinations were made in duplicate; conventional statistical treatment was used for the analysis of the results presented in the Table.

The input of nitrogen changing from 5.295–8.548 mg/day increases the output of total nitrogen from 1.63–3.61 mg/day, this result being directly related to the amount of excreted urea, which rises from 1.90–6.43 mg/day. The differences between the means of creatinine, uric acid and ammonia in the urine of the snails submitted to the two diets, have no statistical significance.

A somewhat similar pattern in the excretion of nitrogenous compounds has been found in normal human subjects submitted to diets of different nitrogen content[12], this being in accordance with POTTS' statement[4] that 'the results of the last 20 years have served to emphasize the similarities in function between the molluscan and vertebrate kidneys'.

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Distribution of the nitrogenous compounds in the urine of the *Strophocheilus*

Diet	Weight of the snails (g)	Input/day		Output of urine/day (ml)	Distribution of the nitrogenous compounds in the urine (mg/100 ml)					
		Nitrogen (mg)	Water (ml)		Total nitrogen	Creatinine	Urea	Uric acid	Ammonia	Non identified nitrogen
Lettuce										
Mean	64.12	5.295	2.45	1.83	89.29	2.67	104.19	17.64	2.65	32.63
± S.D.	16.35	2.636	1.22	0.76	28.75	0.51	28.33	9.36	0.76	
Cabbage										
Mean	73.95	8.548	1.75	1.18	305.57	2.70	544.83	15.05	2.17	38.54
± S.D.	11.27	6.033	1.16	0.78	107.29	0.49	156.86	7.25	0.73	
Statistical significance of the means										
t of Student		1.552	1.276	1.834	6.142	0.129	8.813	0.668	1.393	
Probability *P*		> 0.05	> 0.20	> 0.05	< 0.001	> 0.80	< 0.001	> 0.50	> 0.10	

Further work is now under progress in order to correlate the accumulation of excretory products in the kidney of snails under different diets and conditions of estivation and hibernation.

Résumé. La composition de l'urine du pulmoné terrestre *Strophocheilus oblongus musculus*, spécialement le taux de l'urée, est un reflet des différences du contenu protéique des aliments. Ce fait confirme que le fonctionnement du rein des mollusques et des vertébrés est semblable.

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Synchronization of Mitosis in *Physarum polycephalum* by Coalescence of Postmitotic and Premitotic Plasmodial Fragments[1]

In the coenocytic slime mold, *Physarum polycephalum*, the nuclei undergo mitosis in synchrony[2]. When microplasmodia growing in agitated culture and representing all stages of the mitotic cycle[3] at random are allowed to coalesce on filter paper, the nuclei of the resulting surface plasmodia are mitotically synchronized[4–6]. Postmitotic nuclei which become part, by plasmodial coalescence, of a predominantly premitotic plasmodium, enter the next mitosis along with the nuclei of the latter[7]. We report in the following on mitotic synchronization resulting from fusion between pre- and postmitotic plasmodia in such a ratio that nuclei of both stages were present in approximately equal numbers in the resulting composite plasmodia. Of special interest to us were those combinations in which the premitotic plasmodia, at the time when coalescence was underway, were at a stage of the intermitotic period as close as possible to prophase. We employed for this purpose suspensions of plasmodial fragments which were freshly prepared from mitotically synchronized surface plasmodia.

The organism was grown in form of microplasmodia in agitated culture[8]. Mitotically synchronized surface plasmodia were prepared as described previously[6]. Cultures of microplasmodia from several flasks were pooled, redistributed, and returned to the shaker. One set of surface plasmodia was prepared immediately, and a second set was prepared 1, 2, and 4 h later, respectively, in different experiments. Both sets of plasmodia were made from 1-ml aliquots of identically prepared, concentrated suspensions of microplasmodia[6]. Since the cultures were approaching the plateau of growth at the time of fusion, the increase in the number of microplasmodia during the intervals between preparing the 2 sets of plasmodia was negligible. Immediately upon the first postfusion mitosis, the plasmodia were placed on fresh growth medium. The growth medium added to the first set of plasmodia contained thymidine-^{3}H (5 μc/ml; from Schwarz Bioresearch, Inc.). When the plasmodia of the first set had just completed the second postfusion mitosis (anaphase to late telophase), plasmodia from both sets were floated, along with the supporting filter paper, on balanced salt solution[8] and removed from the filter paper with the help of a spatula. 4 premitotic (each containing approximately N nuclei) and 2 postmitotic (each containing approximately $2N$ nuclei) plasmodia were pooled in 30 ml of balanced salt solution (in 500-ml Erlenmeyer flasks) and fragmented by vigorous shaking for 15 min on an Eberbach alternating shaker (250 reciprocations/min, stroke length 4.5 cm). In order to have an estimate of the effect of fragmentation followed by fusion upon the beginning of mitosis, other premitotic and postmitotic plasmodia were fragmented separately and the fragments were reunited by fusion. After fragmentation the pieces were concentrated by centrifugation at low speed and aliquots of the concentrated suspension were placed on filter paper (this procedure is referred to in the following as 'fusion'). Growth medium was added approximately 2 h later. The actual proportions of postmitotic nuclei/premitotic nuclei in the mixed plasmodia were determined, after addition of growth medium, in autoradiographs (Kodak AR-10 stripping film) prepared from ethanol-fixed smear preparations (incubation in the dark for 3 days). Under these conditions, the heavily labelled postmitotic nuclei of the first set of plasmodia were clearly distinguishable from the premitotic nuclei.

Fragmentation of surface plasmodia caused inhibition of mitosis as seen in Figure 1. Only if the nuclei were

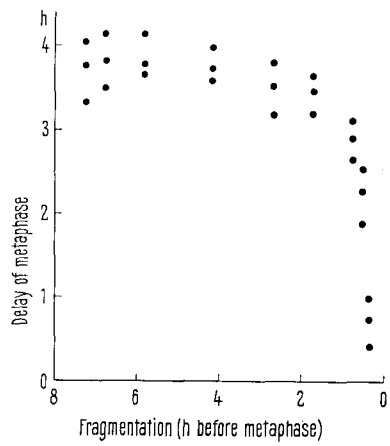

Fig. 1. Delay of mitosis by fragmentation of plasmodia at different times of mitotic cycle. The fragments of each plasmodium were allowed to coalesce in 3 separate groups in different Petri dishes.

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