process which prevented germination, since the 'innocuous' agent only delayed germination slightly when used at the same concentration level as azoester.

We have also found that azoester inhibits growth and sporulation. Inocula of *T. viride* on filter paper were allowed to grow on minimal media for 24 h. The papers were transferred to an azoester solution ($10^{-3}M$), allowed to remain in the solution for 1 h, transferred again to fresh minimal media, exposed briefly to light to stimulate sporulation, and allowed to stand for 24 h in the dark. Growth was markedly inhibited (10–20% of controls) and sporulation was almost completely abolished.

We may thus conclude that glutathione is somehow involved in those processes which result in germination, growth, and sporulation. The use of thiol-oxidizing agents for the investigation of the role of GSH may also aid in elucidating some characteristics of the processes of germination, growth, and sporulation.

It is interesting to compare the structures of the intracellular thiol-oxidizing agents we have discovered (**1** and **2**) with those of the recently reported fungicidal agents **3** and **4**[8].

*Résumé.* Le traitement des spores de *Trichoderma viride* avec le nouveau oxydant intracellulaire des thiols, le composé azoester, nous a conduit à conclure qu'il y a une exigence absolue pour le glutathion pendant le processus de germination. Avec le même composé, azoester, on a demontré que GSH est nécessaire pour la croissance et la sporulation du champignon.

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[11] This research was supported in part by a U.S.D.A. grant under U.S. Public Law No. 480.

## Influence of Dormancy on the Chemical Composition of *Strophocheilus* (Strophocheilidae, Pulmonata, Mollusca)

Physiological and biochemical changes during hibernation and estivation in pulmonates have been investigated by several authors, chiefly with regard to *Helix pomatia*; information on the subject has recently been summarized by Hyman[1]. For representatives of the suborder *Mesurethra*, no information is available. This group is extensively represented in the neo-tropical region by the genus *Strophocheilus*, family *Strophocheilidae*, in relation to which some work has already been done concerning its biology and distribution[2,3]; data on the physiology and chemical composition of different organs and tissues have recently been provided[4,5]. Specimens of *Strophocheilus* are known to go into a state of dormancy, either during hibernation or estivation; such behaviour allowed the authors to carry out the present investigation, with the aim of determining the influence of both hibernation and estivation on the chemical composition of the entire body, without shell, of the snail *Strophocheilus oblongus musculus*.

*Material and methods.* Specimens of *Strophocheilus* were collected in the neighbourhood of Porto Alegre, Rio Grande do Sul (Brazil) and sent to São Paulo, where they were kept in the terrarium of the Zoology Department, University of São Paulo. 3 groups of 10 animals each were studied: one consisted of active animals, kept at 23–24 °C, the other of hibernating animals at 16–17 °C for 45 days, and finally a third group of estivating animals kept at 31–32 °C for 45 days. During this period, relative humidity ranged from 60–80%. All these animals had been, 10 days previous to the experimental procedure described, subdivided in 2 groups, 1 fed with lettuce and the other with cabbage, these plants being known to contain different amounts of calcium and protein[5]; no differences, however, were detected in the chemical constitution of animals kept under different diets, results being pooled for discussion.

At the appropriate time, the animals were sacrificed and the shells carefully removed to avoid any possibility of contamination, especially in relation to calcium. Entire animals, without the shell, were weighed and then dried in an oven regulated at 100 °C, until constant weight was obtained; these remains were then carefully homogenized in a porcelain mortar. The contents of water, sodium, potassium, calcium, magnesium, phosphorus, sulphur, copper, iron, nitrogen, glycogen and total carbohydrates were determined in active animals, as well as in those under estivation or hibernation. Determinations of glycogen and total carbohydrates were necessarily carried out with fresh material. The analytical methods used have already been described in previous papers[4,6,7]; the results were subjected to conventional statistical treatment.

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*Results.* The Table summarizes the results obtained, which are expressed in relation to the dry weight; at the foot of the Table are found the factors which allow the calculations to be made in relation to the fresh weight. This Table presents also the statistical significance of the differences of the means in relation to active, estivating and hibernating animals.

It is noticed that estivating animals lose water ($P < 0.001$), sodium ($P > 0.02$), magnesium ($P < 0.001$), copper ($P > 0.02$), iron ($P < 0.001$) and total carbohydrates ($P > 0.001$), but that they accumulate calcium ($P > 0.001$). No statistical differences have been found in relation to the contents of potassium, phosphorus, sulphur, iodine, nitrogen and glycogen between active animals and those in estivation.

*Strophocheilus* in hibernation lose water ($P > 0.001$), sodium ($P < 0.001$), calcium ($P < 0.001$), magnesium ($P < 0.001$), sulphur ($P > 0.001$), copper ($P < 0.001$), iron ($P < 0.001$) and total carbohydrates ($P > 0.001$), increasing however the contents of potassium ($P > 0.05$) and iodine ($P > 0.001$). In relation to the contents of phosphorus, nitrogen and glycogen, no statistical differences were found between active and hibernating animals.

A comparison between hibernating and estivating animals shows no differences in relation to the contents of water, potassium, phosphorus, iodine, nitrogen and glycogen. Hibernating animals, however, contain less sodium ($P > 0.02$), calcium ($P < 0.001$), magnesium ($P < 0.001$), sulphur ($P < 0.001$), copper ($P < 0.001$) and iron ($P < 0.001$), but more total carbohydrates ($P > 0.01$) than estivating ones. In relation to the results obtained for calcium, the authors would like to express some reservation, due to the ever present danger of contamination with minute shell fragments.

*Discussion.* Before any attempt is made to explain or correlate some of the results obtained with those previously observed in other snails, attention should be called to the fact that *Strophocheilus*, as already mentioned, belongs to the sub-order *Mesurethra*. The *Helicidae*, of the sub-order *Sigmurethra*, are the most highly evolved family of pulmonates[1]; the species *Helix pomatia*, which has been the subject of several physiological and biochemical studies in relation to problems of estivation or hibernation, is a representative of this group. One of the characteristic features of *Helix* on the onset of estivation or hibernation is the secretion of a mucus membrane or an epiphragm, formations which are entirely lacking in hibernating or estivating *Strophocheilus*.

Water loss during dessication takes place, according to PUSSWALD[8], through the body surface, but not in slime secretion, which is inhibited during dessication. In *Strophocheilus* it has been noticed that while in hibernation or estivation, with consequent dessication, the pneumostome is kept open for respiratory purposes and it is likely that a considerable loss of water takes place through the lung; also, no significant mucus secretion has been noticed in such animals, the exposed body parts being barely moist with mucous material.

In the present study whole animals (less the shell) have been analyzed; this makes it rather difficult to compare the present findings with other reports in which particular organs or structures have been studied.

In *Strophocheilus*, dormant specimens present glycogen levels very similar to those found in active animals; however, total carbohydrate levels drop in a significant way, both in hibernation and estivation. These results seem to indicate that the carbohydrates consumed are other than glycogen, or that some glycogen is synthesized during dormancy at the expense of other substances. At this point mention should be made of the fact that in snails, apart from glycogen, another polysaccharide called galactogen, is also found in the animal body, specifically in the albumen gland; in the present work, results expressed as glycogen represent, in fact, glycogen plus galactogen. In *Helix*, however, it has been found that

[8] A. W. PUSSWALD, Z. vergl. Physiol. *31*, 227 (1948).

Influence of dormancy (estivation or hibernation) during 45 days on the chemical composition of the snail *Strophocheilus oblongus musculus* (results expressed in relation to 100 g of the dry weight)

| Condition of the snail | Factor | Water g | Sodium mEq | Potassium mEq | Calcium mg | Magnesium mEq | Phosphorus mg | Sulphur mg | Copper mg | Iron mg | Iodine µg | Nitrogen g | Glycogen mg | Total carbohydrate mg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Active | 9.97 | 89.97 ±1.01 | 33.47 ±1.64 | 23.74 ±3.49 | 2354.5 ±9.4 | 109.78 ±2.31 | 1913.5 ±109.0 | 1319.6 ±130.9 | 18.74 ±3.50 | 23.01 ±0.37 | 42.23 ±1.66 | 13.27 ±1.03 | 2629.2 ±533.9 | 4550.6 ±374.4 |
| Estivating | 4.81 | 79.20 ±1.82 | 28.63 ±4.15 | 27.63 ±3.60 | 2505.9 ±153.1 | 105.51 ±1.82 | 1748.5 ±177.8 | 1351.3 ±35.7 | 14.69 ±0.45 | 20.55 ±0.96 | 44.43 ±8.68 | 14.54 ±1.21 | 2548.4 ±174.0 | 3531.9 ±270.4 |
| Hibernating | 4.81 | 79.24 ±0.87 | 23.94 ±0.75 | 26.71 ±0.64 | 1797.6 ±148.4 | 77.15 ±0.68 | 1822.1 ±36.5 | 1096.2 ±44.1 | 7.64 ±0.46 | 15.56 ±0.75 | 48.40 ±2.24 | 12.95 ±0.27 | 2720.7 ±129.8 | 3939.0 ±74.0 |
| Significance of the differences of the means ($t$ of Student; probability, $P$) | | | | | | | | | | | | | | |
| Activity – Estivation | | 11.567 <0.001 | 2.463 >0.02 | 1.731 >0.10 | 3.645 >0.001 | 3.245 >0.01 | 1.765 >0.10 | 0.442 >0.60 | 2.564 >0.02 | 5.350 <0.001 | 0.554 >0.50 | 1.782 >0.10 | 0.315 >0.70 | 4.889 >0.001 |
| Activity – Hibernation | | 17.925 <0.001 | 11.798 <0.001 | 1.871 >0.05 | 8.353 <0.001 | 30.248 <0.001 | 1.736 >0.10 | 3.574 >0.001 | 7.026 <0.001 | 20.115 <0.001 | 4.936 >0.001 | 0.667 >0.50 | 0.366 >0.70 | 3.608 >0.001 |
| Estivation – Hibernation | | 0.044 >0.90 | 2.486 >0.02 | 0.562 >0.50 | 23.374 <0.001 | 32.614 <0.001 | 0.905 >0.30 | 10.047 <0.001 | 24.449 <0.001 | 9.167 <0.001 | 0.988 >0.30 | 0.572 >0.50 | 1.775 >0.10 | 3.250 >0.01 |

glycogen levels drop during winter and early spring, the highest levels being attained by fall[9].

During dormancy, the loss of certain ions, as observed in *Strophocheilus*, may be explained at least in part, as due to elimination with faeces and urine right at the beginning of estivation or hibernation; it is also possible that some ions may be lost with the mucus produced by the animals, as noticed by DEXHEIMER[10] in relation to calcium incorporated in the mucus produced by *Helix*.

*Résumé.* Pendant l'hibernation, le *Strophocheilus* (Pulmoné, Mollusque) perd de l'eau, du sodium, du calciun, du magnésium, du soufre, du cuivre, du fer et des carbohydrates totaux, mais son taux de potassium et d'iode augmente. En état d'estivation, il y en a perte d'eau, de sodium, de magnésium, de cuivre, de fer et de carbohydrates totaux, mais accumulation de calcium.

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## Receptive Fields of Single Cells of a Marsupial Visual Cortex of *Didelphis virginiana*

Investigations of mammalian visual cortices have revealed a wide variety of response properties, ranging from the rather limited repertoire of the rabbit[1,2] to the relatively sophisticated or at least selective discriminations found for cat[3] and monkey[4]. These species, however, are all placental mammals. The marsupial or pouch-bearing mammals, on the other hand, offer a more primitive neocortex, even to the absence of a corpus callosum. How, then, have the sensory discrimination properties of this parallel, but more slowly evolving mammalian visual cortex developed?

*Materials and methods.* To explore this question, the receptive field and associated response properties of 100 single cells from the visual cortex of *Didelphis virginiana*, the American opossum, were studied. During testing, the animals, 12 young adults, were maintained under light urethane anesthesia in a stereotaxic frame. The pupils were dilated and the corneas were fitted with corrective contact lenses.

An aperture made directly over the left posterior pole of the cortex and resealed with 4% agar gel, gave passage to stainless steel or tungsten microelectrodes (1–40 m$\Omega$) used to record from and to mark single cell response sites. These sites (all in layer IV or shallower) were later localized histologically.

The receptive field of each cell was mapped using a 1°, 85 candle/m$^2$ spot against a 3.5 candle/m$^2$ background. Response to additional visual stimuli, diffuse and discrete, light and shadow and of various edge geometries and areas were also measured.

*Results and discussion.* The receptive fields of 99 of the 100 cell samples studied here could be categorized into 3 geometric classes: (1) 'on' fields; those responding throughout their extent only to the onset of light; (2) 'off' fields; those responding throughout their extent only to the cessation of light; and (3) 'on-off' fields; those responding throughout their extent to both onset and cessation of light. The remaining cell responded only to moving dark edges, uniformly in all directions, but could not be plotted by the flashing stimulus method used for the others. The frequency distribution of these classes, together with their diameters and eccentricities from the reference axis, appear in Table I. The Figure shows the distribution of these classes when projected into visual space relative to the optic nerve head center. No significant relationship was found for any of the groups between receptive field diameter and eccentricity from the reference axis in any meridian, nor were significant differences of field geometry or general response properties between cells of the upper tapetized and lower non-tapetized retina evident.

Among the unique characteristics of this cortical receptive field population was the absence of mutually inhibiting response zones within a given receptive field. Neither the concentric organization, as occurs in the rabbit visual cortex[1], nor the linear division of zones, as in the 'simple' fields reported by HUBEL and WIESEL[3,4] for the cat and monkey visual cortices were found. The presence of antagonistic zones within these fields was tested by comparing local field responses under different retinal adaptation states and by stimulating the periphery of the field while saturating the field center. In all cases the homogeneity of response throughout these fields remained. In addition, area-response determinations showed only simple summation, i.e. as stimulus area was in-

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Table I. Distribution of photically responding cells of a marsupial (*D. virginiana*) visual cortex by receptive field geometry

| Receptive field type | No. | % | Mean diameter | Standard deviation | Range | Mean eccentricities | Standard deviation | Range |
|---|---|---|---|---|---|---|---|---|
| On | 31 | 31 | 20.16° | 8.74° | 5°–44° | 29.24° | 12.81° | 5°–45° |
| Off | 15 | 15 | 19.87° | 9.26° | 4°–40° | 32.93° | 23.83° | 2°–94° |
| On-Off | 53 | 53 | 17.75° | 8.73° | 5°–50° | 29.28° | 12.31° | 5°–56° |
| Unclassed | 1 | 1 | | | | | | |
| | 100 | | | | | | | |