midnight were: 20.2 ± 0.3 °C and 106.5 ± 7.6 μmho · cm$^{-1}$. The turbidity was 136 ± 19 FTU (Formazin turbidity units), the pH ranged from 7.0 to 7.5, and dissolved $O_2$ was 5.3 ± 0.6 ppm. EOD recordings were obtained from single fish and groups of 2 to 5 individuals passing through the electrodes' detection field. Figure 2 shows the EOD frequency distribution of all detected *G. niloticus* during day and nighttime. The daytime average EOD frequencies and their ranges were not significantly different from the nighttime data: day, 253.4 ± 27.9 Hz, range 204–313 Hz; night, 251.5 ± 34.8 Hz, range 196–326 Hz. While fish were passing in groups of 2 to 5 individuals the initial EOD frequency differences ($\Delta f$) among individuals were maintained for as long as the fish stayed in the detection range of the electrodes. These differences were not significantly different with regard to the number of fish passing: groups of 2 (25 observations): $\Delta f$ = 28.6 ± 17.6 Hz, range 5–82 Hz; groups of 3 (14): $\Delta f$ = 26.9 ± 15 Hz, range 6–53 Hz; groups of 4 (9): $\Delta f$ = 14.7 ± 9 Hz, range 4–33 Hz; and groups of 5 (12): $\Delta f$ = 16.8 ± 9.1 Hz, range 8–33 Hz.

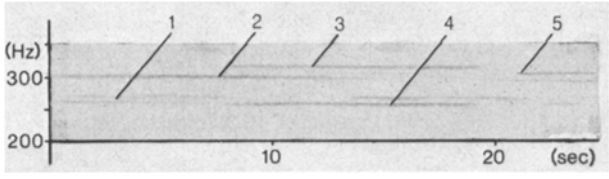


Fig. 1. Sonogram illustrating the electric organ discharge frequencies of 5 *G. niloticus* passing through the detection field of the recording electrodes.

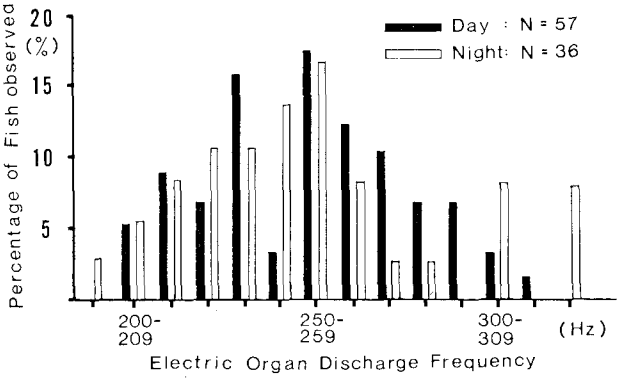


Fig. 2. Range and distribution of electric organ discharge frequencies of *G. niloticus* during day and nighttime.

B) In one of the permanent pools on the island of Irounda EOD recordings and ecological data were taken between 12:00 and 5:00 P.M. at different observation sites ranging in depth from 30 to 100 cm. The water temperature was 19.5 ± 1.0 °C, conductivity 82.9 ± 4.5 μmho · cm$^{-1}$, turbidity 64.2 ± 8.3 FTU, pH range 7.0 to 7.5, and dissolved $O_2$ was 7.7 ± 1.2 ppm. Over a period of 2 weeks, 5 specimens of *G. niloticus* were observed to maintain the same hiding places in partly submerged bushes of *Mimosa aspirata* (during daytime) which they left during the night to prey. The 5 individuals were identified by their characteristic EOD frequencies of 193, 212, 223, 235 and 250 Hz.

*Discussion.* Our field data confirm the wide range of frequencies obtained from *G. niloticus* kept under laboratory conditions. Contrary to nighttime EOD increases in other mormyriform fishes[6] we did not find significant daily EOD variations in *G. niloticus*. LISSMANN[4] reported a decrease in EOD frequencies in 2 captive specimens over a 5 year period when these fish grew in size from 28 and 38 cm to 52 and 54 cm. We caught 2 of the Irounda fish and also related a higher frequency of 250 Hz to the smaller fish (22.5 cm) and a lower frequency of 212 Hz to the larger fish (37 cm). Laboratory recordings from young 6.7 to 9.5 cm *G. niloticus* show an average frequency of 300 Hz at 21 °C under comparable conductivity conditions[7]. Since we recorded the Kalamaloue fish under relatively constant physico-chemical conditions we attribute the observed range of EOD frequencies in part to the fishs' age. Sex differences and possible frequency variations and/or frequency phase shifts during specific behavioral interactions remain to be investigated. Recent laboratory studies on (EOD frequency-) jamming-avoidance[8] showed that *G. niloticus* when stimulated with frequencies close to its own will shift its EOD frequency by at least 4 Hz, a value which is in accordance with our natural observations.

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[7] P. MOLLER, unpublished data.
[8] W. HEILIGENBERG, J. comp. Physiol. *103*, 55 (1975).

## Primary Nucleolus and Amphinucleoli in the Oocytes of *Patella coerulea* L. (Moll. Gast.)

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*Summary.* Electron microscope observations show in the primary nucleolus some granulations with dimensions less (70 Å) than those of the amphinucleoli (90 Å). Even though the primary nucleolus has a high RNA content, this has not a very active turnover except at the periphery, probably in relation to the emission of 'daughter-nucleoli'. The amphinucleoli, even though they do not have RNA which is cytochemically discloseable, possess, however, RNA at a very high rate of turnover.

From the research done especially by JÖRGENSEN[1] and by the researchers of the Institute of Zoology[2] of the University of Messina, it appears that the oocytes of *Patella coerulea* present a complex nucleolar apparatus during their growth. This is constituted by a 'primary nucleolus' more or less distant from the nuclear membrane, colourable in red with Mallory's method and in blue with Dominici's method, with clear evidence of RNA and without nucleolini, and by a variable number of 'amphinucleoli' which generally adhere to the nuclear membrane until they gradually wear themselves out. The amphinucleoli are colourable in blue with Mallory's method and in red with Dominici's method; they show no evidence of RNA and have nucleolini which are more

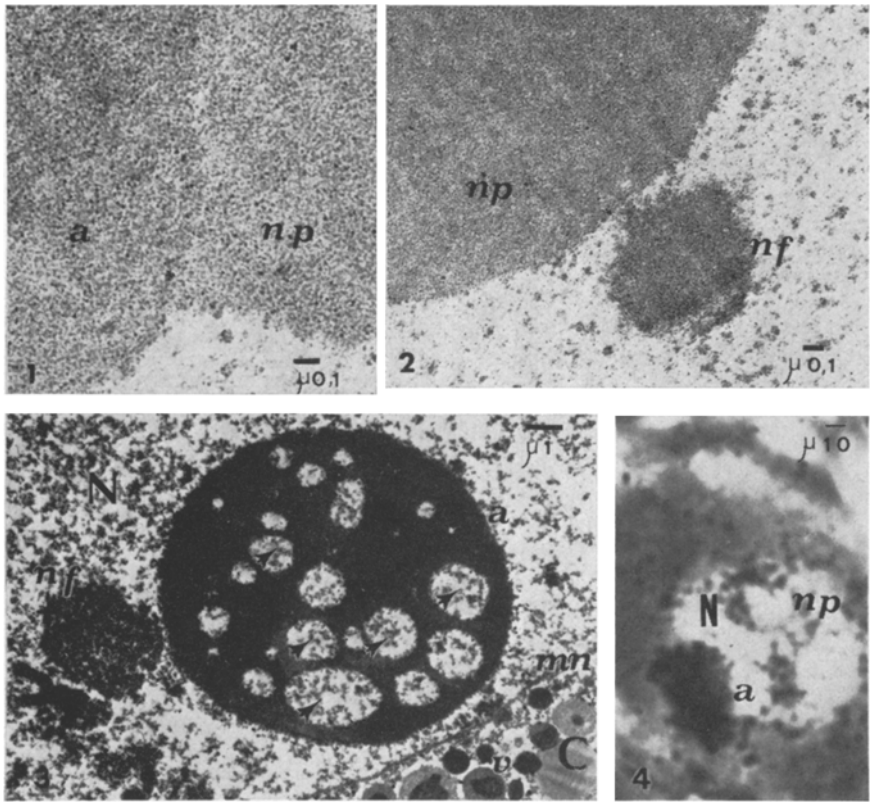


Fig. 1. Zone of contact between the amphinucleolus (a) and the primary nucleolus (np); note that greater and denser granulations exists in the amphinucleolus than in the primary nucleolus. Technique is outlined in the text. × 6,600.

Fig. 2. Peripheric portions of the primary nucleolus (np); note the separation of the daughter-nucleolus (nf). × 6,600.

Fig. 3. Part of the nucleus (N) and of the cytoplasm (C); note in the amphinucleolus (a) the 'clear fibrillar zones' (arrows), the daughter-nucleolus (nf), the nuclear membrane (mn) and the yolk globules (v). × 3,300.

Fig. 4. Oocyte, in whose nucleus (N) there is an amphinucleolus (a) strongly marked with uridin-$H^3$, and a primary nucleolus (np) with its central part unmarked. Technique is outlined in the text. × 440.

numerous the greater the dimensions of the nucleolus. Under the fluorescent microscope[3] and under the electron microscope[4], differences have also been observed between the two types of nucleoli. Other differentiating characteristics have been appreciated by cytochemical profile[5]. Other small nucleoli with RNA have been singled out; they have the capacity to overcome the nuclear membrane and transfer themselves into the cytoplasm[6] (Figures 1–4).

New investigations have enabled us now to verify further differences between these 2 types of nucleoli. With the Unna-Pappenheim's method at different pH (from 5 to 11) according to the technique of GEROLA and VANNINI[7], and with the control by means of the ribonuclease according to BRACHET, a notable concentration of RNA has been confirmed in the primary nucleolus (persistent pyroninophilia at higher pH) and its apparent absence in the amphinucleoli (pyroninophilia only at the lower pH).

By means of autoradiographic investigations, a notable assumption of uridin-$H^3$ was shown by the amphinucleoli (after $1^1/_2$ h of contact with a concentration of 5 μCi/g of animal) especially when they were near the nuclear membrane (Figure 4). This assumption was absent in the central part of the primary nucleolus (Figure 4), but evident at various points of its peripheral layer. For this purpose the material fixed in Bouin and cut in sections was exposed for 5 weeks. The tests done with timidin-$H^3$ did not permit the observation, even after 3 h of contact and with concentration of up to 40 μCi/g of animal, of the assumption of the substance either in the primary nucleolus or in the amphinucleoli.

Under the electron microscope (pre-fixation in glutaric aldehyde at 4%, fixation in $OsO_4$ at 1% on a Millonig buffer; inclusion in araldite-epon, contrasts with uranyle acetate at 5% and with lead citrate according to Reynold) in the primary nucleolus (Figure 1) some granulations were seen with dimensions less (average diameter 70 Å) than the granulations of the amphinucleoli (average diameter 90 Å). These granulations were, as a rule, less dense in the first case than in the second (Figure 1). In any case, during the course of the growth of the oocytes, fibrils of a thickness of 40 and 60 Å were found to be interposed between the granulations. From the peripheral portion of the primary nucleolus, a kind of separation of 'gemmae' or 'daughter-nucleoli' was also observed (Figure 2) having the same ultrastructural characteristics. Then, in the amphinucleoli so-called 'clear fibrillar zones' (Figure 3), which correspond to the nucleolini, appeared. These zones contained, in a very clear matrix, some fibrils with a thickness of from 30 to 60 Å and were surrounded by other fibrils of the same type but very dense in such a manner that they constituted, all together, a kind of limiting membrane.

These new investigations permit us to affirm that in the oocytes of *Patella coerulea*, even though the primary nucleolus has a high RNA content, this has not a very active turnover except at the periphery, probably in

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relation to the emission of the 'daughter-nucleoli'. They also permit us to affirm that the amphinucleoli, even though they do not have RNA which is cytochemically discloseable, do, however, possess, RNA at a very high rate of turnover. There are even seats of analogous processes[8], in the growing oocytes of *Limnaea stagnalis*, the so-called 'para-nucleolus' (without vacuoles and which does not incorporate uridin-H$^3$) and the 'amphinucleolus' (which becomes vacuolate and incorporates uridin-H$^3$). The different sizes and the different density of the granulations shown under the electron microscope in the oocytes of *Patella coerulea* give support to the idea that the ribonucleoproteins have a different ultrastructural expression in the primary nucleolus and in the amphinucleoli.

With reference to the recent research of GOESSENS[9], which demonstrated in the 'centres fibrillaires' of the nucleoli of the tumoral cells of Ehrlich the presence of DNA, although in very small quantity, we are not able to confirm analogous results in the material we undertook to examine. As to the RNA of the primary nucleolus, we have positive indications for its presence in order to prepare the proteic yolk in the cytoplasm, at the present stage, we have no indications for the meaning to be attributed to the RNA of the amphinucleoli.

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## Biphasic Regulation of Transport Through Plant Cell Membranes by Kinetin and its Possible Relation to Directed Transport Between Source and Sink

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*Summary.* The significance of a biphasic dose-response curve of kinetin for the source-sink relation is shown. Such a regulator can create simultaneously both source and sink.

LÜTTGE[2] has recently reviewed the 'source-sink hypothesis' of transport in plants (based on the work of ARISZ[3]). He used the term 'source-sink gradient' to describe how a substance may diffuse from a region of higher concentration (source) to a region of lower concentration (sink) through the symplast. A diffusion region is present between source and sink. However, in order that source and sink should possess some sort of stability, there must not only be a concentration gradient in the diffusion space, but both source and sink must be capable of being enclosed so that submission and uptake of the substance may be regulated, and only concentrations outside these enclosed spaces are relevant for the diffusion. One must therefore search for a mechanism allowing simultaneous regulation at both sites.

The attraction of substances by kinetin[4] was described in terms of a single control point, i.e. that of the sink. The possibility simultaneously to regulate both processes, uptake in the sink and submission in the source, can be found by interpreting 2 previous papers[5,6]. A biphasic dose-response curve was found in experiments on the

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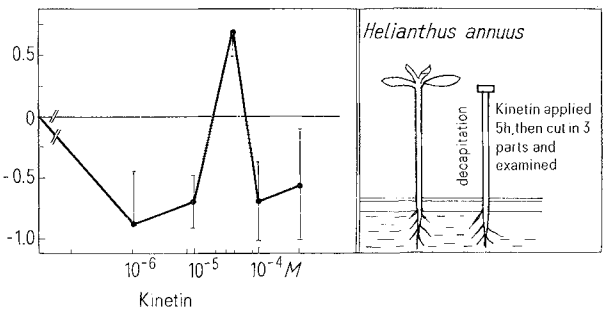


Fig. 1. Short review of the results from a previous paper[5]. Right: Data about the method. Potassium content of hypocotyl segments was measured by means of flame photometry. Left: Dose-response dependence of the potassium content of the hypocotyls upon the concentration of the kinetin solution. No significant difference between the respective segments could be found. Since an excess of kinetin (in comparison with the naturally occurring amount) had to be applied, no natural conditions with respect to gradients can be created. The resulting curve does not simulate natural relations of transport regulation but shows the reaction of cells.

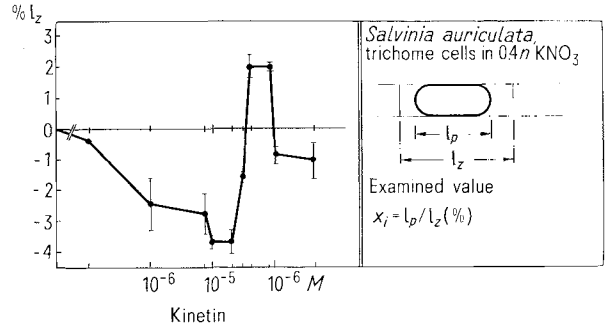


Fig. 2. Short review of the results of a previous paper[6]. Right: Data about the method (plasmometric measurements). Left: Dose-response dependence of the degree of plasmolysis upon the concentration of kinetin in the pretreatment medium (water-3 h). Since the correlation of the plasmolytic behaviour of the cells with changes of their potassium content could easily be shown, this curve can be compared with the curve from Figure 1. Measurements on single cells and on complex parts of plant tissue gave the same results.