

Observations on the response of the dorsal and subventral oesophageal glands of *Globodera rostochiensis* to hatching stimulation

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SUMMARY

Using video-enhanced contrast microscopy, the timing and nature of the response of the oesophageal glands of unhatched *Globodera rostochiensis* to hydration and hatch stimulation were studied. Secretory granule accumulation in subventral glands was solely a response to hydration. Accumulation of granules and increase in size of the gland cell nucleolus both occurred in dorsal glands in response to hydration and were further significantly affected by exposure to potato root difusate (PRD). No secretory material was observed to be voided into the oesophagus or intestine and it is concluded that the oesophageal glands are not involved in the process of eclosion and the limited response to PRD was part of the preparation of the juveniles for a feeding phase after hatching.

RÉSUMÉ

Observations sur la réaction des glandes œsophagiennes dorsales et subventrales de Globodera rostochiensis aux stimuli d'éclosion

Le déroulement et la nature de la réaction des glandes œsophagiennes de juvéniles non encore éclos de *Globodera rostochiensis* à l'hydratation et aux stimuli d'éclosion ont pu être étudiés, grâce à l'utilisation de la vidéo-microscopie à contraste renforcé. Une accumulation de granules sécrétoires dans les glandes subventrales, est la seule réaction à l'hydratation. L'accumulation de granules et l'augmentation de la taille du nucléole des cellules glandulaires se produisent dans la glande dorsale en réaction à l'hydratation :

GDW 1 in 4 by volume; in tests of four weeks duration, the PRD elicited > 85 % hatch.

Batches of soaked cysts were transferred to PRD for various periods between 2 to 48 h (see Fig. 2); control cysts were retained in GDW. Cysts were then cut open and the freed eggs mixed before an aliquot was transferred to GDW on a microscope slide. Eggs were viewed under a Reichert differential interference contrast microscope. The responses to PRD stimulation of the dorsal and subventral glands of unhatched, second stage juveniles and the process of eclosion were recorded at high magnification on 2.5 cm video tapes using video-contrast enhancement (Wyss & Zunke, 1986 *a*) and were analysed where required by single frame evaluation (Wyss & Zunke, 1986 *b*). Hatched second stage juveniles were also examined. An attempt to quantify secretory activity was made by recording, at each period of exposure to PRD, the extent of granule formation in the glands. Four categories were used: a gland was termed "full" when numerous secretory granules were present in all parts of the gland; "half-full" if only

granules (Fig. 1 B); the few exceptions contained only a small number of granules. During the hydration period, the glands showed marked secretory activity and granules accumulated. After seven days soak, about 35 % of the nematodes had dorsal glands which were full of granules and less than one third remained empty; however, there was little additional accumulation of granules during the second week in GDW.

Further secretory activity was observed when unhatched juveniles were transferred to PRD. Secretory material accumulated in the dorsal gland of unhatched juveniles after increasing periods in PRD (Fig. 2 a); this was most marked after 4 h in PRD when the majority of juveniles had full dorsal glands (Fig. 1 C). By 48 h, 80 % of the juveniles had dorsal glands packed full of granules.

Where juveniles were about to commence eclosion, the dorsal gland was full and granules were also present in the distended gland duct (Fig. 1 D). Movement of granules was frequently visible within the gland duct,

Table 1

The diameter (μm) of the nucleolus of the dorsal oesophageal gland of unhatched second stage juveniles of *Globodera rostochiensis* (Twenty values for each time period; S. E. = standard error of mean)

A. Changes in diameter of the nucleolus of unhatched juveniles from dry cysts after transfer to glass distilled water (GDW)

	Period in GDW					
	4 h	6 h	24 h	7 d	11 d	14 d
Mean	2.38	2.37	2.39	3.35	3.31	3.33
S.E.	0.09	0.10	0.08	0.13	0.13	0.15

B. Changes in the diameter of the nucleolus of unhatched juveniles from batches of cysts soaked for 1 wk in GDW and transferred to either potato root diffusate (PRD) or fresh GDW for a further 1 wk

	Period in GDW									
	0	1 h	2 h	3 h	6 h	24 h	48 h	72 h	96 h	7 d
Mean	3.44	3.44	3.36	3.22	3.29	3.44	3.58	3.58	3.58	3.58
S.E.	0.10	0.14	0.15	0.12	0.12	0.14	0.15	0.15	0.19	0.19

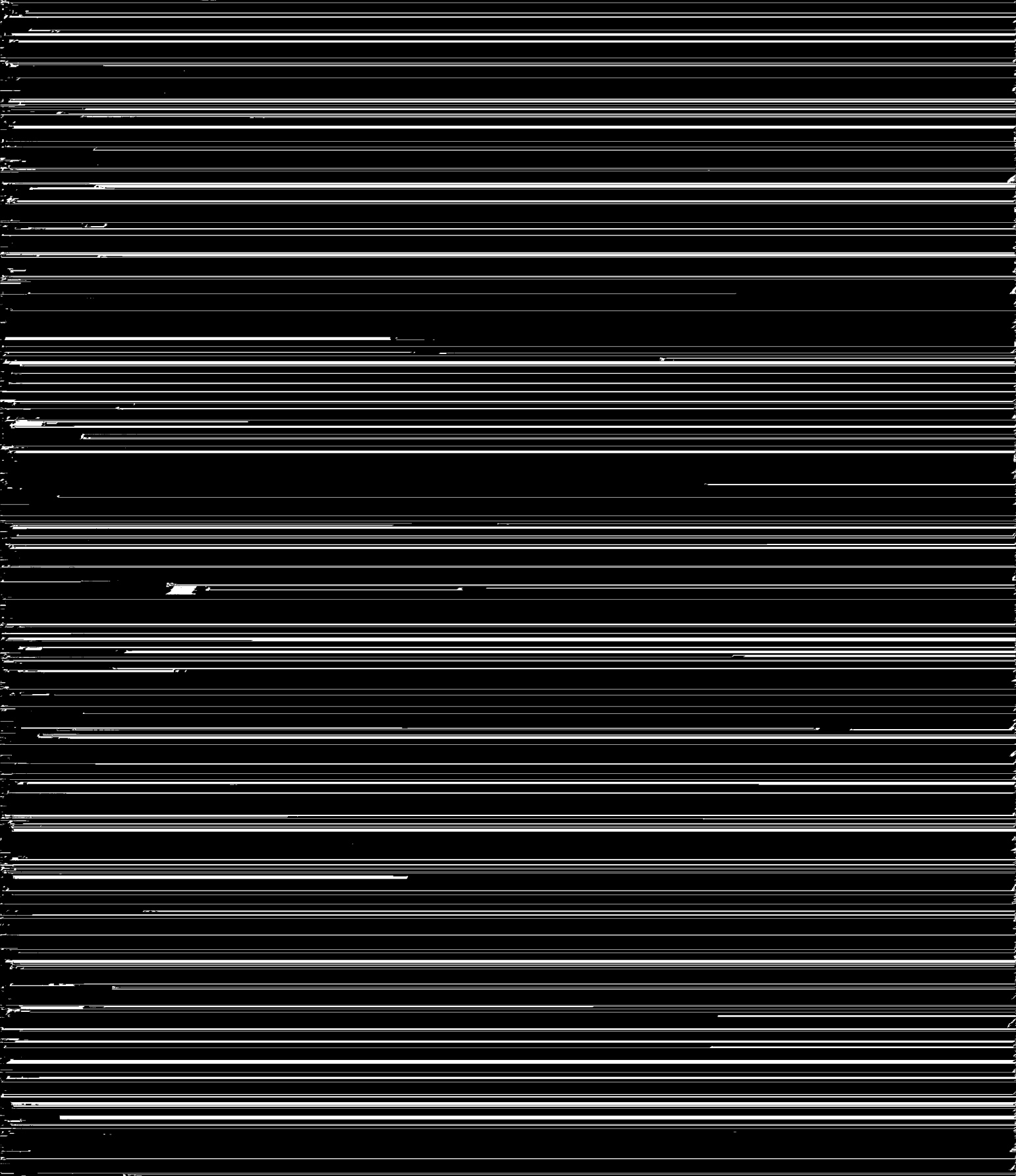
	Period in PRD									
	0	1 h	2 h	3 h	6 h	24 h	48 h	72 h	96 h	7 d
Mean	3.44	3.72	3.65	3.65	3.79	3.94	3.86	3.79	4.01	4.01
S.E.	0.10	0.14	0.17	0.13	0.15	0.12	0.12	0.11	0.12	0.12

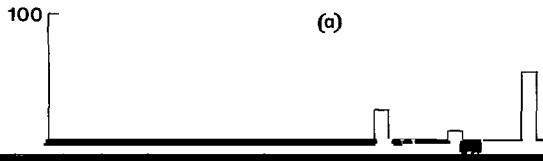
by contrast to the dorsal gland, the secretory activity of the subventral glands appears to be solely a response to hydration rather than PRD stimulation.

Movement of granules within the glands was not seen and, although there is an indication from observations at 1 and 2 h (Fig. 2 b) of a reduction in secretory content

RESPONSE OF THE SUBVENTRAL GLAND NUCLEOLUS

There was no change in the diameter of the nucleolus of either subventral gland at any time during or after eclosion, with or without PRD stimulation. The mean diameter (30 values) was $1.92 \pm 0.08 \mu\text{m}$.





Observations on the response of the dorsal and sub-ventral oesophageal glands of unhatched juveniles of *G. rostochiensis* to hydration and hatch stimulation indicate that the glands are unlikely to be involved in the process of eclosion. The response of the subventral glands was

Physiological changes in the unhatched juvenile are known to occur rapidly after hatch stimulation (Perry, 1986) and only 5 min exposure to PRD is required to trigger the hatching response in *G. rostochiensis* (Perry & Beane, 1982). Although the oesophageal glands are involved in events subsequent to hatching, the dorsal glands respond rapidly to PRD stimulation. Thus, by 4 h in PRD, accumulation of secretory granules resulted in a majority of unhatched juveniles with full dorsal glands with a concomitant significant increase in the size of the gland cell nucleoli. This supports and extends the nature of the bimodal action of PRD (Perry, 1986) to alter eggshell permeability and to stimulate juvenile activity and metabolism; part of the latter role now appears to include a preparation for the invasion and feeding phases following hatching and host location.

Although oesophageal gland secretions are unlikely to play a role in hatching, this does not necessarily preclude enzymic involvement. Enzymes need not emanate from the juvenile; they could be located in the egg fluid or eggshell (Perry, 1987). These possibilities are currently being investigated.

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