tion status of the aged hamster placenta provides useful information regarding the chemical forms and bioavailability of Cd present. In concert with the findings of Samarawickrama and Webb it suggests a mechanism to account for the presence (and absence) of pathology in the fetal system as a response to Cd^{++} administration.

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An immune response-dependent mechanism for the vertical transmission of an entomopathogen

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Summary. An exceptionally efficient mechanism for the vertical transmission of a parasitic gregarine is dependent on the insect host's immune response. Gametocysts of *Ascogregarina chagasi* on the genital accessory glands of adult female sand flies (*Lutzomyia longipalpis*) become encapsulated through hemocyte-mediated immune reactions. Oocysts of *A. chagasi*, ejected into the lumen of the glands owing to pressure exerted by this capsule, become glued to eggshells and are subsequently ingested by larvae. In *L. longipalpis* with an experimentally suppressed encapsulation reaction, fewer accessory glands contained oocysts of *A. chagasi*.
Key words. Humoral encapsulation; sand fly; gregarine; vertical transmission; parasitic life cycle.

Humoral encapsulation is an immune mechanism of dipteran insects, which has been described in *Plasmodium*-infected mosquitoes[1,2] as well as in several other host-parasite combinations[3–6]. The prophenoloxidase-activating cascade, triggered by invasive organisms, releases phenoloxidase which transforms phenolic compounds into quinones. These bind to amino groups of proteins creating a resistant complex of macromolecules, which is deposited around the foreign bodies[5,6]. The involvement of hemocytes in this process has been documented; however, their exact role remains unclear[7,8].

Phlebotomine sand flies (Diptera: Psychodidae) are vectors of the leishmaniases, a group of protozoan diseases which affect humans and occur in many regions of the world. *Lutzomyia longipalpis* is the vector of human visceral leishmaniasis in Latin America[9]. Gregarines are parasites of invertebrates which have been reported in over 20 species of sand flies[10], as well as numerous other arthropods[11]. *Ascogregarina chagasi* (Apicomplexa: Lecudinidae) was originally described in Brazilian sand flies[12], and has since been identified in a laboratory colony of *Lutzomyia longipalpis*, originating in Colombia[13].

Preliminary studies with the above *L. longipalpis* colony verified that 94%–100% of the adults were infected with *A. chagasi*. This high rate of infection was maintained despite the routine removal of dead adults from the rearing containers. It must therefore be attributed to oocyst contamination of eggshells. The earliest larval stage in which oocysts were observed was the third instar. Therefore horizontal transmission in a synchronized laboratory colony could not play a major role in maintaining high infection rates. Irrespective of gonotrophic status, most gamonts and gametocysts of *A. chagasi* in adult female *L. longipalpis* selectively adhere to the accessory glands of the genital apparatus (fig. 1.) Infective-stage oocysts from adherent gametocysts are inoculated through the wall of the accessory glands into their lumen. During oviposition, the accessory gland fluid containing the oocysts is discharged into the common oviduct, smearing

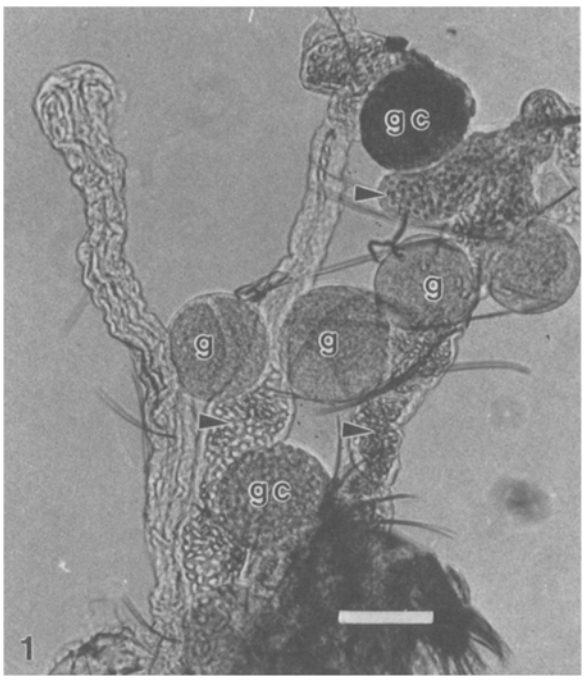

Figure 1. Phase-contrast micrograph of a pair of accessory glands of *L. longipalpis* full of oocysts of *A. chagasi* (arrows) with adherant gamonts (g) and gametocysts (gc). One mature gametocyst (darker) has undergone encapsulation. Scale bar = 100 µm, × 125.

the eggs. As the fluid dries on the eggshells, oocysts become glued to them. Emerged larvae are infected by ingesting the oocysts [12].

Electron micrographs, prepared as described by Walters et al. [14], showed deposition of electron-dense fibrillar matrices around gametocysts. Hemocytes, probably undifferentiated plasmatocytes [15] were often seen in association with the gametocysts, apparently secreting vacuolar material or undergoing lysis on the capsule's surface (fig. 2). The capsule material was PAS negative, but stained light-brown with the Masson-Fontana melanin stain [16]. It was considerably more delicate in appearance than similar structures observed in other dipterans; however, rarely were oocysts found free in the hemocoel, indicating that encapsulation successfully prevented rupturing of gametocysts. Large numbers of oocysts were seen in the lumen of the accessory glands, and numerous empty capsules remained attached to their external surface. These capsules consisted of flocculent electron-dense material enclosing the much-folded membrane of the gametocyst, residual oocysts and cytoplasmic debris (fig. 3).

Having lost its cytoskeleton and cellular organelles, the mature gametocyst of *A. chagasi* is essentially a membranous sac containing oocysts. The membrane displays no discernable ultrastructural features such as a suture, an attachment organ, or a cap, which would indicate a defined orientation for attachment to the accessory gland and expulsion of oocysts. The oocysts themselves are immotile and covered by a thick wall. Therefore it did not seem possible that autonomous mechanisms accounted

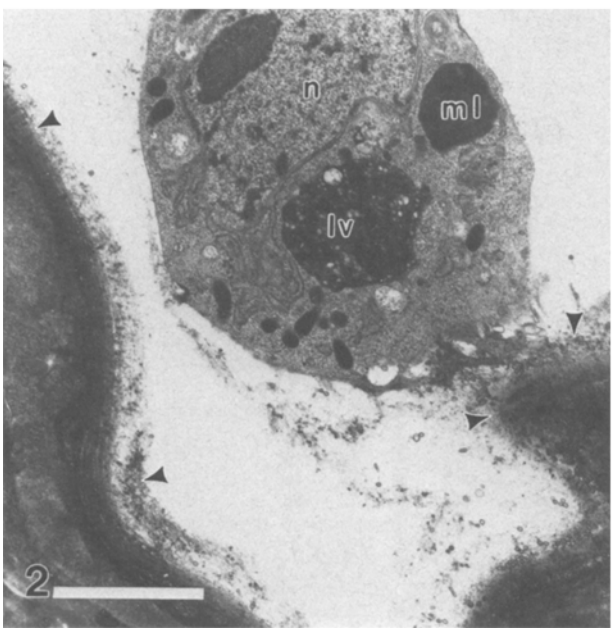

Figure 2. Electron micrograph of a hemocyte, (undifferentiated plasmatocyte) apparently secreting electron-dense material on the surface of an attached *A. chagasi* gametocyst. Nucleus (n), melanin-like granule (ml), lytic vacuole (lv). Arrows indicate fibrilar melanotic capsule. Scale bar = 2 µm, × 10,000.

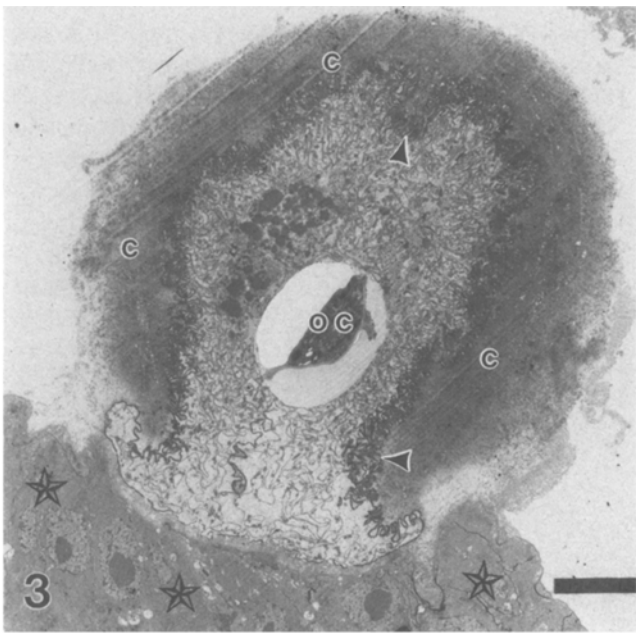

Figure 3. Electron micrograph of an empty, encapsulated gametocyst of *A. chagasi* adherent to the accessory gland wall. Note flocculent capsule material (c) and the exceptionally folded gametocyst membrane (arrows). Residual oocyst (oc), accessory gland cells (asterisks). Scale bar = 10 µm, × 2600.

for the forceful and directional ejection of oocysts. We postulate that the tough melanotic capsule, secreted by the sand fly, restricts the degree by which the gametocyst can expand in response to zygote division and oocyst maturation. Increasing internal pressure causes the membrane to rupture. The point of rupture is adjacent to the accessory gland, where the capsule is thinnest and therefore relatively weak. Consequently the spindle shaped oocysts penetrate the wall and are ejected into the lumen of the gland, while the empty capsule remains attached to the outside of the gland, sealing the resultant wound.

To test this hypothesis, groups of infected *L. longipalpis* females were maintained for six days, beginning 24 h post eclosion, on either phenylthiourea (PTU) or reduced glutathione in a 30% w/v sucrose solution. PTU and reduced glutathione combine with copper ions which are necessary for phenoloxidase action [17], thereby inhibiting reactions leading to encapsulation [18–20]. The mean number of gametocysts adhering to the accessory glands in these flies was the same as in the control groups, and the development of oocysts within them was normal. This shows that the phenoloxidase inhibitors did not interfere with the parasite's life cycle. However, most of their accessory glands harbored no oocysts; the numbers were in almost inverse proportion to those for control groups (chi-square, $p < 0.05$). The intensity of oocyst infections in accessory glands was also much lower in flies fed PTU or glutathione and fewer empty capsules were attached to their outer surface (table). Survival of flies for the duration of the experiments was uniformly above 95%.

Vertical transmission of gregarines to larvae of *L. longipalpis* feeding on decomposing bodies of infected dead adults, although plausible, would be a random process. Moreover, actively-feeding older larvae with well-developed peritrophic membranes are less likely to pass the infection on to the adult stage, because prior to pupation the peritrophic membrane and its entire contents are defecated (Warburg, unpublished). In newly-emerged larvae, before the peritrophic membrane grows, sporozoites of *A. chagasi* are able to establish an infection on the epithelial surface of the gut. Ectoperitrophic trophozoites migrate to the hemocoel of pupae as the midgut epithelium lyses during metamorphosis. Infection in adults is restricted to the hemocoel. By 'harnessing' the sand fly's immune system, the reported mechanism ensures exceptionally efficient vertical transmission. In addition, by providing larvae with large doses of infective oocysts as soon as they begin feeding, the described encapsulation facilitates trans-stadial transmission as well. The mutual adaptations which make this mechanism possible and the apparent lack of deleterious effects on sand fly populations, indicate a long and stable coevolutionary association between *A. chagasi* and *L. longipalpis*.

A. chagasi in *L. longipalpis* female genital accessory glands. Comparison of flies fed 30% sucrose (control) and flies maintained on 30% sucrose and 0.1% of a phenoloxidase inhibitor (PTU or glutathione).

	Control	Glutathione	PTU
No. of flies	54	44	53
No. of infected flies	51	44	51
No. of accessory glands examined	98	88	98
% accessory glands with oocysts in lumen	95	38	22
Mean No. of gametocysts per gland	3.4	3.2	3.5
Mean No. of empty capsules per gland	2.4	1.0	0.9
Mean score of oocysts per infected gland (scale of 1–5*)	4.3	2.5	1.6

* (1–10 oocysts per gland) = 1, (11–25) = 2, (26–50) = 3, (51–100) = 4, (100 and over) = 5.

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