After the addition of a defined mixture of ethyl ester homologues of JH I, II, and III as an internal standard, the extracts were purified by adsorption chromatography on silica gel and by high pressure liquid chromatography. The JHs and the internal standards were then converted to their 10-hydroxy-11-nonafluorohexoxy derivatives[9]. Following purification by high pressure liquid chromatography the samples were subjected to gas chromatography combined with selected-ion-monitoring mass spectrometry by which the individual JHs were identified and quantified[10].

Results and discussion. Only JH II was found in measurable amounts; this was consistent with the report that the corpora allata of *Galleria* produce this homologue exclusively when cultured in vitro[11]. Penultimate instar larvae contained 1–3 pmol JH II/g b.wt but this concentration rapidly decreased to about 0.1 pmol/g by 36 h after the last larval ecdysis (fig. 1). No hormone was detected later in the last instar. This developmental profile of the whole body JH titer is similar to that established with biological assays[12,13], except that the biological tests revealed a small peak of JH at the time of cocoon spinning, at about 140 h of the last instar. According to Peferoen and De Loof[13] the hormone should then rise to about 20 pg/g (i.e. less than 0.1 pmol/g). This quantity could not be detected by our physico-chemical method.

The sharp decline in JH titer at the end of the penultimate and beginning of the last larval instars is obviously caused by a reduction of hormone production and not by an increase in hormone breakdown. This is indicated by the fact that the activity of hemolymph esterases, which play the major role in JH inactivation in Lepidoptera[14], remains at a steady level during this period[15,16] (see fig. 1).

Figure 2 clearly shows that JH production resumes when freshly ecdysed last instar larvae are implanted with brains or are chilled on ice. Within 24 h after either treatment, the JH concentration rose to a value comparable to that found in early penultimate instar larvae. A decrease then occurred around the supernumerary larval molt, similar to the decrease at the time of molt from the penultimate to the last instar. The supernumerary larvae one day after ecdysis contained in average 0.5 pmol JH/g, a concentration virtually identical to that in newly ecdysed last instar larvae. Since neither brain implantation nor chilling have a significant effect on JH esterase activity[17], the increase in JH titer after these treatments must be due to stimulation of the corpora allata rather than a decrease in the rate of hormone degradation. These data provide compelling evidence that the corpora allata of *Galleria* larvae are stimulated by a cerebral allatotropin. The secretion of this factor from the brain in situ apparently ceases after the last larval ecdysis, except when the larvae are exposed to temperatures around 0 °C.

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Morphological differentiation of the growing oocyte of *Ctenomys torquatus* (Rodentia, Octodontidae)

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Summary. The ultrastructural changes observed during the growth phase of oocytes of *Ctenomys torquatus* (Rodentia, Octodontidae) are reported. Interest was particularly centered on the transformation and/or distribution of the components of the endoplasmic reticulum. According to the observations made it is suggested that the endoplasmic reticulum stores some kind of material which may support early stages of development.
Key words. Ctenomys torquatus; oocyte; endoplasmic reticulum; ultrastructure; storage materials.

Mammalian oocytes grow and differentiate after their meiotic prophase has been arrested at the dyctiate stage[1,2]. During this stage oocytes increase their volume by the synthesis of macromolecules and accumulation of organelles[3–7], some of which probably support early embryogenesis, as is the case in lower species[8].

The morphological aspects of oocyte differentiation have been the subject of several light[9,10] and electron-microscopic[11–14] studies. These studies show that oocytes from different mammalian species present similar changes during their growth phase; however, details of these changes may be quite different.

In the present paper the most conspicuous ultrastructural aspects of differentiation of oocytes of *Ctenomys torquatus* are reported. Some of these results have been published as an abstract[15].

Materials and methods. Seven adult female *Ctenomys torquatus* (Rodentia, Octodontidae) were collected in the field at Carrasco, Uruguay. The diploid chromosome number of this population is 2n = 56, as determined by Kiblisky et al.[16]. Ovaries were fixed in 2.5% buffered glutaraldehyde (pH 7.2) at 4 °C; postfixed in 1% buffered OsO_4 and embedded in Durcupan ACM (Fluka). Sections were cut in a Sorval MT2 ultrami-

crotome, stained in uranyl acetate and examined in a Siemens Elmiskop I.

Oocyte stages were determined following the Pedersen and Peters[17] classification for ovarian oocytes of *Mus*. Observations were performed on 49 oocytes: 11 small oocytes (stages 4 and 5a); 16 mid-grown oocytes (stage 5b); 12 fully-grown oocytes (stage 6); and 10 oocytes from antral follicles (stage 7).

Ultrastructural morphometry. Cytoplasmic areas representing 35 μm^2 each were selected from micrographs (× 30,000) of cortical and subcortical regions of oocytes at the stages listed above (12 areas for each stage). The percentage corresponding to the endoplasmic reticulum (ER) was evaluated in each of these areas. The procedure used for the measurement of the ER components was: a) to consider ER vesicles as perfect circles; and 2) to consider ER cisternae as rectangular areas.

Results. In small oocytes (stages 4 and 5a) ER appears in two forms: round vesicles (0.1 µm diameter) and elongated cisternae (fig. 1). In some cases ribosomes were found attached to both structures. ER represents 6 ± 0.5% of the quantified areas (vesicles 5 ± 0.5%; cisternae less than 1%) (fig. 5). Mitochondria are round in shape (0.3–0.5 µm diameter). They show a matrix of low electron density and a few cristae (fig. 1). These organelles as well as the ER components are uniformly distributed in the ooplasm.

In mid-grown oocytes (stage 5b) the population of ER cisternae increases (5 ± 0.5%) whereas the population of vesicles decreases (4 ± 0.5%) (fig. 5). In some cases polysomes were seen attached to their surface (fig. 2). Mitochondria present cristae and electron-dense granules, and their matrix is denser than in preceding stages. Mitochondria form clusters composed of 2 to 4 elements united by an electron-dense substance. These clusters appear associated with the regions of the ER cisternae depleted of polysomes (fig. 2).

In fully-grown oocytes (stage 6) the number and size of the ER vesicles increases (0.2 µm); they represent 20 ± 1% of the evaluated areas (fig. 5). The ER vesicles show a fibrillar content of low electron density (fig. 3) and polysomes are attached to some of them (figs 3 and 4). Most of the ER vesicles are located in the oocytes cortex, where no mitochondria are observed (fig. 3). At this stage mitochondria form a ring-like layer beneath the cortical region which can already be observed by light microscopic examination of thick sections (0.25 µm). In stage 6 as well as in stage 7 (see below) round-shaped mitochondria have an electron-dense matrix and a few cristae.

In oocytes from antral follicles (stage 7) ER vesicles occupy

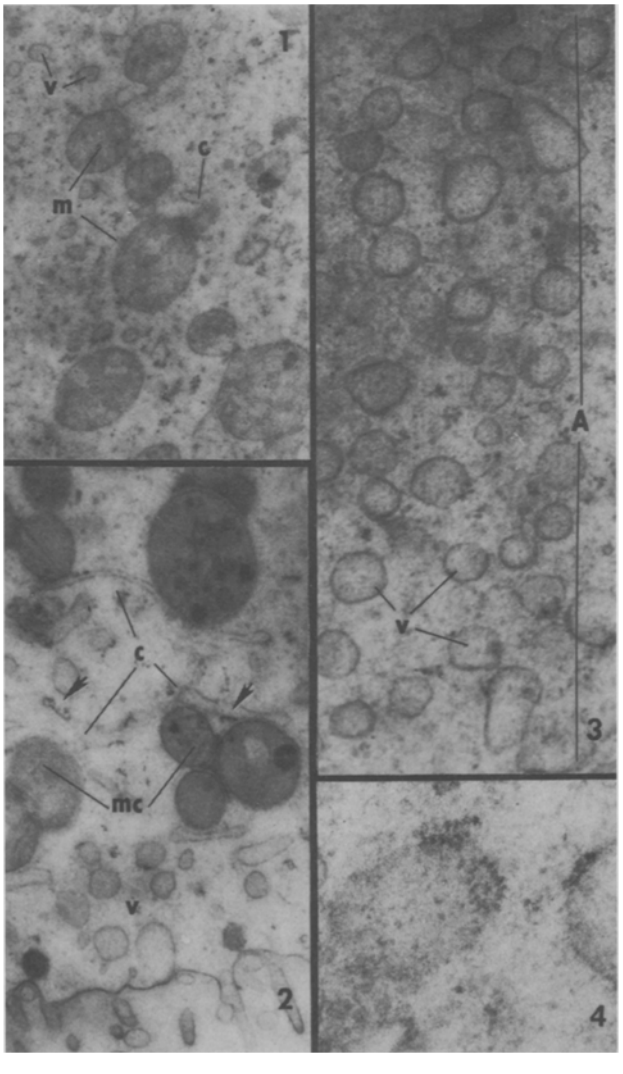

Figure 5.

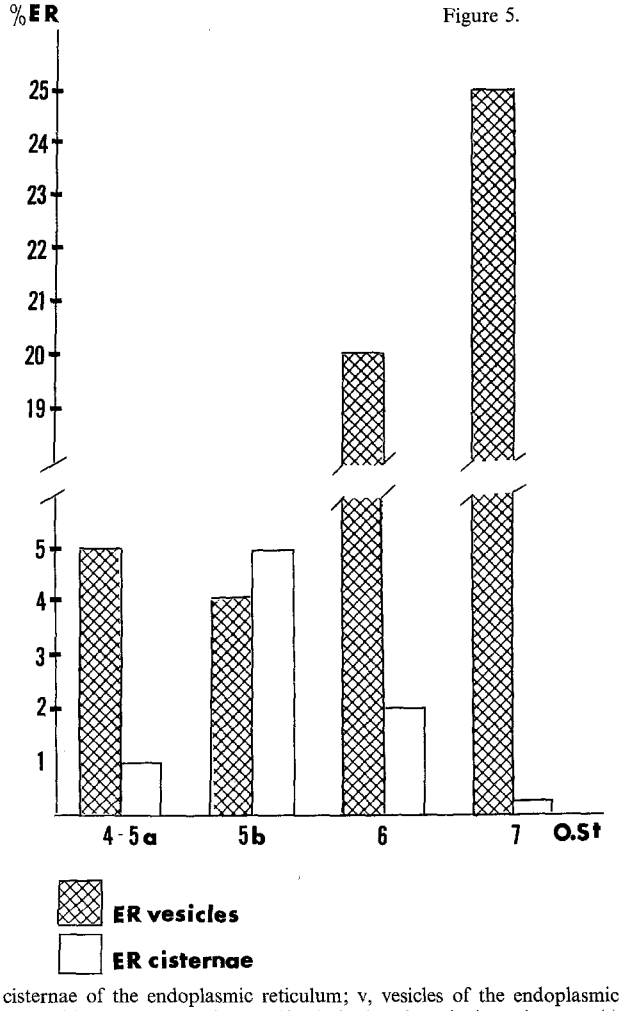

Figure 1. Part of the cytoplasm of a small oocyte (stages 4 and 5a). c, cisternae of the endoplasmic reticulum; v, vesicles of the endoplasmic reticulum; m, mitochondria. × 13,500. Figure 2. The cortical cytoplasm of a mid-grown oocyte (stage 5b). Endoplasmic reticulum cisternae (c) appear associated with mitochondrial clusters (mc). Some cisternae present polysomes attached (arrows). Several endoplasmic reticulum vesicles (V) can also be observed. × 33,500. Figure 3. The cortical region (A) of a fully-grown oocyte (stage 6). Several endoplasmic reticulum vesicles (V) appear grouped in this area. Note the absence of mitochondria. × 30,000. Figure 4. A tangential section of an endoplasmic reticulum vesicle. Polysomes appear attached to its membrane. From a stage-6 oocyte. × 80,000. Figure 5. Diagram representing the percentual area occupied by the endoplasmic reticulum (% ER) at different stages of the oocyte growth (0 st).

25 ± 1% of the quantified areas, but the number of cisternae found is small (less than 0.25%) (fig. 5). ER vesicles and non-clustered mitochondria appear randomly distributed in the cytoplasm. In all stages studied a few Golgi units and scarce multivesicular bodies were observed.

Discussion. The most conspicuous aspects of growing oocytes of *Ctenomys torquatus* are proliferation and morphological transformation of the endoplasmic reticulum components.

In some mammalian oocytes ER is poorly represented[11]; this has been shown to be the case for some species such as rat[18,19], hamster[20] and rhesus monkey[21], but the situation is different in others for example guinea-pig[22], rabbit[23] and sheep[24]. Guinea-pig oocytes[22] present smooth vesicles with a lipid-like content; rabbit oocytes[23] show large rough ER vesicles with a floccular content as well as rough and smooth small vesicles grouped in the cortical region. In sheep oocytes[24] large and irregularly shaped ER vesicles with a smooth surface have been described. In the three last-mentioned species the accumulation of substances inside the components of the endoplasmic reticulum has been related to the existence of a storage mechanism.

Similarly, it can be suggested that the increase and morphological transformation of the ER elements in growing oocytes of *Ctenomys torquatus* correspond to the synthesis and accumulation of some kind of storage materials.

In other species, stored materials have been described as being composed of nonmembranous lamellar or fibrillar structures (rat, mouse, hamster, human)[12,25]. Nonmembranous lamellar structures associated with the ER have been described in *Thomomys townsendii*[26], in *Acomys cahirinus*[27] 'ribosomic fibrils' associated with the ER were observed. With regard to this point it is interesting to remark that species which store materials as nonmembranous lamellar or fibrillar structures present a poorly developed ER, whereas species which do not show these materials present a well-developed ER.

These facts led us to think that storage materials in mammalian oocytes may exist in at least three forms: 1) nonmembranous structures not associated with ER; 2) nonmembranous lamellar or fibrillar structures associated with ER; and 3) materials contained inside the components of the ER, as is the case with *Ctenomys torquatus* oocytes.

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Comparison of the effects of imidazo[1,2-a]pyridine-2-carbamates and benzimidazole-2-carbamates on the development of *Hymenolepis nana* in *Tribolium confusum*

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Summary. The anthelmintic properties of several imidazo[1,2-a]pyridine carbamates and benzimidazole carbamates against *Hymenolepis nana* are compared. The results of this study, coupled with previous work, indicate that methyl 6-(trichloroethenyl)-imidazo[1,2-a]pyridine-2-carbamate has the potential of being a broad spectrum anthelmintic, effective against both nematodes and cestodes.

Key words. Anthelmintic; benzimidazole carbamates; *Hymenolepis nana;* imidazo[1,2-a]pyridine carbamates.

Several 6-substituted imidazo[1,2-a]pyridine-2-carbamates have shown antinematocidal activity[2-4]. Here we report that some of these compounds also seem to have an anticestocidal effect. To test these compounds we used the *Tribolium confusum-Hymenolepis nana* system. The results obtained with imidazo[1,2-a]pyridine-2-carbamate are compared to those reported previously for this parasite and similarly substituted benzimidazole-2-carbamates (tables 1 and 2). Also an additional benzimidazole carbamate, fenbendazole (**IIc**), not tested previously, is included in the present study[1]. Student's t-test was used to assess statistical differences between experimental and control groups. The probabilities are given when they were less than 0.05.

The flour beetles infected with *H. nana* eggs were fed continuously from day 1 to day 10 or 18 post infection (p.i.) on mixtures of nine parts flour and one part drug, the concentration used previously in studies with benzimidazole carbamates[5,6]. Control beetles received only flour. On day 10 p.i.,