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Title: ___ THE COMPARATIVE HISTOLOGY AND HISTOCHEMISTRY OF THE

_____ DIGESTIVE TRACT OF THE LAND SNAILS _____

_____ ALLOGONA TOWNSENDIANA, LEA (HERBIVOROUS) _____

_____ AND HAPLOTREMA VANCOUVERENSE, LEA (CARNIVOROUS) ___

Redacted for Privacy

Abstract approved _____
                     (Ernst J. Dornfeld)

A herbivorous terrestrial snail, Allogona townsendiana, was compared by both histological and histochemical methods to a sympatric carnivorous species, Haplotrema vancouverense, with respect to localization of digestive enzymes.

The distributions of alkaline phosphatase, acid phosphatase, non-specific esterases, aminopeptidase, and general lipid were described for selected segments of the digestive tracts.

Differences in distribution of alkaline phosphatase were evident in the buccal cavity, esophagus, stomach, and rectum. Allogona showed generally higher activity in the musculature of the stomach and rectum while Haplotrema had high apical activity. Both species showed high apical activity in the intestinal loop epithelium.

Acid phosphatase was commonly low in areas of high alkaline phosphatase activity except in the cytoplasm of the calcium cells in the digestive gland of both species.

Non-specific esterase activity was most intense in _Haplotrema_. No activity was observed in calcium or mucous cells for either species. The hematoxylin-staining cells of the salivary glands showed no loss of activity following E-600 treatment suggesting that A-esterase and possibly C-esterase are secreted by these cells. In general, E-600 treatment caused the greatest loss of activity at the lumen and striated border of the digestive tract which indicates the location of B-esterase. E-600 resistant activity corresponded to the small yellow granules of the digestive cells.

The greatest difference in site and intensity of enzyme activity was observed for aminopeptidase. _Haplotrema_ showed very high activity in the buccal epithelium, digestive gland, and intestinal loop. _Allogona_ showed high activity only in the intestinal loop.

The staining reaction for general lipid was considerably greater in all regions of the digestive tract in _Allogona_. Salivary gland cells showed no reaction in either species. No significant correlation could be made between lipid concentration and E-600 inhibition.

THE COMPARATIVE HISTOLOGY AND HISTOCHEMISTRY OF THE
DIGESTIVE TRACT OF THE LAND SNAILS
ALLOGONA TOWNSENDIANA, LEA (HERBIVOROUS)
AND HAPLOTREMA VANCOUVERENSE, LEA (CARNIVOROUS)

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I. INTRODUCTION

Several general reviews of gastropod anatomy and histology have been published (Pelseneer, 1906; Cooke, 1927; and Baecker, 1932; among others). The most intensive cytological attention has been focused on the pulmonate Roman snail Helix pomatia. Early authors who were principally concerned with the histology of the digestive tube (esophagus, stomach, and intestine), include Monti, 1900; Spiro, 1911; Jordan and Begeman, 1921; and von Haffner, 1923.

The digestive tract in stylomatophorans generally consists of a buccal mass which contains a chitinous radula. The esophagus leaves the buccal mass dorsally and passes the food into an extensible stomach. A pair of fused salivary glands lies along the mid-esophagus (crop) and empties digestive enzymes into the buccal cavity through two salivary ducts. A lobed digestive gland secretes digestive enzymes into the lumen of the stomach by ducts emptying into the mid-region. The thin-walled intestine is usually long and passes as an S-shaped loop through the digestive gland tissue, where it leaves as a relatively short rectum. The anus of pulmonate snails opens lateral to the pneumostome in the mantle collar at the edge of the shell. Plate 1, Figure 1 illustrates the digestive tract of Helix pomatia in simplified diagram.

Several cell types have been reported in the pulmonate digestive

tract. Discussions of the functional role of these cells are found throughout the literature. Of principal note are the following:

1) chitinogenic cells located in the buccal epithelium, which secrete chitin at their free borders;

2) odontoblasts located in the radular sac, which produce the radular teeth;

3) ciliated epithelium lining the entire digestive tract and most ducts;

4) mucus secreting cells located primarily in the stomach, intestine, and rectum;

5) salivary gland cells;

6) calcium (lime) cells and digestive (secretory-resorption) cells of the digestive gland.

In 1925 and 1928 Krijgsman reported on the secretory rhythms of the "digestive cells" in Helix pomatia. Relying on histological observations of vacuole formation, Krijgsman demonstrated polyphasic secretory and excretory activity. In describing the four morphological stages occurring in the nucleus and cytoplasm of the digestive cells, Krijgsman laid the cytological foundation for all subsequent histophysiological and cytochemical studies.

In 1946 Carriker and Bilstad investigated the histology of the alimentary system in the aquatic basommatophoran Limnaea stagnalis. This paper described the secretory histology of the ciliated gland cells, digestive cells, salivary cells, and lime cells. The digestive and salivary gland cells were previously known to secrete enzymes from the work of Pacaut and Vigier (1906). Gabe and Prenant (1948)

followed this with a brief report on the cytology of the salivary

glands of the same species.

Thiele, in 1953, published a comparative histological study of

the digestive gland (Mitteldarmdrüse) in twenty pulmonate, five

prosobranch, and two opisthobranch genera. This detailed study

described the three functional stages of the calcium cells. The

third functional stage had previously been considered as a separate

cell type (Keulenzelle, Frenzel, 1885).

Sumner (1965) produced a detailed cytological review of the

digestive gland cells of H. pomatia in which he described 1) thin,

undifferentiated cells, 2) digestive cells, 3) calcium cells, and

4) excretory cells. Sumner agreed with Kirjgsman (1928) that the

calcium cells stages I, II, and III were degenerating cells and

supported Thiele's (1953) hypothesis that these cells are excretory

in function.

Hydrolytic enzyme activity in the digestive juices of Helix has

been characterized extensively by biochemical assay. Rosen (1937)

reported intracellular protease activity in the midgut epithelium of

Helix. Although peptidase activity was observed in the gut contents,

no protease activity was found. This was later confirmed by Thirlwell

et al. (1963) who detected no unequivocal evidence for protease in

Helix.

Carriker (1946) made semi-micro tests for amylase and proteinase

in the salivary gland secretions of Lymnaea stagnalis. In 1950,

Holden and Tracey, working with tobacco leaf components and defined

substrates, demonstrated high levels of intracellular and

extracellular hydrolytic action on many specific carbohydrate sub-
strates in Helix. This information coupled with the diversity of
digestive tract structure and feeding habits observed in gastropods
appeared to indicate that some extraordinary mechanisms of enzyme
synthesis existed in these species.

Billet (1954), using 8-hydroxyquinoline glucuronide substrate,
discovered a high B-glucuronidase activity in digestive gland and
crop homogenates of Helix. This information was instrumental in the
histochemical localization of B-glucuronidase activity in the distal
tips and cell bodies of digestive gland secretory-resorptive cells
(Billet and McGee-Russell, 1955).

A soluble extract prepared from the isolated digestive tract and
its contents was shown by Myers and Northcote (1958) to contain
quantitative amounts of proteinases, lipases, and twenty different
carbohydrases.

Thirlwell et al. (1963) compared the specific activities of
several hydrolytic enzymes occurring in the digestive juices of Helix
pomatia and Achatina fulica, the Giant African Snail. Enzyme activi-
ties towards oligo- and polysaccharides, arylsulfates, and B-glucosides
were determined.

Florkin and his co-workers (Florkin and Lozet, 1949) have pro-
duced conflicting evidence concerning the snail's production of
certain classes of hydrolytic enzymes. Experiments with bacteria
isolated from the gut fluids of Helix pomatia have revealed high
cellulase and catheptase activity.

Guardabassi and Ferreri (1953), using histochemical localization

techniques, compared alkaline phosphatase activity in the digestive tracts of active and estivating H. pomatia. They also investigated the problem of fat absorption throughout the digestive epithelia employing Baker's formol-Sudan black technique.

Nakazima (1956) investigated alkaline and acid phosphatase activity in marine lamellibranchs, gastropods, and polyplacophores (chitons).

Early histophysiological approaches to enzyme problems have been re-evaluated using the newer techniques of cryostat sections, specific substrates, and simultaneously coupling azo dyes. With these new techniques have come improved intracellular localizations and greater accuracy in correlating enzyme activity with subcellular organelles.

The work of Rosenbaum and Ditzion (1963) on the digestive gland cells of starved and feeding Helix pomatia has employed new techniques for qualitative visualization of alkaline and acid phosphatase, B-glucuronidase, non-specific and E-600 resistant esterase, amino-peptidase, phospholipid, and glycogen.

With the exception of Achatina fulica, the Giant African Snail, all references cited have concerned herbivorous species. In his excellent review, Arvy (1962) commented on the extensive histophysio-logical literature compiled for the herbivorous gastropods. Arvy added, however, that little is known about the histochemical activity of carnivorous species, especially with regard to aminopeptidases. In view of the sparsity of information on carnivorous gastropods, the present study was undertaken to compare the histology and histo-chemistry of the digestive tract of a carnivorous terrestrial snail

(<u>Haplotrema</u>) with that of a related herbivorous species (<u>Allogona</u>) with which it shares the same habitat.

II. MATERIALS AND METHODS

Allogona townsendiana (Lea) and _Haplotrema vancouverense_ (Lea) were collected in Skagit County, Washington near Mount Vernon. Specimens of _H. vancouverense_ were also obtained in Benton County, Oregon on Wood's Creek near Philomath (Plate 2, Figure 2).

These two species of terrestrial snails, indigenous to the Northwest, are found on leaf-littered woodland slopes especially in the vicinity of sword ferns. _A. townsendiana_ is strictly herbivorous while _H. vancouverense_ is carnivorous. These snails were selected because of their sympatric distribution and their close similarity in size and diurnal activity. The taxonomic position of these snails is as follows:

Class - Gastropoda

Subclass - Pulmonata

Order - Stylommatophora

Suborder - Sigmurethra

Family - Polygyridae

Allogona townsendiana (Lea)

Family - Haplotrematidae

Haplotrema vancouverense (Lea)

Field collected snails were held in covered dishes on moist paper toweling at 50°F. Prior to killing, actively feeding snails were removed to room temperature and allowed to continue feeding for 24-48 hours. _A. townsendiana_ was fed fresh lettuce and _H._

vancouverense small earthworms.

Histological Methods

Cold aqueous Bouin's fluid was the principal histological fixa-
tive; 10% neutral formalin and Carnoy's solution were also used.
Paraplast embedded tissues were sectioned at 6, 8, and 10 microns and
stained with Harris' hematoxylin and eosin or Masson's trichrome.
Fresh frozen, calcium-formol post-fixed cryostat sections were also
stained with Harris' hematoxylin for histological reference.

Histochemical Methods

Fixation

Two fixation methods were employed for cryostat sectioning:
1) quick-freezing in gum tragacanth, followed by mounting of sections
on cold slides and post-fixing in cold (-20°C) acetone, and 2) pre-
fixing in cold (4°C) calcium-formol for 18-24 hours before quick
freezing in gum tragacanth on cryostat planchets.

The digestive gland with its associated intestinal loop presented
a special sectioning problem due to its extreme friability. Although
frozen sections were prepared with acetone post-fixation, most samples
of digestive gland were prefixed in cold calcium-formol for 18 hours,
rinsed in two changes of distilled water, and embedded in 15% gelatin
for one to two hours at 37°C. After cooling at 4°C for 20 minutes, a
small cylinder of gelatin containing the specimen was removed with a
cork borer and quick frozen in an International-Harris CTD cryostat.

Enzyme Localization

Alkaline phosphatase was demonstrated by the calcium cobalt method of Gomori (1952) using sodium-B-glycerophosphate as substrate, and the azo dye method recommended by Barka and Anderson (1963) with sodium alpha-naphthyl acid phosphate substrate; fast violet B dye was added as the coupling agent in 0.1 M sodium barbiturate buffer at pH 9.2. Incubation times were one to three hours for the Gomori technique and 30 minutes to three hours for the azo dye.

Acid phosphatase was detected by the lead sulfide method of Gomori (1952) using sodium-B-glycerophosphate as substrate in 0.1 M tris-maleate buffer at pH 5, and the pararosanilin azo dye method of Barka (1960) with sodium alpha-naphthyl acid phosphate as substrate in tris-maleate buffer adjusted to pH 6.5. Incubation times were similar to those for alkaline phosphatase above.

Non-specific esterase activity was visualized by the alpha-naphthyl acetate substrate method recommended by Barka and Anderson (1963), using hexazonium pararosanilin as the coupler in 0.15 M phosphate buffer adjusted to pH 7.3. Incubation times ranged from three minutes to three hours at 24°C. A parallel series of sections was pre-treated for one hour with the organo-phosphate inhibitor E-600 (diethyl-P-nitrophenyl phosphate) at a concentration of 10^{-5} M in 0.1 M tris-maleate buffer at pH 7.2.

Aminopeptidase was distinguished according to the L-leucyl-B-naphthyl-amide substrate method of Burstone and Folk (1956), employing fast blue B as the coupling dye in 0.1 M acetate buffer at pH 6.5. Cold acetone post-fixed sections were incubated 30 minutes to three hours.

Control sections for all histochemical methods were incubated in a parallel series which contained no substrate.

Other Histochemical Methods

Oil red O isopropyl alcohol, after the method of Lillie and Ashburn (1943), was used for general lipid staining on active and estivating snail tissues. A 20 minute incubation was used on fresh cryostat sections followed by a hematoxylin counterstain.

III. HISTOLOGICAL OBSERVATIONS

Buccal Cavity

The buccal mass which houses the radula is bulbar and heavily
muscularized in both species. The epithelium increases in height from
very low cuboidal (12-14μ) in the subradular membrane to tall columnar
(35μ) in the supraradular roof (Plate 13, Figure 42). Beneath the
radular support, or collostyle, an inverted T-shaped pouch is lined
by high columnar cells. These cells secrete chitin at their free
borders. The subradular cells produce a thin radular pad beneath the
denticles. The lateral walls of the buccal cavity above the radula
have a thin chitinous covering which gradually thickens directly above
the radula. The thickest layer of chitin is located in the T-shaped
pouch below the collostyle, where it may exceed 100μ. Histochemical
attention was focused on the mid-buccal supraradular epithelium. This
epithelium is continuous with the lining of the esophagus.

The basic morphology of the buccal cavities of the two species is
similar. The Haplotrema radula, however, differs considerably from
those of the common herbivorous types. The Haplotrema radula possesses
fewer and larger teeth which serve to tear earthworms into small,
easily ingestible segments.

Esophagus

The esophagus of both species may be divided into pre-, mid-, and
post-esophageal anatomical segments. The mid-esophageal region, or
crop, beneath the salivary glands is greatly expandable. The esophagus

of _Allogona_ averages about 14 mm in length and gradually enlarges into

the tubular stomach. In the collapsed condition the mid-esophagus is

about 2 mm in diameter (Plate 3, Figure 5). Four major and several

minor longitudinal folds extend into the lumen. The ciliated columnar

epithelium reaches a height of 55μ; the epithelium contains scattered

mucus secreting flask cells.

As may be noted in Plate 3, Figure 6, the _Haplotrema_ esophagus

has a smaller diameter than that of _Allogona_. Its longitudinal folds

are more numerous, and in the mid-esophagus region secondary folds are

present.

The longitudinal folds on both species contain connective tissue

and traces of longitudinal muscle, as well as occasional large nerve

cells.

Salivary Gland and Ducts

In _Allogona_ the salivary gland lies dorsal to the esophagus about

midway between the buccal mass and the beginning of the stomach

(Plate 2, Figure 3). The paired yellow glands are united mid-dorsally

and extend ventrally to cover most of the lateral aspect of the mid-

esophagus. Paired salivary ducts pass anteriorly (4-5 mm) to enter

the rear of the buccal cavity. The salivary glands are approximately

8 mm in length. The ducts ramify throughout the gland and are lined

by large secreting cells.

In _Haplotrema_ the paired salivary glands are united both dorsally

and ventrally by a continuous connective tissue sheath to form a white,

doughnut shaped structure around the mid-esophagus (Plate 2, Figure 4).

In the salivary glands of both species, three functional stages
of the secreting cells may be observed:

1) cells, approximately 25µ X 50µ, with cytoplasmic granules

   which color brownish-pink with hematoxylin and eosin, nucleus

   central;

2) cells, approximately 25µ X 50µ, with reticular cytoplasm

   which colors intensely blue with hematoxylin, nucleus central;

3) cells, approximately 50µ X 85µ, containing eosinophilic

   secretion droplets, nuclei displaced peripherally or

   unidentifiable.

The smaller ducts within the glands (20µ diameter) are lined with very
low cuboidal or squamous epithelium; they join to form larger ducts
(90-100µ diameter) with low columnar epithelium. The extrinsic
salivary ducts are densely lined with columnar cells possessing long
cilia. Their nuclei often appear very close to the duct lumen as seen
in Plate 3, Figure 7.

Stomach

In freshly killed _Allogona_ the stomach appears as an enlarged
tube and is clearly distinguished by its prominent rows of internal
longitudinal folds. Specimens preserved and hardened in FAA solution
tend to become buckled just posterior to the entrance of the digestive
gland ducts at the gastro-duodenal junction.

The _Haplotrema_ stomach, which arises abruptly from the long and
narrow posterior esophagus, is crescentic.

In both species the stomach wall possesses a band of circular

muscle just inside the outer connective tissue sheath. The high

columnar ciliated cells (50-60μ) are greatly compressed at their base

and flare outward to the free border. In addition to the ciliated

cells, a few mucous cells appear near the lumen.

Intestine and Digestive Gland

In both _Allogona_ and _Haplotrema_ the duodenum extends anteriorly

beneath the stomach before narrowing into the region of the intestinal

loop which is embedded in the anterior lobe of the digestive gland.

The thin walled intestinal loop follows an elongated S-shaped path

from the duodenum to its emergence from the gland into the rectum.

The average length of the intestinal loop, exclusive of the duodenum

and rectum, is 30 mm.

The longitudinal folds of the _Haplotrema_ intestinal loop are more

numerous than those of _Allogona_, but in the turgid intestine only

dorsal and ventral folds persist. The highest ciliated columnar

epithelium (about 100μ) is found just below the crests of the folds.

The cilia are approximately 7μ in length and arise from a well

defined striated border. Scattered longitudinal muscle fibers lie

in the connective tissue between the circular muscle layer and the

epithelial basement membrane.

Two types of secreting cells are scattered throughout the intesti-

nal epithelium: (1) elongated cells filled with eosinophilic granules,

and (2) thin, hematoxylin staining cells whose reaction is so intense

that the nucleus is commonly obscured (Plate 3, Figure 8).

In the fresh condition the digestive gland of _Haplotrema_ is of a

rich, brown color somewhat darker than that of <u>Allogona</u>. The diges-
tive gland sends ducts to the stomach and then extends posteriorly
into the small coils of the shell; the anterior lobe surrounds the
loop intestine. Histologically, the digestive gland is a complex
compound tubuloalveolar structure. The individual cells which make
up the tubule walls are of two distinct types, the digestive or
secretory-resorption cells, and the calcium cells. The more numerous
digestive cells are long (about 100μ) and extend from the basement
membrane to the lumen. The roughly triangular and generally recessed
calcium cells are broader and shorter than the digestive cells and
contain what have been termed calcium spherules. These colorless
granules completely fill the cell cytoplasm (Plate 4, Figures 9 and
10).

The cytoplasm of the digestive cell contains various inclusions:
small colorless granules at the tips of the cells near the lumen,
larger yellow granules distributed throughout the cytoplasm, and
commonly one or two large brown vacuolated granules near the nucleus.

<u>Rectum</u>

The rectal canal of <u>Allogona</u> measures approximately 18 mm in
length. The measurement is made between the exit of the small intes-
tine from the digestive gland and the anus. The <u>Haplotrema</u> rectum is
somewhat shorter, 10-15 mm.

The mid-rectal epithelium of both species is composed of low
columnar cells (25μ in height) which line 25 to 30 low longitudinal
folds. These cells possess cilia 5μ in length. Numerous mucous cells

are located in the epithelium giving the epithelium a vesicular
appearance. When filled with secretion products, these mucous cells
are larger than the epithelial cells and are probably responsible for
the notable epithelial crowding. The muscle and connective tissue
components continue from the intestine without significant change.
The rectum is, however, closely associated with the heavy musculature
of the mantle.

IV. HISTOCHEMICAL OBSERVATIONS

Histochemical comparisons of the various regions of the digestive tract principally concerned the localizations of the following enzyme systems: alkaline phosphatase, acid phosphatase, non-specific esterase, E-600 resistant esterase, and aminopeptidase.

In evaluating histochemical activity between species, the following levels were distinguished: (-) negative, (--+) to (-+) slight or weak activity, (+) well localized activity, (++) high activity, (+++) very high activity with attendant diffusion. The comparative histochemical results are summarized in Table I.

Alkaline and Acid Phosphatase

Buccal Cavity

Allogona. Alkaline phosphatase activity in the supraradular epithelium was found in the apical portions of the epithelium and the striated border. The reaction was of low intensity (-+) (Plate 5, Figure 11). Acid phosphatase showed higher activity (+) especially well localized between the nucleus and striated border (Plate 10, Figure 30).

Haplotrema. Alkaline phosphatase activity was very high (+++) in the striated border, apical region, and around the nucleus. In the basement membrane lower activity (+) was observed (Plate 5, Figure 12). Acid phosphatase was decidedly lower (-+) (Plate 10, Figure 31).

Esophagus

Allogona. Alkaline phosphatase activity was generally low, being
(- to -+) in the basal areas of the epithelium surrounding the nuclei
(Plate 5, Figure 13). Acid phosphatase was also low (--+), being
localized just above the nuclei (Plate 10, Figure 32).

Haplotrema. Alkaline phosphatase reaction was very heavy (+++)
in the apical and striated border. In the basal region the activity
was light (-+) (Plate 5, Figure 14). Acid phosphatase activity was
similar to that of Allogona (- to -+).

Salivary Gland and Ducts

Allogona. Alkaline phosphatase activity was positive (+) in the
reticular cells (Plate 6, Figure 15). Acid phosphatase reactions
occurred in the nuclei and cytoplasm of both test and control sections,
indicating negative results. The luminal epithelium of the salivary
ducts showed very high activity (++) for alkaline phosphatase (Plate 9,
Figures 26 and 27) and acid phosphatase (+) (Plate 11, Figure 34).

Haplotrema. Alkaline phosphatase activity was positive (+) in
secreting stages near the duct lumen. Acid phosphatase activity for
the same cells was negative (-) (Plate 6, Figure 16). In the salivary
duct epithelium, the alkaline phosphatase activity was high (++) in-
cluding the basement membrane region (Plate 6, Figures 17 and 18).
Acid phosphatase activity was weak (-+).

Stomach

Allogona. Alkaline phosphatase was localized very strongly (+++)

in the basement membrane region and muscle. Apical activity in the
epithelium was comparatively negative (-) (Plate 7, Figure 19). Acid
phosphatase showed low activity (-+) in the muscle and connective
tissue (Plate 10, Figure 33).

Haplotrema. A very light (-+) alkaline phosphatase level in the
basement membrane and muscle regions, however, high activity (++) was
localized in the apical and striated borders of the epithelium
(Plate 7, Figure 20). Acid phosphatase was diffuse and weak (-+).

Digestive Gland

Allogona. The digestive cells, or secretory-resorptive cells,
showed moderate activity (+) at the tubule lumen for alkaline phos-
phatase with both Gomori and azo acid techniques. Very high alkaline
phosphatase activity (+++) was visualized in the cytoplasm of the
calcium cells by simultaneous coupling azo dye methods (Plate 7,
Figure 21). Azo acid phosphatase activity was less positive (+) and
heavily localized in the calcium cells. Digestive cells were (--+)
(Plate 11, Figure 36).

Haplotrema. Luminal activity in the digestive cells was (+) for
alkaline phosphatase and always somewhat heavier than Allogona
(Plate 7, Figure 19). Acid phosphatase was diffuse and weak (-+) in
the digestive cells. Calcium cells showed high (+++) activity for
alkaline phosphatase (Plate 7, Figure 22) and (++) activity for acid
phosphatase (Plate 11, Figure 37).

Intestine

Allogona. Alkaline phosphatase activity was high (++ to +++) in

the apical region of the epithelial cells, especially in the striated

border; mucous cells were negative. A slight reaction was noted in

the basement membrane region (-+) (Plate 8, Figures 23, 24, and 25).

Acid phosphatase followed the same pattern but was observed to be very

slight (--+) (Plate 10, Figure 33).

Haplotrema. Alkaline phosphatase (+++) activity was localized in

the apical striated border of the epithelium (Plate 9, Figures 28 and

29). Acid phosphatase activity was slight (-+) apically.

Rectum

Allogona. Alkaline phosphatase activity was high (+++) in the

muscle and connective tissue. The epithelial reaction was diffuse and

weak (--+) (Plate 12, Figure 38). Acid phosphatase activity was low

(-+) in the basement membrane and muscle and negative (-) in the

epithelium (Plate 12, Figure 40).

Haplotrema. The alkaline phosphatase reaction was (-+), being

heaviest in the apical cell region. The activity was diffuse in the

basal cytoplasm and basement membrane (Plate 12, Figure 39). Acid

phosphatase was negative (-).

Non-Specific and E-600 Resistant Esterase

Buccal Cavity

Allogona. Non-specific esterase activity was high (++ to +++)

in the apical and luminal regions of the supraradular epithelium
(Plate 13, Figure 41). High activity was also noted in the subradular
and cavity floor epithelium. E-600 resistant activity was (+) in the
apical region, but absent from the lumen and striated border (Plate 13,
Figure 42).

Haplotrema. Non-specific esterase was usually heavier than in
Allogona, being highly concentrated at the striated border and lumen
(+++) (Plate 13, Figure 43). E-600 resistant activity persisted in
the apical region but was absent at the lumen (Plate 13, Figure 44).

Esophagus

Allogona. The moderate (+) non-specific esterase activity was
localized in the apical portion of the epithelium (Plate 14, Figure 45).
The Allogona esophagus was negative (-) for E-600 resistant activity
(Plate 14, Figure 46).

Haplotrema. High non-specific esterase activity (+++) was
diffused throughout the epithelium (Plate 14, Figure 47). E-600
treatment left (-+) activity in the apical area (Plate 14, Figure 48).

Salivary Gland and Ducts

Allogona. The hematoxylin-staining reticular cells showed low
non-specific esterase activity (--+ to -+). All other areas of the
gland were negative. E-600 treatment did not significantly reduce
this activity (Plate 15, Figures 49 and 50). The salivary duct
epithelium external to the gland showed weakly diffuse esterase
activity (--+) which was negative (-) following E-600 treatment.

Haplotrema. The non-specific esterase activity in the reticular cells showed higher activity than Allogona (+) and E-600 treatment did not appreciably reduce the stain intensity (Plate 15, Figures 51 and 52). The salivary duct epithelium was weakly positive outside the gland (−+). E-600 treatment caused the loss of all esterase activity in the salivary ducts.

Stomach

Allogona. A positive non-specific esterase reaction with some diffusion (++) was heaviest in the apical region and the striated border. Some activity (−+) was visualized in the region of the base-ment membrane. E-600 resistant activity was negative (−) in Allogona (Plate 16, Figures 53 and 54).

Haplotrema. Non-specific esterase activity was similar to that of Allogona described above (++), however, the E-600 resistant activity was well localized in the lower apical regions of the epithelium; no E-600 resistant activity was found near the basement membrane (Plate 16 Figures 55 and 56).

Digestive Gland

Allogona. Non-specific esterase activity was high (++) in the digestive cells and negative in calcium cells. The E-600 resistant esterase activity was weaker (−+) and localized primarily at the lumen in the digestive cells (Plate 17, Figures 57 and 58).

Haplotrema. The non-specific esterase activity was equal to that of Allogona (++) for the digestive cells. Calcium cells were negative.

Treatment with E-600 resulted in some loss of cytoplasmic activity but not as marked as in Allogona (Plate 17, Figures 59 and 60).

Intestinal Loop

Allogona. Non-specific esterase was localized principally in the striated border (+) with some diffused activity throughout the cytoplasm (--+). Treatment with E-600 resulted in negative activity (Plate 18, Figures 61 and 62).

Haplotrema. High non-specific esterase activity (+++) was visualized in the apical and striated border regions. E-600 resistant esterase activity remained moderately positive (+) (Plate 18, Figures 63 and 64).

Rectum

Allogona. Positive (+) non-specific esterase activity was located in the apical border of the rectal epithelium. E-600 treatment, however, completely inhibited the reaction (Plate 19, Figures 65 and 66).

Haplotrema. Non-specific esterase activity was higher than in Allogona (+ to ++). In addition to apical activity, some reaction was noted in the connective tissue and near the basement membrane. E-600 treatment reduced the activity by about one-half (Plate 19, Figures 67 and 68).

Aminopeptidase

Buccal Cavity

Allogona. Negative (Plate 20, Figure 69).

Haplotrema. A strong (++) reaction was evident in all regions of the buccal epithelium (Plate 20, Figure 70).

Esophagus

Allogona. Negative (Plate 20, Figure 71).

Haplotrema. Weak (--+ to -+) activity was diffused throughout the cell cytoplasm (Plate 20, Figure 72).

Salivary Gland and Ducts

Allogona. Salivary gland cells were negative; however, the salivary duct epithelium external to the gland showed very low activity (--+) (Plate 21, Figure 73).

Haplotrema. Salivary gland cells showed negative activity and the salivary duct epithelium was weakly positive (--+) similar to Allogona.

Stomach

Allogona. Negative (Plate 21, Figure 74).

Haplotrema. Very weak, diffuse aminopeptidase activity (--+) was distributed throughout the epithelium (Plate 21, Figure 75).

Digestive Gland

Allogona. Very slight activity (--+) was evident in digestive cell cytoplasm (Plate 22, Figures 76 and 77) and calcium cells were negative.

Haplotrema. Very high positive activity (+++) was observed in digestive cells. Great diffusion occurred in long incubations (Plate 22, Figures 78 and 79). Due to this diffusion it was difficult to observe the calcium cells.

Intestinal Loop

Allogona. Well localized positive activity (++) was observed in intestinal epithelium in sharp contrast to the (--+) digestive gland reaction. The greatest reaction was localized in the apical cell region (Plate 22, Figures 76 and 77).

Haplotrema. Generally a higher activity was present than in Allogona (+++) with much more diffusion (Plate 22, Figures 78 and 79).

Rectum

Allogona. Low, diffuse epithelial activity was observed (--+) without concentrated localization (Plate 23, Figure 80).

Haplotrema. Activity was somewhat higher than in Allogona (-+) and weakly diffuse throughout the cytoplasm (Plate 23, Figure 81).

Oil Red O

Buccal Cavity

Allogona. A light, diffuse stain (- to --+) was observed in the
buccal epithelium characterized by fine droplets which became larger
at the beginning of the esophagus (Plate 23, Figure 82).

Haplotrema. The reaction was generally greater than in Allogona
with very fine droplets appearing in the apical regions (-+) (Plate 23,
Figure 83).

Esophagus

Allogona. Increasing numbers and size of oil red O positive
droplets occurs as the esophagus leaves the buccal mass. A very abun-
dant reaction with large droplets in both apical and basal regions
(+++) was observed in the pre-esophagus. In the mid-esophagus the
cells on the crests of the folds have conspicuously fewer positive
droplets. This condition continues into the stomach in both species.
(Plate 24, Figure 84).

Haplotrema. Small positive droplets appear in the apical area.
The stain (+) is only slightly more intense than that of the buccal
epithelium, but well defined (Plate 24, Figure 85).

Salivary Gland and Ducts

Allogona and Haplotrema both showed essentially negative reactions
for oil red O in the glandular secreting cells. A few fine positive
droplets were associated with the connective tissues. The salivary

ducts displayed a light (--+) epithelial reaction in both species (Plate 24, Figures 86 and 87).

Stomach

Allogona. Little apical reaction occurred in the longitudinal folds. Large positive (++) droplets were found in both apical and basal epithelial regions (Plate 25, Figure 88).

Haplotrema. A low basal concentration (--+) of fine positive droplets was observed. The apical reaction was also low, but showed slightly larger granules (Plate 25, Figure 89).

Digestive Gland

Allogona. Medium to large positive droplets were concentrated in the basal regions of the digestive cells (+++). The luminal tips of the digestive cells showed little reaction. Calcium cells were negative, however, excretory cells (degenerating calcium cells) showed some peripheral reaction (Plate 25, Figure 90).

Haplotrema. Positive droplets were sparce throughout the digestive cells (+); the basal regions contained the largest droplets. Again the excretory cells showed a peripheral reaction (Plate 25, Figure 91).

Intestinal Loop

Allogona. The basal region of the cells showed some reaction (--+) similar to the buccal epithelium (Plate 26, Figure 92). No activity was noted in the mucous cells for either species.

<u>Haplotrema</u>. A slight apical (--+) reaction and a very low basal concentration was observed similar to <u>Allogona</u> (Plate 26, Figure 93).

Rectum

Some very slight positive reaction could be recognized; however, the rectal epithelium for both species was comparatively low (--+ to -) (Plate 26, Figures 94 and 95).

TABLE I. COMPARATIVE SUMMARY OF HISTOCHEMICAL RESULTS

Allogona townsendiana

Organ	Alkaline Phosphatase	Acid Phosphatase	Non-specific Esterase	Amino-peptidase	Oil Red O
Buccal	-+	+	++	-	--+
Esophagus	--+	(-)-+	+(+)	-	+++
Sal. Gld.	+	-	(-)-+	-	-
Sal. Duct	++	+	--+	--+	--+
Stomach	+++ ct mus	--+	++	-	++
Dig. Gld.	+ dig +++ ca	--+ dig + ca	++ dig	--+ dig	+++ dig
Int. Loop	+ apical	-+	+	++	--+
Rectum	- + ct mus	-	+	--+	--+

Haplotrema vancouverense

Organ	Alkaline Phosphatase	Acid Phosphatase	Non-specific Esterase	Amino-peptidase	Oil Red O
Buccal	+++	-+	++	++	-+
Esophagus	+++ apical	(-)-+	+++	(-)-+	+
Sal. Gld.	+	-	+	-	-
Sal. Duct	++	-+	-+	--+	--+
Stomach	++ apical	-+	++	--+	-+
Dig. Gld.	+++ dig +++ ca	--+ dig ++ ca	++ dig	+++ dig	+ dig
Int. Loop	+++ apical	-+	+++	+++	--+
Rectum	-+	-	+(+)	-+	--+

```
   -   negative                            ca, calcium cell
 --+  to  -+  slight or weak activity      ct, connective tissue
   +   well localized activity             dig, digestive gland
  ++   high activity                       mus, muscle
 +++   very high activity with diffusion
```

V. DISCUSSION

As early as 1880, Barfurth published a review of the histophysiology of the gastropod "hepatopancreas." Since that time, a growing interest in the digestive physiology of Mollusca has stimulated extensive research. In 1953, Guardabassi and Ferreri summarized the histological and histochemical literature and later, in 1962, Arvy reviewed the most recent histochemical findings.

Reports of histochemical enzyme localizations have not been without contradiction. Many of the problems apparently stem from individual differences even among closely related species. Yet another problem in interpretation has been created by previous lack of efficient tissue preparation techniques and highly specific substrates and inhibitors.

Histologically, the digestive tracts of the herbivorous Allogona and the carnivorous Haplotrema show no major differences. The modification of the radula in Haplotrema, however, is very striking. In contrast to Allogona, the carnivorous radula is deeply grooved medially and contains fewer rows of long needle-sharp teeth. The convex radula of the herbivorous Allogona is composed of small, close-set teeth. Baker (1930) held that the crescentic jaw and central radula of Haplotrema are clearly vestigial and that the general anatomy suggests a close relationship to the Streptaxidae and Rhytididae. Differences in the chromosome caryotypes (Ford, 1962) indicate a significant cytological divergence of the families.

In general, gastropods show alkaline phosphatase activity along the entire digestive tract. Very high activity has been reported

along the intestinal basement membrane and in the musculature

(Guardabassi and Ferreri, 1953). Allogona fits this pattern in that

the stomach showed the greatest basal activity (a very light apical

reaction) but the intestinal loop region always had high apical

activity with the basal areas being only moderate. On the other hand,

in Haplotrema alkaline phosphatase activity was always highest in the

apical epithelium of both stomach and loop intestine. Other major

differences in activity were noted in the buccal epithelium (+++ in

Haplotrema, -+ in Allogona) and the mid-esophagus (+++ apical in

Haplotrema, --+ in Allogona). In the rectum, activity for both

species decreased, activity being limited to the connective tissue and

muscle in Allogona. Mucous cells were negative for alkaline phospha-

tase in both tracts.

Alkaline phosphatase results in the digestive gland agree with

those of Rosenbaum and Ditzion (1963). The digestive cells of

Haplotrema were somewhat more reactive. The presence of alkaline

phosphatase in the cytoplasm of calcium cells is also supported by

the work of Muller (1965) who worked on the schistosome bearing snail

Australorbis glabratus.

It is interesting to note that the salivary duct epithelium shows

more alkaline phosphatase activity than the secreting cells of the

gland. In all cases the duct became intensely positive as the

epithelium rose to columnar height. This increased reaction might

be associated with the metabolism of ciliary activity.

Acid phosphatase activity was considerably weaker in activity

throughout the digestive tract. Highest activity was observed in the

calcium cells of the digestive glands in both Allogona and Haplotrema.
Sumner (1965) suggested a secretory function for the calcium cells in
Helix by demonstrating a high RNA and Golgi apparatus concentration in
the cytoplasm. Barka (1962), in making an analysis of the localization
of acid phosphatase activity in cell structures, reported this enzyme
to be associated with pinocytotic and secretory (reversed pinocytosis)
processes. Birns (1960) also found acid phosphatase activity in the
pinocytotic vacuoles in Chaos chaos. Rosenbaum and Ditzion (1963)
showed an increase in acid phosphatase activity in the calcium cells
of feeding Helix. With regard to acid phosphatase in mucous secreting
goblet cells, Muller (1965) takes exception to Zacks' (1955) report
of negative activity in A. glabratus. Wagge (1951) also reported
negative acid phosphatase activity for the mucous cells of Helix
aspersa. Allogona and Haplotrema were negative.

The most widespread enzyme activity was observed for non-specific
esterase. Three major groups of enzymes are capable of hydrolyzing
the substrate alpha-naphthyl acetate, A-esterase, B-esterase and
C-esterase. The A-esterases hydrolyze acetates at a faster rate than
the longer chain types while B-esterases hydrolyze butyrates at an
equal rate or faster than acetates (Pearse, 1960). C-esterases
hydrolyze indoxyl acetates and may represent cathepsin activity. It
is difficult to separate the activities of these somewhat overlapping
enzymes. However, Pearse (1960) and Shnitka and Seligman (1961) have
recommended the use of the organophosphate inhibitor E-600 (diethy-p-
nitrophenyl phosphate) for inhibition of B-esterase activity which
may include lipase. A-esterases are not inhibited at 10^{-8} M.

Cholinesterase is also capable of hydrolyzing alpha-naphthyl acetate and therefore must be considered as a possible component of the E-600 sensitive esterase activity.

The principal separation using E-600 inhibition was therefore A-esterase (and possibly C-esterase) as resistant enzymes from B-esterase (lipase).

Most segments of the digestive tract except the calcium and mucous cells showed non-specific esterase activity. The hematoxylin positive reticular cells of the Haplotrema salivary glands showed somewhat more activity than those of Allogona. Treatment with E-600 did not appreciably reduce the activity in the salivary gland cells of either species, but did cause a general reduction of reaction in all other tissues. It is suggested that the primary enzyme of the salivary glands is of the A-esterase (perhaps including C-esterase) type in both species.

Marked differences between the species were observed in the esophagus and intestinal loop; the activity in Haplotrema was always more intense. E-600 treatment caused complete inhibition of esterase activity in the esophagus, stomach and rectum of Allogona. A very slight reaction remained in the intestinal loop. E-600 treatment in Haplotrema did not cause complete inhibition in any tissues studied. In comparison to Allogona, generally higher levels of resistant activity were noted for the esophagus, stomach, intestine and rectum.

Since the greatest loss of activity was at the lumen and striated border of the digestive tract, B-esterase and perhaps lipase activity is indicated in these regions. The loss of activity in the digestive

glands corresponds to the general area of the yellow granules previously reported for Helix by Rosenbaum and Ditzion (1963).

Results of the oil red O tests for distribution of general lipid showed Allogona to have comparatively more lipid in all areas of the digestive tract. The greater amounts of lipid localized in the digestive tract epithelia of Allogona suggest that fat digestion and absorption occurs more rapidly and intensely than in Haplotrema. In short term starved animals, reduction of the number and size of lipid droplets occurred throughout the digestive tract in both species. The greatest loss was observed in the esophagus and in the basal regions of the digestive cells. This agrees with the findings of Sumner (1965) for Helix pomatia.

No specific correlation between lipid absorption and E-600 labile esterase could be made. In general, E-600 appeared to have a greater effect on the esterase activity in Allogona than in Haplotrema.

Localizations of aminopeptidase activity showed the greatest variation between the herbivorous and carnivorous species. Differences were observed in both site and intensity of reaction. Allogona was essentially negative for aminopeptidase activity in the buccal, esophageal, and stomach epithelia as well as the salivary gland cells. Haplotrema, on the other hand, showed high aminopeptidase activity in the buccal epithelium, digestive cells, and loop intestine. The epithelium of the esophagus, stomach and rectum showed slight (--+ to -+) activity in Haplotrema, but no or only a trace of activity in Allogona. The salivary duct epithelium showed slight (--+) activity in both species.

Striking differences in aminopeptidase activity were observed in the digestive gland, the digestive cells of Haplotrema being extremely active (+++) as compared to Allogona (--+). The intestinal loop epithelium showed high activity in both species. In Allogona a very sharp contrast was evident between the digestive gland (--+) and the embedded intestinal loop (++). The digestive gland and intestinal epithelium showed equally high activity (+++) in Haplotrema. Rosenbaum and Ditzion (1963) were able to demonstrate some slight aminopeptidase activity in the digestive cells of H. pomatia in both starved and feeding animals, but no activity was observed in calcium cells. In the case of Haplotrema, extended incubation caused great diffusion throughout the digestive gland, connective tissue and musculature. Therefore it was difficult to ascertain that calcium cells were entirely negative.

Van Weel (1959, 1960) demonstrated that the midgut gland of gastropods produces carbohydrases, proteases (including extracellular peptidases and dipeptidases), and esterases; however the amount appears to depend on the natural diet and the amount of food available. In working with the Giant African Snail (carnivorous when sexually mature) van Weel (1960) noted, however, that the enzyme production did not show a "fitting" adaption to a change in diet. The snails fed on starch produced more proteinase than did those that were fed protein.

Both the herbivorous and carnivorous snails apparently have the capacities for producing the enzymes studied. This is consistant with the biochemical observations of Thirlwell et al. (1963) who compared the digestive juices of lettuce fed Helix pomatia

(herbivorous) and _Achatina_ _fulica_ (carnivorous). The levels and over-all pattern of hydrolytic enzymes in _Achatina_ were shown to compare favorably to those of _Helix_. Differences occur primarily in the amount and distribution of both digestive enzymes and those enzymes associated with cellular metabolism (alkaline and acid phosphatase).

VI. SUMMARY

1. The digestive tract of a herbivorous terrestrial snail
   (<u>Allogona</u> <u>townsendiana</u>, Lea) was compared by both histo-
   chemical methods to a carnivorous species (<u>Haplotrema</u>
   <u>vancouverense</u>, Lea) with which it shares the same habitat.

2. The distributions of the following histochemical reactions
   were described for each species: alkaline phosphatase, acid
   phosphatase, non-specific esterase, E-600 resistant esterase,
   aminopeptidase, and general lipid.

3. Differences in distribution of alkaline phosphatase occurred
   in the buccal cavity, esophagus, stomach and rectum. <u>Allogona</u>
   showed high activity in the musculature and connective tissue
   of the stomach and rectum while <u>Haplotrema</u> had high apical
   activity in these areas.

4. Both species showed well localized apical activity for
   alkaline phosphatase in the intestinal epithelium.

5. The cytoplasm of the calcium cells of the digestive gland
   showed high activity for both alkaline and acid phosphatase
   in both species. Digestive cells were only moderately
   positive at the tubule lumen for alkaline phosphatase and
   very low for acid phosphatase.

6. Acid phosphatase activity was in general lower than that of
   alkaline phosphatase with the exception of the <u>Haplotrema</u>
   buccal epithelium, which showed well localized supranuclear
   activity.

7. <u>Haplotrema</u> showed higher non-specific esterase activity throughout the digestive tract. The greatest difference between the species was observed in the esophagus and intestinal loop; activity in the epithelia of these organs was more intense in <u>Haplotrema</u>.

8. E-600 inhibition completely abolished the non-specific esterase activity in the esophagus, stomach and rectum of <u>Allogona</u>. Complete inhibition of esterase activity was not demonstrated in any tissue in <u>Haplotrema</u>, although reduced reactions were observed.

9. The salivary gland cells of both species showed non-specific esterase activity, which was not inhibited by treatment with 10^{-5} M E-600. It was suggested that A-esterase and perhaps C-esterase are the most active enzymes in this gland.

10. The most striking differences in enzyme concentration and localization was observed for aminopeptidase. <u>Haplotrema</u> showed very high activity in the buccal epithelium, digestive gland and intestinal loop. <u>Allogona</u> showed high activity only in the intestinal loop. The localization and intensity of aminopeptidase appeared to be correlated with the final stages of protein digestion.

11. General lipid staining was considerably higher in all regions of the <u>Allogona</u> digestive tract. No reaction was observed in the salivary gland cells for either species. The results suggest that lipid digestion and absorption occur more rapidly and intensely in <u>Allogona</u>.

12. The distribution and concentration of the enzymes studied
    were considered in relationship to the natural diets of the
    two species.

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APPENDIX

APPENDIX

PLATE ABBREVIATIONS

A	anus
Buc	buccal mass
C	calcium cell
Cr	crop (mid-esophagus)
D	digestive cell
E	eosinophilic cells
Dig Gld	digestive gland
Eso	esophagus
H	hematoxylin staining cells
Int	intestine
L	lumen
M	muscle
Rec	rectum
Sal Duct	salivary duct
Sal Gld	salivary gland
St	stomach

Plate 1

Figure 1. A simplified diagram of the digestive tract of
Helix pomatia (redrawn after Cooke, 1927).

Plate 2

Figure 2.   <u>Allogona townsendiana</u>, Lea is pictured at top
            left. The shell is brown with lighter striae.
            <u>Haplotrema vancouverense</u>, Lea is pictured at
            lower right. The shell is light greenish-
            yellow. About natural size.

Figure 3.   <u>Allogona</u>. A dissection of the anterior seg-
            ment of the digestive tract including buccal
            mass and stomach. The salivary glands are
            united above the mid-esophagus. X 2.

Figure 4.   <u>Haplotrema</u>. A dissection of the anterior
            segment of the digestive tract including
            buccal mass and stomach. Note the doughnut-
            shaped salivary gland surrounding the mid-
            esophagus. X 2.

2

−Sal Gld

3

+Sal Gld

4

Plate 3

Figure 5. Esophagus of Allogona anterior to the salivary
           gland. Harris' hematoxylin and eosin. X 50.

Figure 6. Esophagus of Haplotrema anterior to the sali-
           vary gland. Harris' hematoxylin and eosin.
           X 50.

Figure 7. Salivary gland and ducts of Allogona. Harris'
           hematoxylin and eosin. Cell types include
           hematoxylin staining (H) and eosinophylic (E).
           X 200.

Figure 8. Intestinal loop epithelium of Allogona showing
           eosinophilic and hematoxylin-staining cells.
           X 800.

Plate 4

Figure 9. Cross section of a digestive gland tubule
            of Allogona. Harris' hematoxylin and eosin.
            X 200.

Figure 10. Cross section of a digestive gland tubule
            of Haplotrema. Harris' hematoxylin and
            eosin. X 200.

Plate 6

Figure 15. Salivary gland of _Allogona_ stained for alka-
line phosphatase (Gomori). Hematoxylin-
staining cells (H) show a positive reaction.
X 200.

Figure 16. Salivary gland of _Haplotrema_ stained for
alkaline phosphatase (Gomori). X 200.

Figure 17. Salivary duct of _Haplotrema_ stained for
alkaline phosphatase (Gomori). Note the
high luminal activity in both species.
X 200.

Figure 18. Salivary duct of _Haplotrema_ with substrate
omitted from alkaline phosphatase reaction
(Gomori). X 200.

11

12

13

14

Plate 6

Figure 15. Salivary gland of _Allogona_ stained for alkaline phosphatase (Gomori). Hematoxylin-staining cells (H) show a positive reaction. X 200.

Figure 16. Salivary gland of _Haplotrema_ stained for alkaline phosphatase (Gomori). X 200.

Figure 17. Salivary duct of _Haplotrema_ stained for alkaline phosphatase (Gomori). Note the high luminal activity in both species. X 200.

Figure 18. Salivary duct of _Haplotrema_ with substrate omitted from alkaline phosphatase reaction (Gomori). X 200.

<u>Plate 7</u>

Figure 19.   Stomach of <u>Allogona</u> stained for alkaline
phosphatase (azo). Note the extremely high
activity in the muscle. X 300.

Figure 20.   Stomach of <u>Haplotrema</u> stained for alkaline
phosphatase (azo). Note the high apical
activity in contrast to <u>Allogona</u>. X 300.

Figure 21.   Digestive gland of <u>Allogona</u> stained for alka-
line phosphatase (azo). Heavy staining in
the calcium cells (C) with lighter, more
diffuse activity in the digestive cells (D).
X 1000.

Figure 22.   Digestive gland of <u>Haplotrema</u> stained for
alkaline phosphatase (azo). Similar to
<u>Allogona</u> but higher luminal activity in the
digestive cells (D). X 1000.

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D

D

C

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C

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Plate 9

Figure 26. Salivary duct of _Allogona_ showing extremely
high luminal activity for alkaline phospha-
tase (Gomori). X 800.

Figure 27. Salivary duct of _Allogona_ with substrate
omitted from the alkaline phosphatase
reaction (Gomori). X 800.

Figure 28. Intestine of _Haplotrema_ showing high apical
activity for alkaline phosphatase (Gomori).
X 200.

Figure 29. Intestine of _Haplotrema_ showing false-
positive nuclear activity for alkaline
phosphatase (Gomori) in the control. X 200.

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Plate 11

Figure 34.   Salivary duct of _Allogona_ showing high luminal
             activity for acid phosphatase (azo). X 1000.

Figure 35.   Intestine of _Allogona_ showing weak supranuclear
             activity for acid phosphatase (azo). X 200.

Figure 36.   Digestive gland of _Allogona_ showing acid phos-
             phatase (azo) activity to be localized in the
             calcium cells (C) with only a slight reaction
             in the granular components of the digestive
             cells (D). The intensity of the reaction is
             greater than is indicated in the color repro-
             duction. X 200.

Figure 37.   Digestive gland of _Haplotrema_ showing a distri-
             bution of acid phosphatase (azo) similar to
             Allogona (Figure 3). X 1000.

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Plate 12

Figure 38.   Rectum of Allogona stained for alkaline
             phosphatase (Gomori). High activity is
             seen in the muscle layer (M). X 800.

Figure 39.   Rectum of Haplotrema stained for alkaline
             phosphatase (Gomori). A higher apical
             reaction than observed in Allogona but
             little muscle activity. X 800.

Figure 40.   Rectum of Allogona stained for acid
             phosphatase (Gomori). Note the low
             basal activity. X 200.

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Plate 13

Figure 41.   Buccal epithelium of *Allogona* showing high
             non-specific esterase activity in the apical
             and striated border regions. X 800.

Figure 42.   Buccal epithelium of *Allogona* showing the
             complete loss of esterase activity in the
             striated border following treatment with
             E-600. X 400.

Figure 43.   Buccal epithelium of *Haplotrema* showing a
             more intense non-specific esterase activity
             than seen in Allogona. X 800.

Figure 44.   Buccal epithelium of *Haplotrema* showing the
             complete loss of esterase reaction in the
             striated border following treatment with
             E-600. X 800.

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Plate 14

Figure 45.   Esophagus of Allogona showing a finely
              particulate distribution of non-specific
              esterase in the apical cell region.   X 200.

Figure 46.   Esophagus of Allogona showing the complete
              inhibition of esterase activity.   X 200.

Figure 47.   Esophagus of Haplotrema showing a higher
              non-specific esterase reaction than seen
              in Allogona.   X 200.

Figure 48.   Esophagus of Haplotrema showing the esterase
              activity remaining after treatment with E-600.
              X 200.

Plate 15

Figure 49. Salivary gland of Allogona showing non-specific
            esterase activity in the hematoxylin-staining
            reticular cells (H). X 200.

Figure 50. Salivary gland of Allogona showing no apparent
            loss of esterase activity following E-600
            treatment. X 200.

Figure 51. Salivary gland of Haplotrema showing an intense
            non-specific esterase activity in the hema-
            toxylin-staining reticular cells (H). X 200.

Figure 52. Salivary gland of Haplotrema showing no
            observable loss in esterase activity after
            E-600 treatment. X 200.

Plate 16

Figure 53. Stomach of Allogona showing a diffuse apical
            non-specific esterase activity. X 200.

Figure 54. Stomach of Allogona showing the complete
            inhibition of esterase activity following
            E-600 treatment. X 200.

Figure 55. Stomach of Haplotrema showing non-specific
            esterase activity localized below the striated
            border. X 200.

Figure 56. Stomach of Haplotrema showing no significant
            loss of esterase activity following E-600
            treatment. X 200.

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Plate 17

Figure 57.   Digestive gland of <u>Allogona</u> showing diffuse
             non-specific activity in the digestive cells
             (D) near the lumen; calcium cells are negative.
             X 200.

Figure 58.   Digestive gland of <u>Allogona</u> showing reduced
             luminal esterase activity in the digestive
             cells following E-600 treatment. Activity
             remains in the granular components. X 200.

Figure 59.   Digestive gland of <u>Haplotrema</u> stained for
             non-specific esterase. Similar to <u>Allogona</u>.
             X 200.

Figure 60.   Digestive gland of <u>Haplotrema</u> showing a loss
             of general cytoplasmic esterase reaction
             following E-600 treatment. Activity is
             localized in granular components. X 180.

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Plate 18

Figure 61. Intestinal loop of <u>Allogona</u> showing a very low
apical activity for non-specific esterase.
X 200.

Figure 62. Intestinal loop of <u>Allogona</u> showing complete
inhibition of esterase activity following
treatment with E-600. X 200.

Figure 63. Intestinal loop of <u>Haplotrema</u> showing a high
apical non-specific esterase reaction. Mucous
cells are negative. X 200.

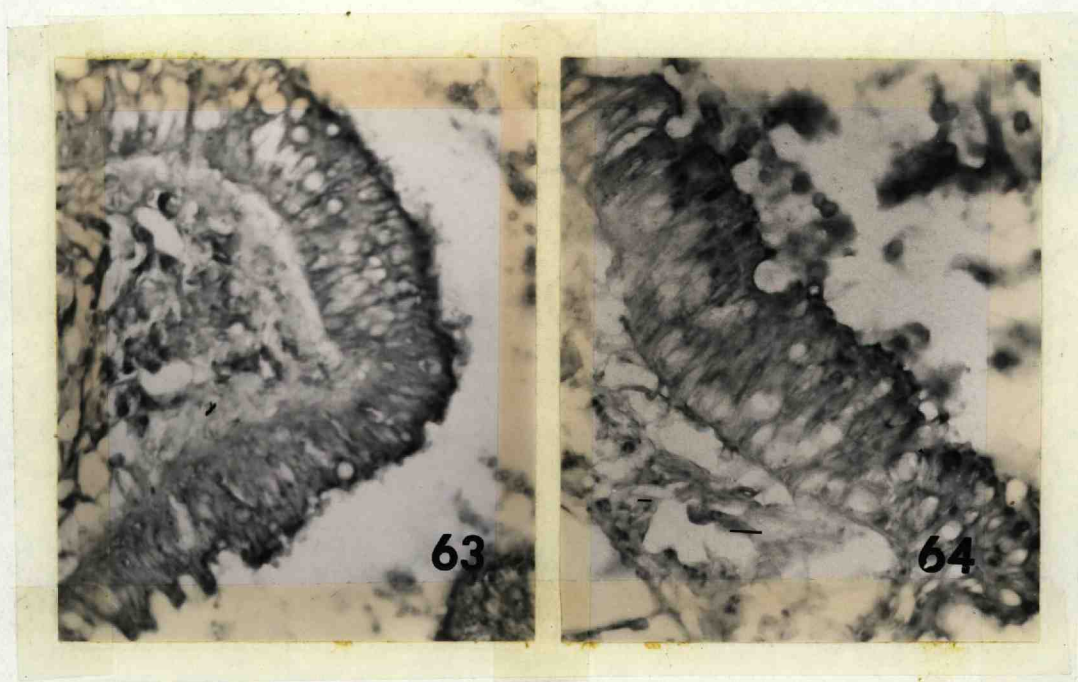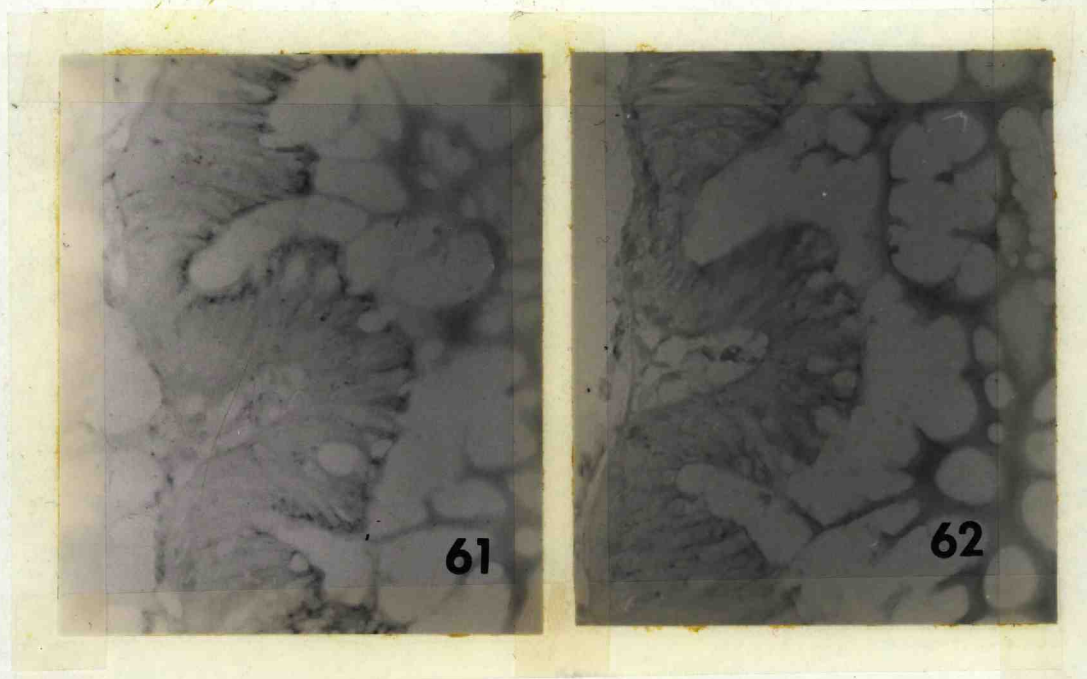Figure 64. Intestinal loop of <u>Haplotrema</u> showing some
E-600 resistant apical activity. X 200.

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Plate 19

Figure 65. Rectum of _Allogona_ showing low apical non-
           specific esterase activity. X 200.

Figure 66. Rectum of _Allogona_ showing complete esterase
           inhibition following E-600 treatment. X 200.

Figure 67. Rectum of _Haplotrema_ showing low apical non-
           specific esterase activity. X 200.

Figure 68. Rectum of _Haplotrema_ showing only slightly
           reduced activity following E-600 treatment.
           X 200.

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Plate 20

Figure 69. Buccal epithelium of *Allogona* showing a nega-
tive aminopeptidase reaction. X 1000.

Figure 70. Buccal epithelium of *Haplotrema* showing a
very high luminal aminopeptidase activity.
X 1000.

Figure 71. Esophagus of *Allogona* showing a negative
aminopeptidase reaction. X 300.

Figure 72. Esophagus of *Haplotrema* showing a weak,
diffuse activity for aminopeptidase. X 300.

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Plate 21

Figure 73.   Salivary duct of _Allogona_ showing weak,
             diffuse aminopeptidase activity. X 1000.

Figure 74.   Stomach of _Allogona_ showing negative amino-
             peptidase activity. X 1000.

Figure 75.   Stomach of _Haplotrema_ showing weakly positive
             aminopeptidase reaction. X 1000.

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Plate 22

Figure 76. Intestinal loop and digestive gland of _Allogona_
showing a moderately high apical aminopeptidase
activity. X 300.

Figure 77. Intestinal loop and digestive gland of _Allogona_.
Aminopeptidase control. X 300.

Figure 78. Intestinal loop and digestive gland of _Haplotrema_
showing high aminopeptidase activity in both
tissues. X 300.

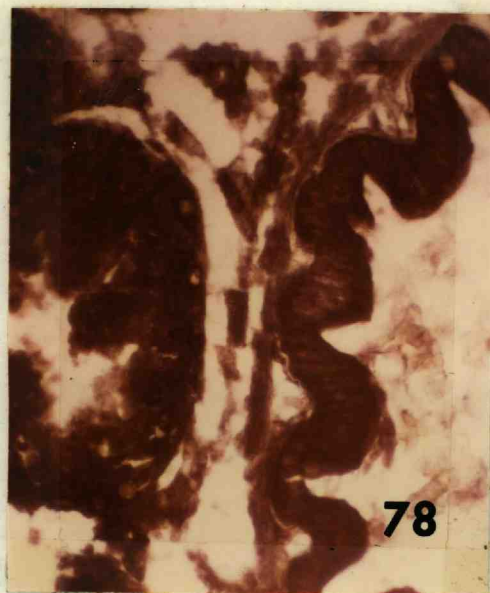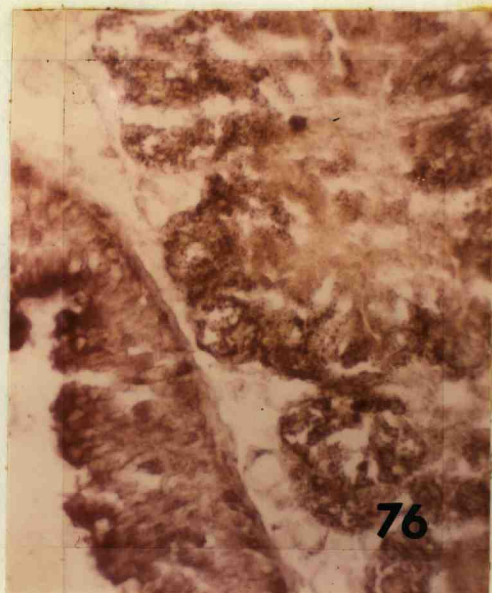Figure 79. Intestinal loop and digestive gland of _Haplotrema_.
Aminopeptidase control. X 300.

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Plate 23

Figure 80. Rectum of Allogona showing a very light,
            diffuse aminopeptidase reaction. X 300.

Figure 81. Rectum of Haplotrema showing a diffuse,
            moderately positive reaction for amino-
            peptidase. X 300.

Figure 82. Buccal epithelium of Allogona showing the
            diffuse distribution of small oil red 0
            positive droplets. X 1000.

Figure 83. Buccal epithelium of Haplotrema showing a
            greater apical concentration of oil red 0
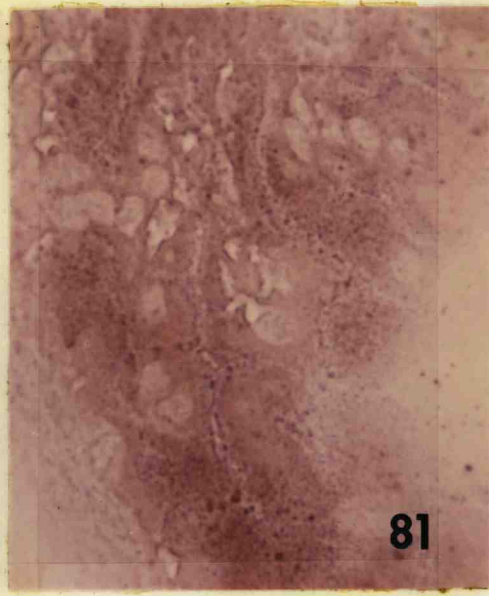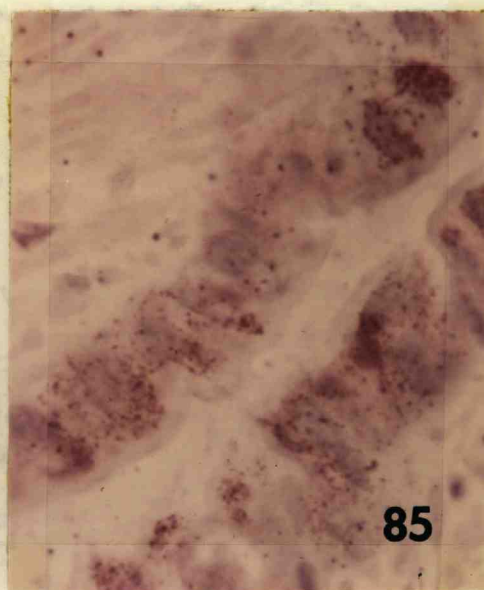   droplets than seen in Allogona. X 1000.

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<u>Plate 24</u>

Figure 84.   Esophagus of <u>Allogona</u> showing a high concen-
tration of positive oil red 0 droplets dis-
tributed throughout the cytoplasm. X 300.

Figure 85.   Esophagus of <u>Haplotrema</u> showing fine apical
oil red 0 positive droplets. X 1000.

Figure 86.   Salivary duct of <u>Allogona</u> showing a weakly
diffuse reaction for general lipid with
oil red 0. X 1000.

Figure 87.   Salivary duct of <u>Haplotrema</u> showing a weak
oil red 0 reaction similar to <u>Allogona</u>
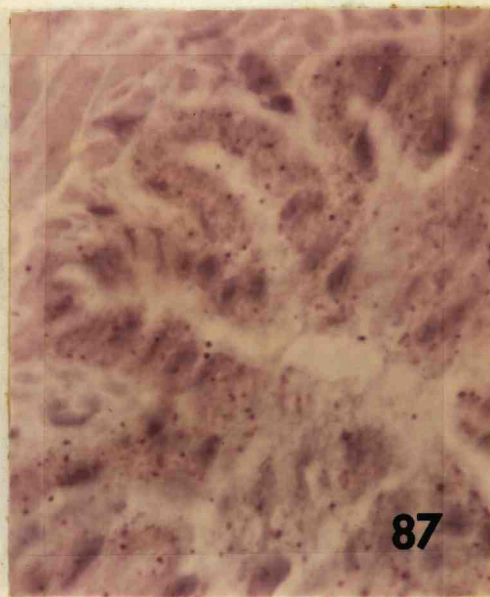(Figure 86). X 1000.

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Plate 25

Figure 88. Stomach of *Allogona* showing large oil red O
            positive droplets in the apical and basal
            cell regions. X 1000.

Figure 89. Stomach of *Haplotrema* showing a low concen-
            tration of fine oil red O positive droplets.
            X 1000.

Figure 90. Digestive gland of *Allogona* showing a high
            basal concentration of large oil red O
            droplets. Note the positive droplets in
            the degenerating calcium cells (C). X 1000.

Figure 91. Digestive gland of *Haplotrema* showing fewer
            oil red O positive droplets than in *Allogona*
            (Figure 90). X 1000.
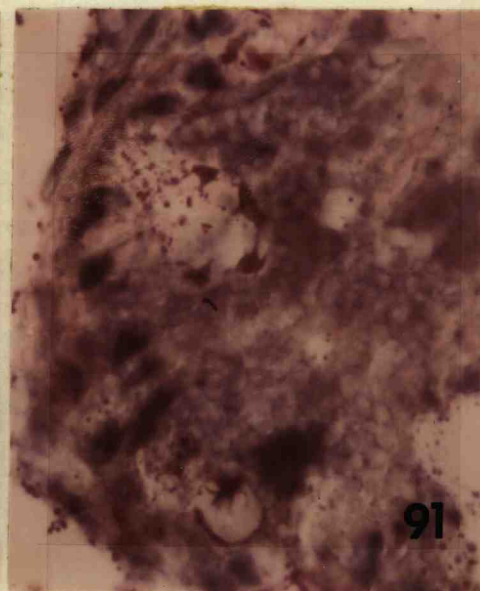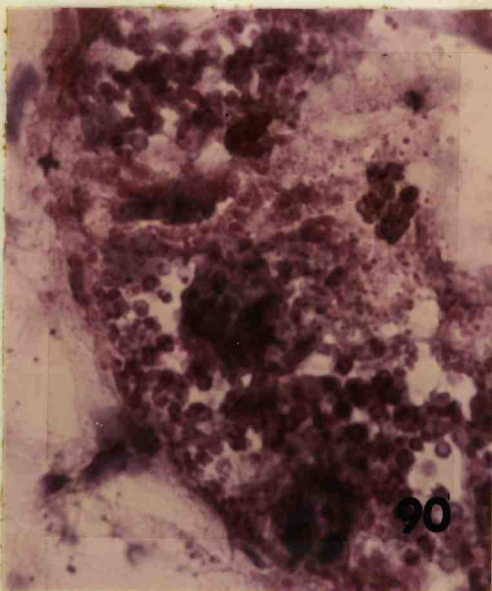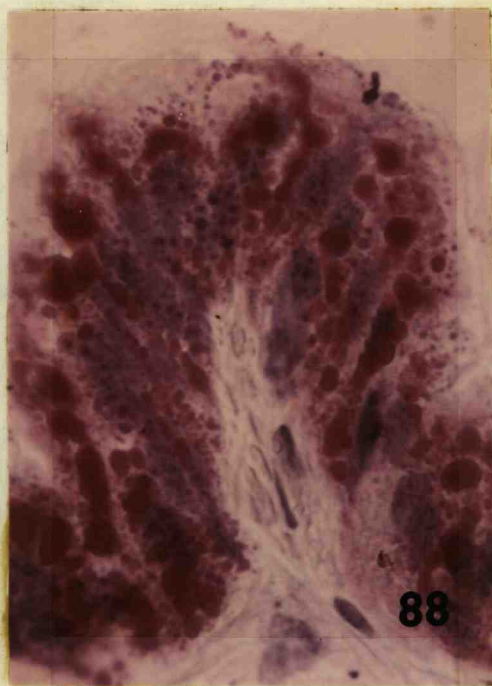
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Plate 26

Figure 92. Intestinal loop of Allogona showing very
            slight oil red O stain. Note the negative
            reaction in the mucous cells. X 3000.

Figure 93. Intestinal loop of Haplotrema showing fine,
            diffuse oil red O reaction. Scattered basal
            cells appear to be positive. X 1000.

Figure 94. Rectum of Allogona showing a weakly positive
            apical reaction for oil red O. X 1000.

Figure 95. Rectum of Haplotrema showing a negative
            oil red O reaction. X 1000.

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