# NORMAL POSTMORTEM CHANGES IN THE BROWN SHRIMP, *PENAEUS AZTECUS*[1]

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## ABSTRACT

A study was carried out to determine the normal rates and patterns of gross and histologic postmortem changes in the brown shrimp (*Penaeus aztecus* Ives). Experimental shrimp were held at 10°, 20°, or 30°C in water-saturated air or in seawater at a salinity of 30‰. Observations were made at 0, 2, 4, 8, 12, 24, 48, and 72 h postmortem.

The first change observed grossly was the onset of a rigorlike stiffening of the abdominal musculature. This stiffening was noted at 2 h postmortem at 30°C, but disappeared by 12 h postmortem. The condition appeared later and persisted longer at the lower temperatures.

Histologically, the tubule epithelium of the hepatopancreas was the first tissue to show autolytic change. The autolysis in the remaining tissues examined occurred in the following order: foregut and midgut epithelium, heart, neurons and nerve fibers, antennal gland epithelium, gill epithelium, epidermis, muscle, and lastly connective and cuticular tissue elements. In all tissues the rate of autolysis was temperature-dependent.

Shrimp from the Gulf of Mexico represent one of the most valuable fishery products of the United States. Their popularity as a food item and for use as sportfishing bait in some coastal areas has resulted in recent studies aimed at developing methods of artificially culturing these animals. Despite the enormous value of shrimp as a seafood, little is known about their histology and the rates and patterns of postmortem change.

Postmortem biochemical changes in the muscle of brown shrimp (*Penaeus aztecus* Ives) were reported by Flick and Lovell (1972). They reported that the compounds ATP, ADP, AMP, IMP, and glycogen decreased with time postmortem, while inosine, hypoxanthene, and lactic acid increased. The latter compounds were suggested as being partly responsible for flavor deterioration of ice-stored shrimp. Tissue pH values increased from 7.4 to 8.2 after 10 days in ice-stored shrimp (0°C), and, according to these authors, even with advanced bacterial spoilage, increases in pH are usually observed in fish and shellfish. Shrimp tails remained tender and soft during the entire storage period of 10 days (at 0°C) and did not exhibit any of the characteristics commonly associated with rigor mortis (Flick and Lovell, 1972).

In the only histologic study of postmortem change in an invertebrate animal, Sparks and Pauley (1964) reported the normal postmortem changes in the oyster, *Crassostrea gigas*. The digestive tubules of the oyster underwent the most rapid autolytic change in dead oysters held at 14°-16°C, while the Leydig tissue, gut, stomach, mantle, gill and palps autolyzed somewhat less rapidly. The gonads were the most resistant of all oyster tissue to autolysis with ova and sperm appearing relatively normal even after all other tissues had undergone extensive autolysis.

There are certainly a number of factors which influence the rate of autolysis in a dead animal. Some of these factors include water temperature, dissolved oxygen concentration, pH, bacterial flora of the water and of the animal, and the physiological condition of the animal at the time of death. It has been demonstrated in man and other animals that postmortem changes occur in a regular and irreversible pattern and at a relatively constant rate from one individual to another when factors causing variation in the rate and pattern are considered (Sparks and Pauley, 1964). Differentiation of histological changes due to disease from those due to postmortem autolysis or poor fixation is possible once the normal rates and patterns

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of postmortem changes under various conditions are known.

The present study was undertaken to determine the normal rates and patterns of postmortem change in penaeid shrimp as an aid in distinguishing gross and histologic changes due to autolysis from changes due to disease.

## MATERIALS AND METHODS

Juvenile brown shrimp averaging 50 mm in total length (tip of rostrum to tip of telson) were obtained live from a commercial bait dealer and were held in 500-liter fiberglass tanks for several days prior to being killed. Control shrimp were killed by immersion in fixative. The remaining shrimp were killed by placing the shrimp between wet towels in an enamel tray for 30 min. The shrimp were removed after 30 min and placed into 100-ml glass jars. Two groups at three temperatures (10°, 20°, and 30°C) were studied: one in air and the other in seawater. Shrimp held in air were introduced wet into test jars and the jars were sealed. Shrimp held in water were introduced into the test jars and enough Instant Ocean[3] artificial seawater (at 30 ‰ salinity) was added to fill the jars. Jars were held in wire baskets at midlevel in constant temperature baths.

Samples for antemortem examination were taken at 0 h while those for postmortem examination were taken at 2, 4, 8, 12, 24, 48, and 72 h. Four control shrimp were taken for study and two shrimp (one from seawater and one from air) were taken from the 10°, 20°, and 30°C baths at each of the remaining sampling times.

General appearance, color, odor, and condition of the hepatopancreas were noted at each sampling period. Tissues for microscopic examination were preserved in 10% buffered Formalin, prepared for microscopy with standard paraffin embedding and sectioning methods, and stained with hematoxylin and eosin.

## RESULTS

### Gross Observations

The first change observed was the onset of a rigorlike condition of the abdomen which appeared at about 2 h after death at 30°C and at 4 and 24 h at 20° and 10°C, respectively. The abdomen became flaccid at 12 and 48 h after death in shrimp held at 30° and 20°C, but at 10°C the abdomen remained rigid at 72 h after death (Table 1).

TABLE 1.—Time of onset of a rigor-like stiffening of shrimp abdominal musculature at 10°, 20°, and 30°C in air and seawater.

| H postmortem | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 10°C | | 20°C | | 30°C | |
| | Air | Water | Air | Water | Air | Water |
| 0 | – | – | – | – | – | – |
| 2 | – | – | + | – | + | + |
| 4 | – | – | + | – | + | + |
| 8 | – | – | + | + | + | + |
| 12 | – | + | – | + | – | – |
| 24 | + | + | + | + | – | – |
| 48 | + | + | – | – | – | – |
| 72 | + | + | – | – | – | – |

+ = stiff
– = flaccid

Color change and the appearance of spoilage odor were first observed at 4 h after death at 30°C. The general appearance of the shrimp changed from the usual semitransparent to a whitish-opaque at about the same time the first trace of odor was detected (Tables 2 and 3). At 20° and 10°C the first color change and appearance of odor were noted at 12 h and 24 h, respectively. At all three temperatures the color of the shrimp changed from opaque to an orange-red and finally to red with some blackened areas (Table 2). The intensity of spoilage odor increased along with the color change (Tables 2 and 3).

Fluid leakage from the hepatopancreas was first observed at 4 h at 30°C and at about 8 and 12 h postmortem at 20° and 10°C. Enzymatic digestion of hepatopancreas and surrounding tissues was grossly evident at 12 h at 30°C as

TABLE 2.—Times of postmortem color change of whole shrimp at 10°, 20°, and 30°C in air and seawater.

| H postmortem | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 10°C | | 20°C | | 30°C | |
| | Air | Water | Air | Water | Air | Water |
| 0 | – | – | – | – | – | – |
| 2 | – | – | – | – | – | – |
| 4 | – | – | – | – | 0 | 0 |
| 8 | – | – | – | – | 0 | 0 |
| 12 | 0 | – | – | 0 | LR | LR |
| 24 | LR | 0 | LR | LR | Rb | Rb |
| 48 | LR | LR | R | Rb | Rb | R |
| 72 | Rb | R | Rb | R | Rb | Rb |

– = normal
0 = opaque
LR = orange to light red
R = red
Rb = red with blackened edges of cuticle or blackened appendages

[3] Reference to trade names in this publication does not imply endorsement of commercial product.

TABLE 3.—Time of appearance of postmortem spoilage odor in whole shrimp held at 10°, 20°, and 30°C in air and seawater.

| H postmortem | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 10°C | | 20°C | | 30°C | |
| | Air | Water | Air | Water | Air | Water |
| 0 | − | − | − | − | − | − |
| 2 | − | − | − | − | − | − |
| 4 | − | − | + | − | + | + |
| 8 | − | − | ++ | − | + | + |
| 12 | − | − | ++ | − | ++ | ++ |
| 24 | + | + | ++ | ++ | ++ | ++ |
| 48 | ++ | + | +++ | +++ | +++ | +++ |
| 72 | ++ | ++ | +++ | +++ | +++ | +++ |

− = normal
+ = odor

indicated by a loosening of the junction of the cephalothorax and abdomen. By 48 h the junction was very loose and by 72 h the tissues of the junction appeared mostly liquified. At 10° and 20°C the same process was observed but at a proportionately slower rate.

## Histological Observations

Since the same patterns of autolysis were seen in shrimp held at all three temperatures, the differences being a function of time (Table 4), only the histological results from the 30°C portion will be presented in the text. The only significant histological differences between groups held in air and water noted were the more rapid tissue decomposition due to increased bacterial action in animals held submerged in seawater.

### Digestive Tract

According to Roberts (1966), the digestive tract in shrimp is composed of three divisions: (1) the foregut, which includes the mouth, esophagus, stomach, and associated glands; (2) the midgut and hepatopancreas; and (3) the hindgut. Of these organs the hepatopancreas, the foregut, and midgut were studied in detail. The hindgut was not studied.

#### Hepatopancreas

The glandular hepatopancreas is the first organ to undergo autolytic change (Figure 1a). This organ is a compound tubularacinar exocrine gland composed of tubules which end in blind sacs or acini. The tubules and acini are lined with a simple low to high columnar epithelium (Figure 1b). Autolysis of the epithelium of this

TABLE 4.—Rate of postmortem histologic change in shrimp held in air or seawater at three temperatures.

**a.** Hepatopancreas.

| H postmortem | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 10°C | | 20°C | | 30°C | |
| | Air | Water | Air | Water | Air | Water |
| 0 | 0* | 0 | 0 | 0 | 0 | 0 |
| 2 | 2.5* | 2.5 | 2.5 | 2.5 | 3 | 3.5 |
| 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 8 | 4 | 3.5 | 4.5 | 5 | 4.5 | 4.5 |
| 12 | 3.5 | 4 | — | — | 5 | 5 |
| 24 | 5 | 4 | 5 | 5 | 5 | 5 |
| 48 | 5 | 5 | 5 | 5 | 5 | 5 |
| 72 | 5 | 5 | 5 | 5 | 5 | 5 |

**b.** Midgut epithelium.

| H postmortem | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 10°C | | 20°C | | 30°C | |
| | Air | Water | Air | Water | Air | Water |
| 0 | 0* | 0 | 0 | 0 | 0 | 0 |
| 2 | 1* | 1 | 1 | 1 | 1-2 | 1 |
| 4 | 2 | 1 | 1-2 | 1-2 | . | 2 |
| 8 | 4 | . | 4-5 | . | 3 | 4 |
| 12 | . | 3 | 2 | 4 | 5 | 4 |
| 24 | 5 | 4 | — | 5 | 5 | . |
| 48 | 5 | 5 | 5 | 5 | — | 5 |
| 72 | 5 | 5 | 5 | 5 | 5 | 5 |

**c.** Abdominal muscle.

| H postmortem | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 10°C | | 20°C | | 30°C | |
| | Air | Water | Air | Water | Air | Water |
| 0 | 0* | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 1 | 1 | 2 | 1 | 1.5 | 1 |
| 8 | 0 | 1 | 2 | 2 | 2 | 2 |
| 12 | 1 | 1 | 2.5 | 2.5 | 3 | 3 |
| 24 | 3 | 3 | 3.5 | 3 | 3.5 | 3.5 |
| 48 | 2.5 | 3 | 4 | 4 | 3.5 | 4.5 |
| 72 | 4 | 3.5 | 4.5 | 4 | 4.5 | 4 |

**d.** Epidermis.

| H postmortem | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 10°C | | 20°C | | 30°C | |
| | Air | Water | Air | Water | Air | Water |
| 0 | 0* | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 1 | . | — | 2 | 2 |
| 8 | 1 | 1 | . | 2 | 3 | 3 |
| 12 | 2 | 2 | 3 | 3 | 4 | 4 |
| 24 | 3 | 3 | 3 | 3 | 4 | . |
| 48 | 3 | 3 | 4 | 4 | 5 | 5 |
| 72 | 5 | 5 | 5 | 5 | 5 | 5 |

. No observation made.
* Average assigned values from a scale of 0 to 5 denoting the general histological appearance of the tissue or organ.
0 = Normal histologic appearance, like the control, no postmortem change.
1 = Slight change, scattered pyknotic nuclei, slight staining differences.
2 = More advanced cellular change with increases in nuclear pyknosis, karyrhexis, karyolysis, some cytolysis; loss of normal appearance or structure of the tissue or organ.
3 = Further advanced change with no normal appearing areas.
4 = Advanced autolytic change, tissue or organ represented by cellular debris or by its fibrous or cuticular stroma.
5 = Complete autolysis, tissue or organ no longer demonstrable.

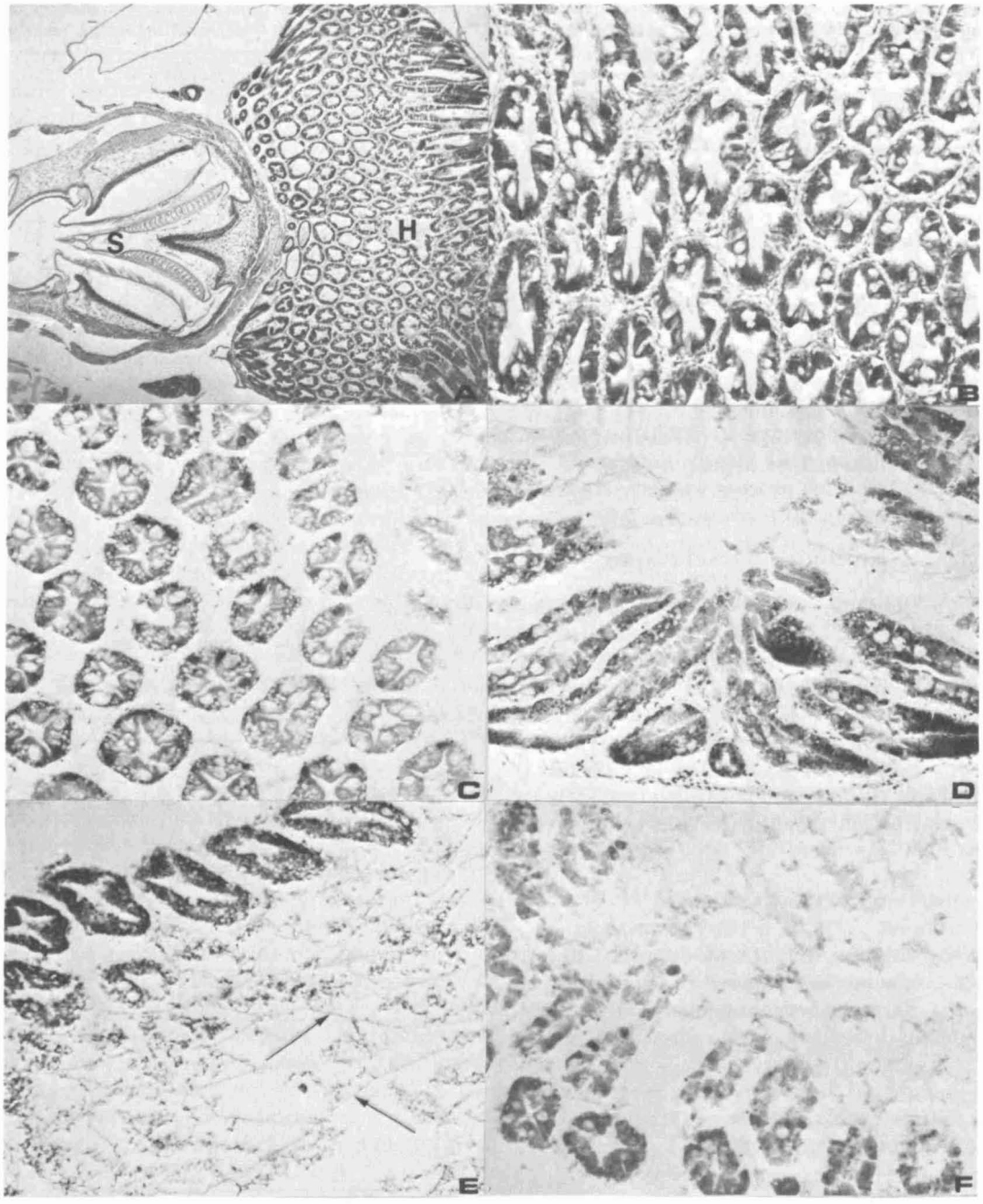


FIGURE 1.—**a.** Normal stomach (S) and hepatopancreas (H). 25×. **b.** Normal hepatopancreas. 120×. **c.** Hepatopancreas at 2 h postmortem showing edematous swelling between adjacent tubules. Autolysis is more advanced nearer the center of the organ (upper right) than at the periphery (left). 110×. **d.** Hepatopancreas at 4 h postmortem showing tubules on longitudinal section. Note the progression of autolytic change in the tubules from the periphery of the organ (bottom) to the autolyzed center (top). 110×. **e.** Hepatopancreas showing near complete autolysis (4 h postmortem). Note network of connective tissue stroma (arrows) remaining after autolysis of tubule epithelium. 120×. **f.** Hepatopancreas at 8 h postmortem showing advanced autolysis. Intensely pyknotic nuclei are present in the remaining epithelial cells near the periphery of the organ. Tissue debris and remnants of the connective tissue stroma are present nearer the organ's center (upper right). 120×.

organ proceeds so rapidly that by 2 to 4 h postmortem, the epithelium of tubules near the center of the organ showed advanced autolysis. These tubules showed desquamation and cytolysis of the lining epithelium and replacement with eosinophilic debris (Figures 1c and 1d). Nearer the periphery of the organ, the condition of the tubules and tubule epithelium appeared progressively more normal, with the most normal appearing tubules and acini at the periphery (Figures 1c and 1d). In the band of tissues between the normal appearing periphery and the lysed core, all stages of cell death were observed. A thin band of tissue in this area contained tubules whose epithelial cells possessed scattered pyknotic nuclei and had slight cytoplasmic staining differences (Figure 1e). Deeper to this layer the epithelial cells of tubules and acini possessed scattered pyknotic nuclei and had slight cytoplasmic staining differences (Figure 1e). The cytoplasm of these cells was highly vacuolated and stained variably with hematoxylin and eosin but generally much less basophilicily than normal (Figure 1c). At this time the spaces between adjacent tubules and acini had become swollen (Figures 1c and 1d). Slightly deeper to this layer epithelial cell nuclei had undergone karyorrhexis or karyolysis and disappeared. Many of the cells of this area had lysed and the cellular debris stained red with eosin. The supportive stroma of the hepatopancreatic tubules remained intact in some areas after the epithelium had autolyzed, thereby masking the former site of the hepatopancreatic tubules (Figure 1e).

By 8-12 h postmortem even the tubules and acini at the periphery of the organ showed advanced autolytic change, and the tissue debris and remnants of supportive stroma in the center of the organ were liquified (Figure 1f). The connective tissue capsule of the organ had become ruptured and few recognizable tubules were present. Past 12 h no trace of the hepatopancreas was present, and surrounding tissues had also been partially or completely digested, presumably by enzymes released from the autolyzed hepatopancreas.

### Foregut and Midgut

Autolytic changes in the foregut, particularly the epithelium of the stomach (Figure 1a), proceeded at approximately the same rate as changes in the hepatopancreas. Nuclear changes within epithelial cells were observed at 2 h postmortem with considerable change by 4 h. By 8 to 12 h the epithelium of the stomach had undergone nearly complete autolysis and had disappeared, leaving only the cuticular elements of the stomach lining intact. The cuticular elements of the esophagus and stomach persisted for the duration of the study (72 h).

The midgut extends from the pyloric stomach to the sixth abdominal segment where it joins with the hindgut (Roberts, 1966). It is without a lining cuticle. The first autolytic change in the midgut was observed in the lining epithelium at 2 to 4 h, when the epithelial cells began to show changes such as scattered pyknotic nuclei, changes in staining reaction from a pale basophilic reaction to a more eosinophilic one, and the "blebing" of the apical ends of epithelial cells into the gut lumen (Figure 2a). The epithelium usually remained attached to the basement membrane at 2 h, but in some areas portions of the midgut epithelium had been sloughed into the gut lumen (Figures 2b and 2c). Sloughed epithelial cells were rounded and had intensely pyknotic nuclei and a uniform eosinophilic cytoplasm. At this time the gut lumen usually contained a fibrous, eosinophilic coagulum (Figure 2b). The gut wall basal to the lining epithelium showed no appreciable changes by 4 h.

By 8 to 12 h the midgut epithelium had been sloughed into the gut lumen (Figure 2d). The epithelial cells in the gut lumen were rounded, and some had pyknotic nuclei, but they were mostly anucleate. Many of the epithelial cells had lysed and left behind amorphous masses of eosinophilic debris (Figure 2d). Changes in the cellular elements of the wall of the midgut became apparent by 8-12 h. These changes consisted primarily of a decrease in nuclear number in the muscle and connective tissue cells present and pyknosis of those nuclei remaining (Figure 2d). In general, the cytoplasm of the cells present showed increased eosinophilia.

No trace of the lining epithelium was present after 24 h (Figure 2e). The coagulum, which was present in the gut lumen of some animals at 2-8 h, was still present. Also present in the gut lumen were large numbers of bacteria (Figure 2e). No nuclei were present in the gut wall, and the cellular elements remaining stained intensely with eosin.

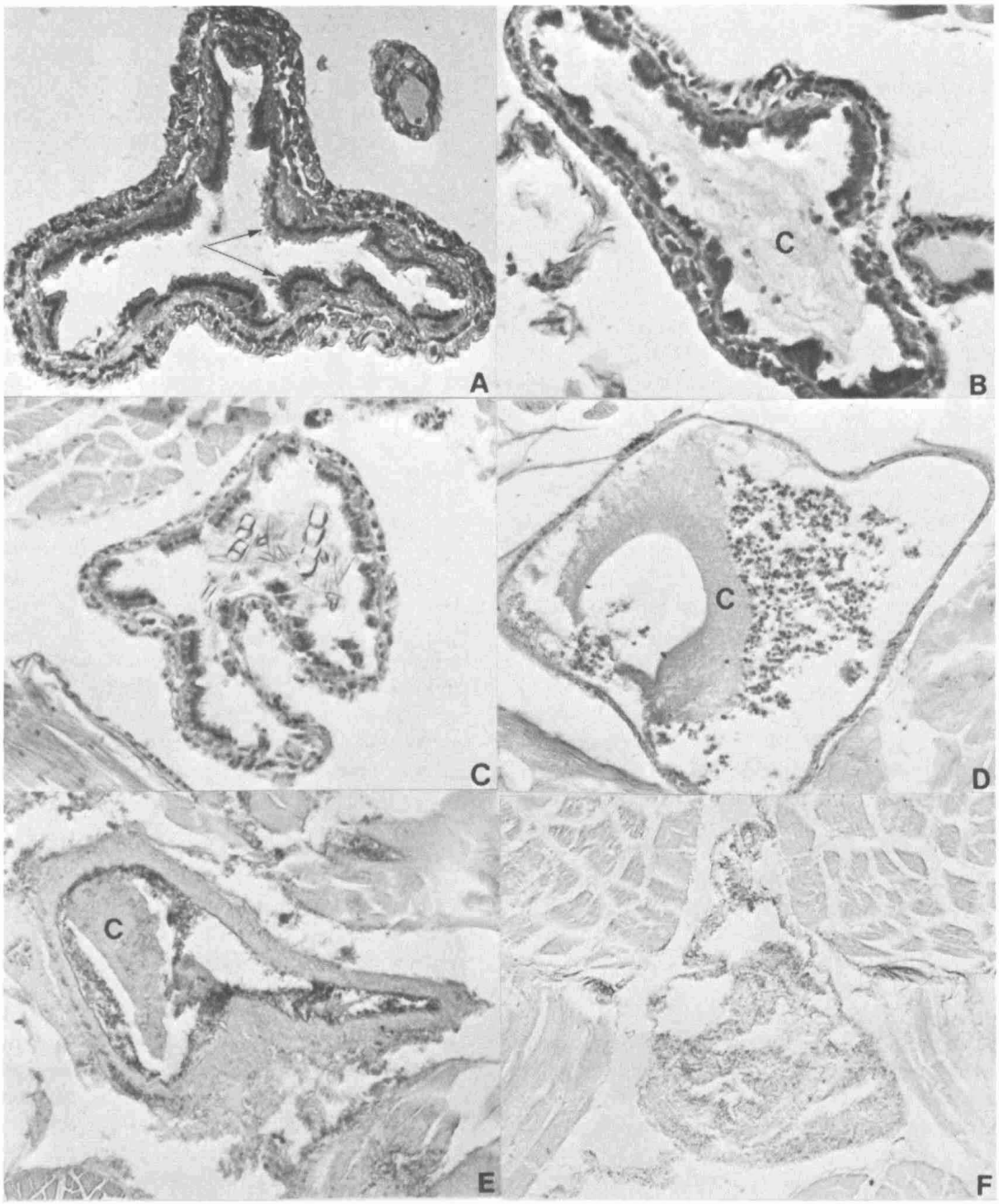


FIGURE 2.—**a.** Cross section of midgut at 2 h postmortem. The appearance is near normal except for the "blebing" of the apical ends of some of the epithelial cells (arrows) and a few pyknotic nuclei. 250×. **b.** Midgut showing more advanced autolytic change at 2 h postmortem. Some epithelial cells have been sloughed into the gut lumen where an eosinophilic coagulum (C) has formed. 240×. **c.** Midgut at 4 h postmortem. Most of the epithelial cells possess pyknotic nuclei, and some of the cells have been sloughed into the gut lumen. 210×. **d.** Midgut at 8 h postmortem. Sloughed epithelial cells are rounded and are either anucleate or have pyknotic nuclei. An eosinophilic coagulum is present. 160×. **e.** Midgut at 24 h postmortem. An eosinophilic coagulum is present in the gut lumen as are numerous bacteria. No trace of the gut epithelium remains. The muscle and fibrocyte cells of the gut wall are anucleate. 190×. **f.** Site of midgut at 48 h postmortem. Bacteria and debris have filled the gut lumen. Only fibrous elements of the gut wall remain. 150×.

By 48 h, the gut wall had become thin and was frequently interrupted. The gut lumen was filled with bacteria and other debris (Figure 2f). By 72 h, all traces of the gut, including the gut wall, had disappeared leaving the former site of the gut marked only by masses of bacteria and amorphous eosinophilic cellular debris.

## Heart and Major Vessels

In shrimp the heart lies immediately dorsal and slightly caudad to the large hepatopancreas. Only the thin connective tissue coverings of the two organs separate them. Hence, autolysis of the hepatopancreas and release of its proteolytic enzymes results in a rapid destruction of the rather loose tissues of the shrimp heart (Figure 3a). The hepatopancreas showed considerable autolytic change by 4 h postmortem leaving the heart barely recognizable (Figure 3b). By 8 h the heart was not distinguishable from the other tissue debris present at the heart's former location in the cephalothorax. Vessels in the vicinity of the hepatopancreas and heart also disappeared by 4-8 h, but vessels elsewhere, such as in the abdomen, persisted much longer, some still recognizable after 24 h. However, by 48 h vessels were not usually demonstrable anywhere in the body of a shrimp.

## Musculature

Shrimp locomotory muscle is striated and presents a histologic appearance that is similar to that of vertebrate striated muscle (Figure 4a). The muscles of the cephalothorax in the vicinity of the hepatopancreas underwent rapid autolytic change, apparently due to digestion by enzymes released on lysis of the hepatopancreas. Further from the hepatopancreas, the rate of autolytic change in the muscle was much slower. The earliest observed postmortem change in the muscle was at 4 h when some individual muscle fibers had a slightly "frayed" appearance. There was a pronounced swelling, presumably edematous, between adjacent muscle fibers (Figure 4b). By 8-12 h, muscle cell nuclei had become pyknotic. After 24 h muscle cells had become anucleate, highly eosinophilic, and the edematous swelling between adjacent muscle cells had decreased. Cross striations within muscle fibers were especially evident (Figures 4d, 4e, and 4f).

In some, but not all, of the shrimp studied, bacterial growth was evident between muscle bundles, especially in the vicinity of the gut. The presence of large numbers of bacteria greatly increased the rate of tissue deterioration (Figure 4c), while muscle not heavily invaded by bacteria remained recognizable as muscle tissue at 72 h (Figure 4f).

## Integument

The integument, consisting of epidermis and an overlying cuticle, underwent rapid degeneration in the area of the cephalothorax that surrounds the hepatopancreas, leaving only

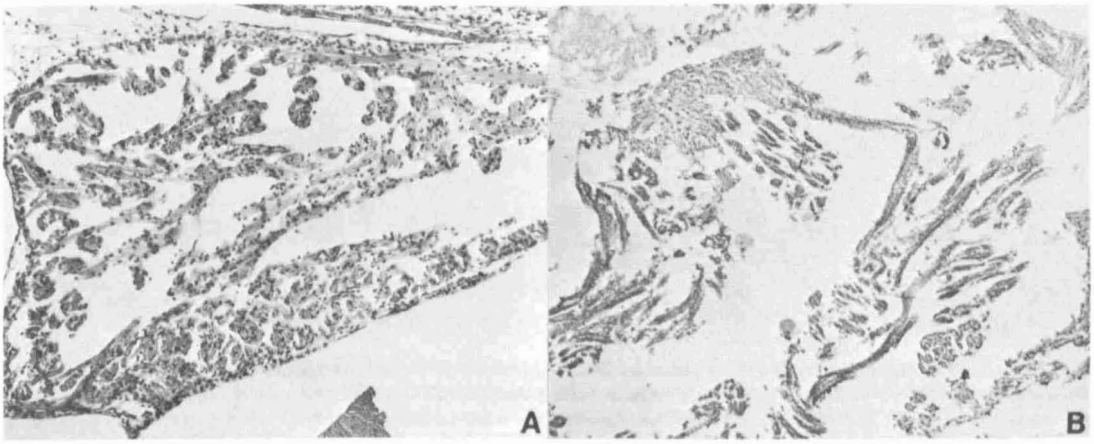


FIGURE 3.—**a.** Normal heart. 110×. **b.** Heart at 4 h postmortem showing considerable autolytic change and loss of structural detail. 100×.

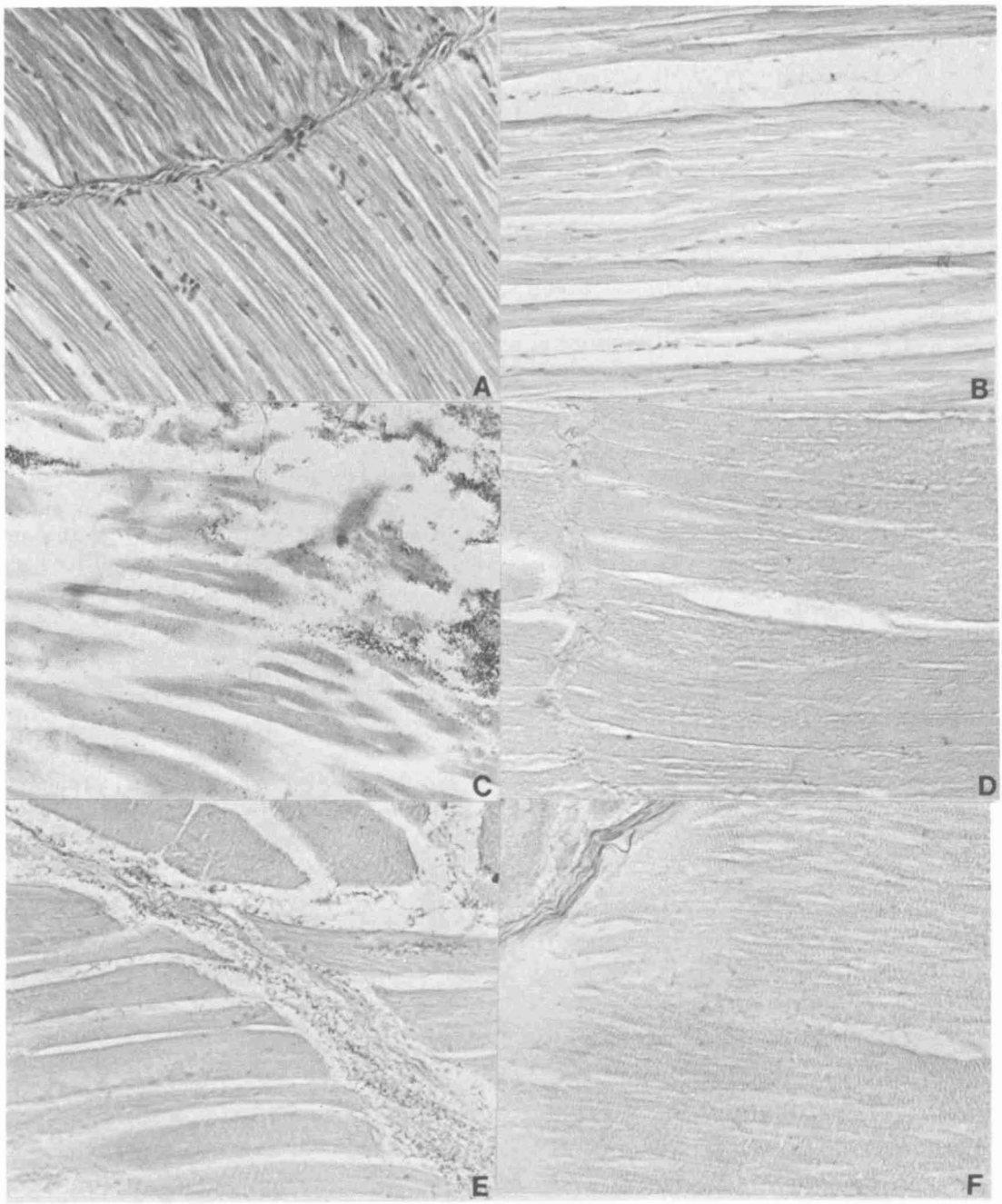


FIGURE 4.—**a.** Normal abdominal muscle. 220×. **b.** Muscle at 4 h postmortem showing edematous swelling between muscle bers. Sarcoplasmic staining reaction is more eosinophilic than normal and there has been a decrease in the number of nuclei although few pyknotic nuclei are shown. 150×. **c.** Muscle showing advanced autolytic change due to the presence of large amounts of bacteria (12 h postmortem). 190×. **d.** Muscle at 24 h postmortem. Edematous swelling has decreased, but the muscle fibers have become anucleate. Note the prominence of cross striations. 240×. **e.** Muscle at 48 h postmortem. 240×. **f.** Muscle fibers with prominent cross striations are still recognizable at 72 h postmortem. 250×.

the cuticle remaining by 4 h. Distant from the hepatopancreas, the epidermis showed pyknotic nuclei and cell rounding by 4 to 8 h (Figures 5a and 5b). A slight hemocytic response was present at this time in the subepidermal tissue layers representing the only inflammatory-like response observed in the study.

The epidermis had frequently become detached from the overlying cuticle by 12 to 24 h postmortem and many of the epidermal cells had lysed, with those remaining having pyknotic nuclei (Figure 5c). By 24 to 48 h nearly all traces of the epidermis had been lost and in some animals examined only cellular debris or clumps of bacteria marked its former location (Figure 5d). Though usually interrupted, the cuticle was the most resistant structure to autolytic change and showed only slight histological change by 72 h.

## Gills

The shrimp respiratory system consists of paired gills in the branchial chambers of the cephalothorax. The structure of the gills is dendrobranchiate (Barnes, 1963). The gills are covered by a thin cuticle underlain by a thin epithelium and other supportive cells (Figure 6a).

A peritrichous ciliated protozoan (Figure 6b), presumably a commensal on shrimp (especially common on the gills but also found elsewhere on

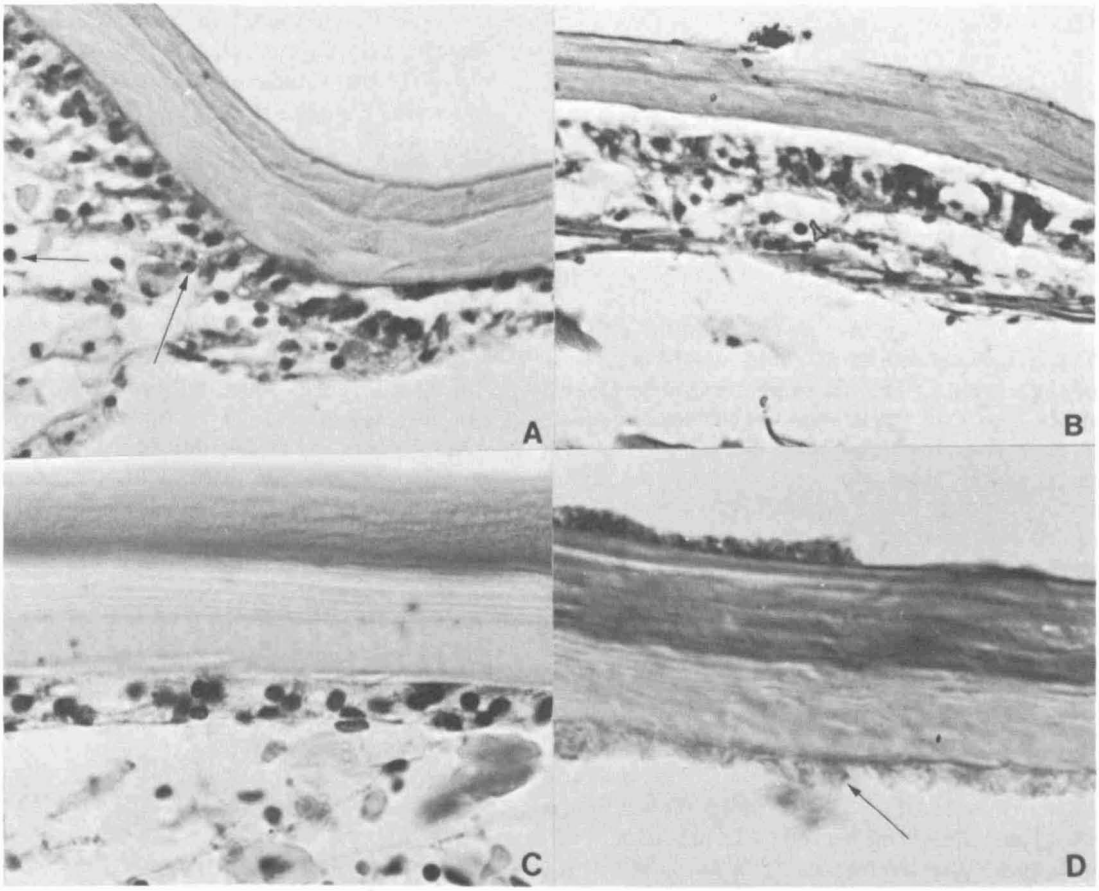


FIGURE 5.—**a.** Integument consisting of epidermis and overlying cuticle at 4 h postmortem. Some of the epidermal cells possess pyknotic nuclei. A few hemocytes are present in the subepidermal tissues (arrows). 480×. **b.** Integument at 8 h postmortem. Inflammatory cells are present in the subepidermal tissue. There is an increase in nuclear pyknosis in the epidermis and in the subepidermal tissue. 300×. **c.** Epidermis and cuticle at 24 h postmortem. All of the epidermal cells have intensely pyknotic nuclei, as does all the subepithelial tissue. 600×. **d.** Integument at 48 h postmortem. The cuticle is present, but the epidermis is represented by debris (arrows). 750×.

the body surface), increased rapidly in numbers for 2 to 4 h after death of the shrimp. They were absent by 8 h postmortem.

The cellular elements of the gills underwent fairly rapid autolytic change. By 8-12 h scattered pyknotic nuclei were present (Figure 6c). By 24 h the cellular elements of the gills were for the most part anucleate, with some portions of the gills having only eosinophilic debris within the lamellar cuticle (Figure 6d). By 48 h the thin cuticle of gill lamellae had begun to deteriorate and hence the gill lamellae sectioned transversely began to lose their typical "dumbbell" appearance (Figure 6e). By 72 h the gills were usually no longer demonstrable histologically, but in one of four animals examined portions of the gills were still evident (Figure 6f).

### Nerve Tissue

The nervous system of shrimp is composed of a large ventral nerve cord and segmental ganglia from which smaller nerve branches originate to innervate the tissues. At the anterior end of the ventral nerve tract is the supraesophageal ganglion, which anteriorly receives the large optic nerve tracts.

Neuron perikaryons in the ganglia (Figure 7a) underwent the most rapid autolytic change of the various elements of shrimp nerve tissue. After 2 to 4 h, these cells showed considerable rounding, pyknotic or karyolytic nuclei, and a change in cytoplasmic staining from highly basophilic to a lesser basophilic to almost eosinophilic (Figure 7b). By 8 h no trace of neuron perikaryons was evident.

The nerve tracts of the ventral nerve, its branches, and the optic nerves autolyzed less rapidly than did neuronal perikaryons. However, nerve cell processes (axons and dendrites) within the nerve tract autolyzed more rapidly than did the supportive neurolemmal and glial cells, and were no longer demonstrable histologically by 12 to 24 h (Figure 7c). The supportive glial cells of the nerve tracts persisted without noticeable change to 8 to 12 h, but these cells became anucleate or underwent autolysis after 24 h, and their former presence was represented only by debris and an occasional pyknotic nucleus (Figure 7d).

After 24 h postmortem, the basic structural arrangement of the nerve tract remained recognizable due to the persistence of neurolemmal fibers (Figures 7d and 7e), which persisted to 72 h at the sites of the optic nerve and ventral nerve tracts.

### Antennal Gland

The antennal gland of crustaceans had been demonstrated to be important in ion regulation (Robertson, 1959). The antennal or hemocoelic excretory gland in shrimp is located in the cephalothorax above the supraesophageal ganglion (Young, 1959). The gland is composed of a collection of tubules and a bladder (Figures 8a and 8b). By 4 h some sloughing of tubule epithelium was evident (Figure 8b), but for the most part the histologic appearance of the organ remained normal. At 12 h, however, most of the nuclei of the tubule epithelium were intensely pyknotic (Figure 8c), and by 24 h the organ had disappeared or had become difficult to recognize (Figure 8d). No trace of the gland was found after 48 h postmortem.

### Gonadal Tissue

Since the animals used in this study were immature juvenile shrimp, the gonads were small, poorly differentiated and were located in the cephalothorax lateral and slightly caudad to the hepatopancreas. The terminal ampule of male shrimp was poorly developed and in female shrimp the ovarian lobe, which extends into the abdomen in older shrimp, had not yet developed.

The rate of autolysis in the gonads of the shrimp studied was rapid, due to their close proximity to the hepatopancreas. Gonadal tissue was not recognizable histologically after 4 to 8 h postmortem.

## DISCUSSION

The rigorlike stiffening observed in this study may represent true rigor mortis. Sparks (1972) postulated that rigor mortis or a similar phenomenon may occur in some invertebrates with well organized skeletal systems and associated skeletal muscles. He based his opinion on the observation that many arthropods, which are flaccid after somatic death, subsequently

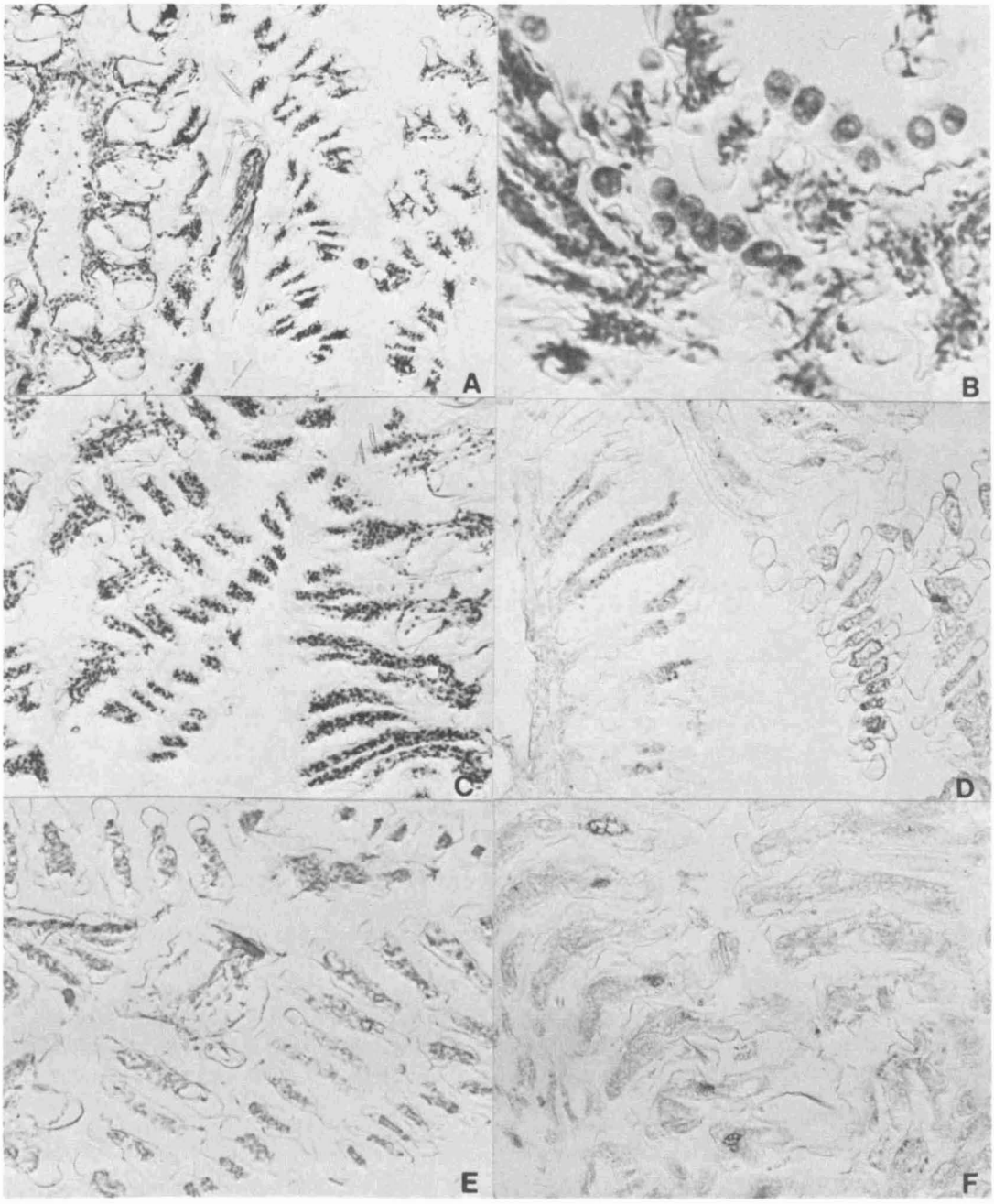


FIGURE 6.—**a.** Normal gills. 120×. **b.** An unidentified ciliated protozoan abundant on the gills at 4 h postmortem. 240×. **c.** Gills at 12 h postmortem. Note the absence of the protozoan and the presence of pyknotic nuclei in the cellular elements. 130×. **d.** Gills at 24 h postmortem. Except for a few pyknotic nuclei only the cuticle and cellular debris remain. 160×. **e.** Gills at 48 h postmortem. The tissue is still recognizable as gills; however, the lamellae are losing their usual "dumbbell" appearance and contain only eosinophilic cellular debris. 190×. **f.** Gills at 72 h postmortem. Gills were recognizable histologically only from this one of four animals examined. 120×.

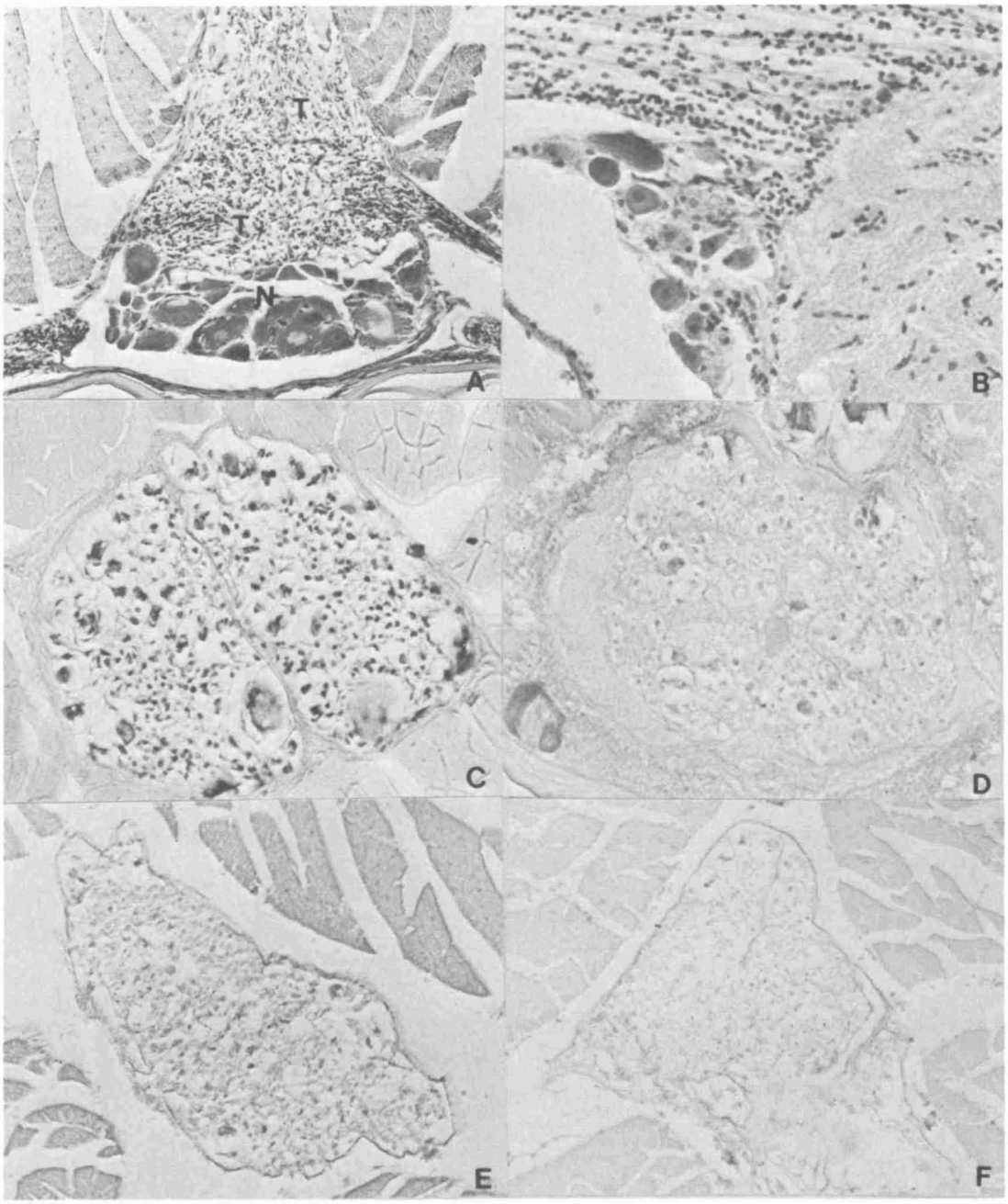


FIGURE 7.—**a.** Cross section of an abdominal segment ganglion on the ventral nerve (0 h control). Neuron perikaryons (N) are present ventral to the large ventral nerve tract (T). 110×. **b.** Sagittal section of an abdominal segment ganglion at 4 h postmortem. The neuron perikaryons are rounded and have pyknotic nuclei. Nerve cell processes, neurolemmal and glial cells in the nerve tract show no apparent autolysis. 220×. **c.** Cross section of the ventral nerve at 12 h postmortem. Nerve cell processes are not evident and neurolemmal and glial cells possess pyknotic nuclei. 200×. **d.** Cross section of the ventral nerve at 24 h postmortem. Only supportive fibrous tissue elements and eosinophilic debris remain. 190×. **e.** Cross section of ventral nerve at 48 h postmortem. Fibrous elements are still present. 200×. **f.** Ventral nerve in cross section at 72 h postmortem. The fibrous elements of the nerve are still present. 120×.

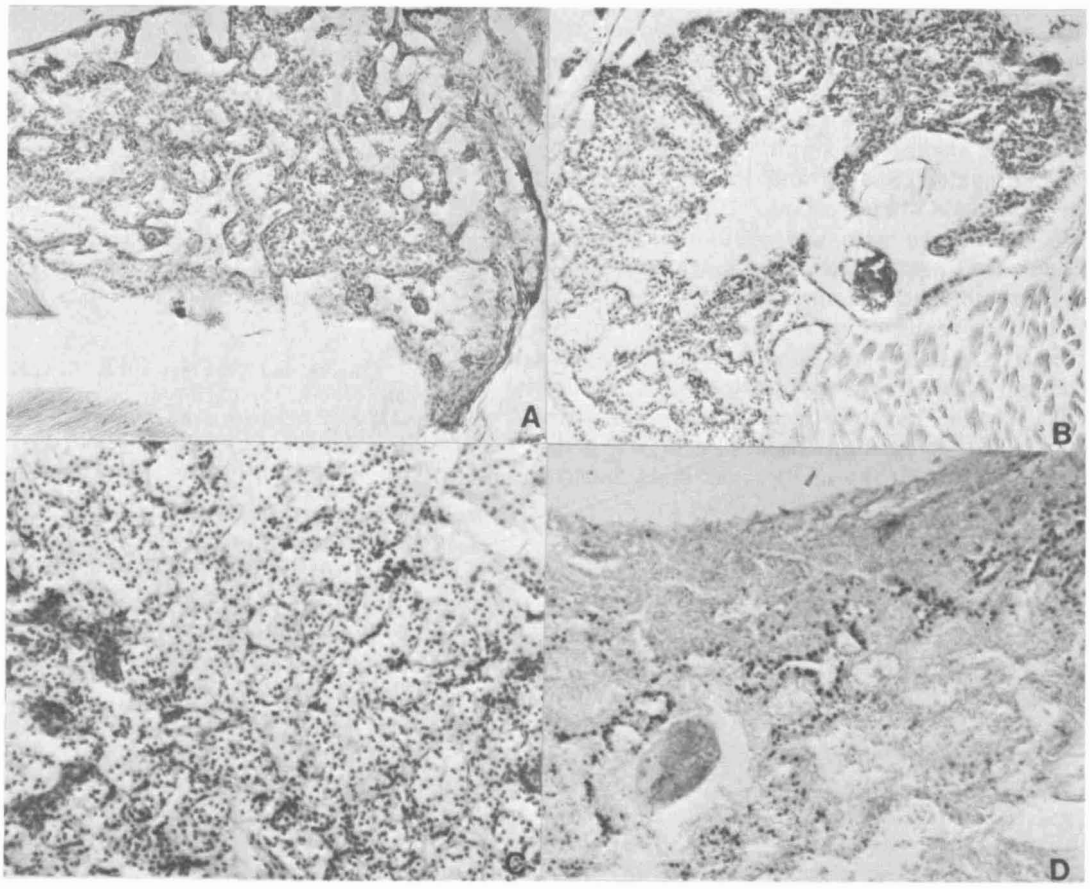


FIGURE 8.—**a.** Normal antennal gland. 100×. **b.** Antennal gland at 4 h postmortem. A few epithelial cells have been sloughed into the tubule lumens. 100×. **c.** The tubule epithelium of the antennal gland at 12 h postmortem showing intense nuclear pyknosis. 150×. **d.** Antennal gland at 24 h postmortem. The tubule epithelium has lysed filling the tubule lumens with eosinophilic debris and nuclear remnants. 150×.

become rigid. Whether this was due to desiccation of the tissues or actual rigor of the muscles was not determined. In the present study, freshly killed juvenile shrimp became rigid in sealed glass jars containing water-saturated air and when totally submerged in water. Desiccation was not possible. The time of onset of rigidity was, as in vertebrates, temperature-dependent, occurring earlier at higher temperatures than at lower temperatures.

Flick and Lovell (1972) in studying postmortem biochemical changes in penaeid shrimp reported that shrimp tails remained soft and did not exhibit any of the characteristics commonly associated with rigor mortis during a storage period of 10 days at 0°C. Perhaps the effect of freezing or near-freezing temperature on shrimp muscle either masks or inhibits the onset of physical rigor.

The rate of autolysis of the hepatopancreas is extremely rapid. The organ is a large, multifunctional organ believed to produce the bulk of enzymes used in the digestive process in shrimp and to have some absorptive and storage function. The hepatopancreas connects to the midgut near its origin from the pyloric stomach. The gut is a short, nearly straight tube, and, hence, enzymatic digestion must occur as rapidly as possible if the shrimp is to utilize its food efficiently. Even careful handling of shrimp to avoid stress before fixation, opening of the cuticle over the hepatopancreas, or excision and bisection of the gland to enhance fixation, frequently failed to provide adequate penetration and fixation of the organ when Formalin fixatives were used. The remaining tissues of shrimp are generally adequately fixed for light microscopy with Formalin, provided

that small pieces of tissue are used or that the cuticle is opened on smaller shrimp that are fixed whole.

The relative rates and patterns of postmortem change in shrimp are similar to those described for the oyster (Sparks and Pauley, 1964) and for mammals (Cruickshank, 1912; Smith and Jones, 1966). In mammals, oysters, and shrimp, tissues that produce large amounts of proteolytic enzymes such as the mammalian pancreas and lining epithelium of the stomach, oyster digestive tubules, and shrimp hepatopancreas and gut epithelium autolyze the most rapidly. Tissues that autolyze nearly as rapidly are high lipid containing tissues such as nerve tissue. In the shrimp and in mammals, muscle, connective, and epidermal tissues undergo the least rapid autolysis.

## LITERATURE CITED

BARNES, R. D.
1963. Invertebrate zoology. W. B. Saunders Co., Phila., 632 p.

CRUICKSHANK, J.
1912. The histological appearances occurring in organs undergoing autolysis. J. Pathol. Bacteriol. 16:167-184.

FLICK, G. J., AND R. T. LOVELL.
1972. Post-mortem biochemical changes in the muscle of Gulf shrimp, Penaeus aztecus. J. Food Sci. 37:609-611.

ROBERTS, N. L.
1966. Morphology and histology of the stomach of the white shrimp, *Penaeus fluviatilis* (Say, 1817). Ph.D. Thesis, Univ. South. Mississippi, Hattiesburg, 78 p.

ROBERTSON, J. D.
1959. Osmotic and ionic regulation. *In* T. H. Waterman (editor), The physiology of crustacea, Vol. 1, p. 317-339. Academic Press, N.Y.

SMITH, H. A., AND T. C. JONES.
1966. Veterinary pathology. 3d ed. Lea & Febiger, Phila., 1192 p.

SPARKS, A. K.
1972. Death and postmortem change. *In* A. K. Sparks, Invertebrate pathology noncommunicable diseases, p. 1-19. Academic Press, N.Y.

SPARKS, A. K., AND G. B. PAULEY.
1964. Studies of the normal postmortem changes in the oyster, *Crassostrea gigas* (Thunberg). J. Insect Pathol. 6:78-101.

YOUNG, J. H.
1959. Morphology of the white shrimp, *Penaeus setiferus* (Linnaeus 1758). U.S. Fish. Wildl. Serv., Fish. Bull. 59:1-168.