THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN INSECTS

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(Plates 1-3)

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Summary

The distribution of alkaline phosphatase in insect tissues has been studied by a histochemical technique. The enzyme is widely distributed in the alimentary tract, storage tissue, nervous tissue, in parts of the reproductive system, in certain muscles, and some glands. It thus appears to be involved in a variety of functions. Many examples of both the histological and cytological distribution are explained by the relation of the enzyme to mechanisms of transport across cell boundaries. But a function of this kind is not evident in such sites as muscles and nerves. The presence of deposits of inorganic phosphate in the alimentary tract and malpighian tubules of some species is recorded.

I. Introduction

The tremendous current interest in the biochemistry of phosphates and phosphatases is largely due to their unique role in energy transfer and in enzymatic syntheses (Kalckar 1947). A special impetus to the study of phosphatases was given by the discovery of comparatively simple methods for their histological detection. Yet there is only one brief report (Bradfield 1946) on the localization of alkaline phosphatase in insects, which concluded that the enzyme is located in cells most active in the synthesis of fibrous proteins. In vertebrates the phosphatases are widely distributed (see for example, Gomori 1941; Bourne 1943*a*) and this and other evidence (Moog 1946) suggests that they are concerned with other functions as well. Since a wider distribution of phosphatases in insect tissues than that reported by Bradfield has been indicated by several microchemical studies (Drilhon 1943; Drilhon and Busnel 1945; Nakamura 1940), it seemed desirable to investigate in greater detail the localization of the enzymes in a number of insect species.

II. Material and Methods

Tissues of a number of insects were examined by the well-known Gomori-Takamatsu technique, and the schedule recommended by Gomori (1941) was followed in detail. Sodium glycerophosphate (B.D.H.) was used as the substrate. The species examined were: *Ctenolepisma longicaudata* Esch., *Blattella germanica*

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(L.), *Periplaneta americana* (L.), *Locusta migratoria* (L.), *Tenebrio molitor* (L.), *Tineola biselliella* Hum., *Pieris rapae* (L.), and *Lucilia cuprina* Wied.

No differences were observed in the distribution of alkaline phosphatase between any of the several specimens (at least three) of each species examined. A large number of *B. germanica* were studied and no example of variation in the distribution of the enzyme was found.

The specificity of the technique has been thoroughly discussed by Danielli (1946). He showed that, provided controls omitting the substrate are stained simultaneously to distinguish preformed phosphate, there can be no doubt of the specificity of the reaction.

III. Observations

(i) *General.*—The majority of insect tissues have, in one species or another, been found to be positive for alkaline phosphatase. Exceptions are the dermal tissues, the heart, and the pericardial nephrocytes (Table 1). Usually the nuclei gave a stronger reaction than the cytoplasm. Nucleoli frequently were positive also in the controls indicating the presence of inorganic phosphate. Usually the enzyme was confined to one region of the cytoplasm, especially the periphery or free border of the cell. The reaction was generally diffuse and probably never confined to cytoplasmic granules. Where granules occurred they were always positive in the controls also, indicating the presence of inorganic phosphate. It was, of course, possible that some enzyme was also located in the granules, but its presence was obscured.

A comparison between the distribution of alkaline phosphatase and of ascorbic acid in insects (Day 1949) reveals that these two substances were rarely present together in cells. When they did occur together in the same cells, e.g. in fat body of *Blattella,* they generally occupied different positions in the cells. Exceptions to this generalization were found in the nervous tissue.

(ii) *Dermal Tissue.*—Hypodermal tissue, except for sense cells (see below), was negative in all species studied. Occasional nuclei of the hypodermis of *Ctenolepisma* and of *Locusta* were positive but the majority contained no demonstrable alkaline phosphatase.

(iii) *Alimentary Canal.*—Foregut. The epithelial cells of the pharynx of several insects gave a strongly positive reaction. In *Ctenolepisma* (Plate 1, Fig. 1) the pharyngeal epithelium in the head region stained uniformly, the reaction being especially marked in a sense organ just below the supraoesophageal ganglion. In *Locusta* (Plate 1, Fig. 2) the epithelium was uniformly stained, while in *Tenebrio* only the lumen border of the epithelium was positive.

Midgut. In many species there was no obvious reaction in the midgut epithelium, but in *Ctenolepisma* the cytoplasm was faintly positive, the nuclei strongly so, and in *Blattella* a few small positive areas were seen between the nucleus and the striated border in both the epithelium of the midgut and its caeca.

TABLE 1

HISTOLOGICAL DISTRIBUTION OF ALKALINE PHOSPHATASES IN INSECT TISSUES

Species	Dermal Tissues Blood Heart	Alimentary Canal			Malpighian Tubules	Tissue				Glands (Salivary and Silk)	Oenocytes	Reproductive Systems
		Foregut	Midgut	Hindgut		Storage	Respiratory	Muscle	Nervous			
Ctenolepisma	N Dermal tissues only	+ (Plate 1, Fig. 1)	+	+	−	N	−	+ −	+			
Blattella	−	+	+ Especially muscularis	+ (Plate 3, Fig. 13) Rectum (Plate 2, Fig. 7)	+ Muscles rarely (Plate 3, Fig. 15)	+ (Plate 2, Fig. 8)	−	+	+	+ (Plate 2, Fig. 11)	−	+ (Plate 2, Fig. 10)
Periplaneta embryo	−	−	−	−	−	+	−		+			+
Periplaneta adult	−	+	+ (Plate 1, Fig. 3)		−	−	−	−	+			
Locusta	N Dermal tissues only	+ (Plate 1, Fig. 2)	−	+ Rectum (Plate 3, Fig. 14)	−	+ (Plate 2, Fig. 9)	+ In rectum only	+ −	+ (Plate 2, Fig. 12)		−	+
Tenebrio larva	−		−	+		−	−	+ −				
Tenebrio adult	−	−	Faintly positive reaction	+	+	−	−	−			−	
Pieris larva	−		N			N	N	+ −		+	+ (Plate 3, Fig. 16)	
Tineola larva	−	−	+ Columnar cells only		+	−	−	−		+		
Lucilia larva	−		+ (Plate 1, Fig. 4)	+	−	−	−	+ −	+			
Lucilia pupa	−		+ (Plate 1, Fig. 5)		−	−	−	−	+			
Lucilia adult	−		+	+ Especially rectal glands (Plate 1, Fig. 6)	+	−	−	−	+	+	−	+

− indicates alkaline phosphatase absent. + indicates alkaline phosphata se present. No mark indicates the tissue was not studied. N indicates the positive reaction is confined to the nucleus.

The most unexpected location for alkaline phosphatase found in this investigation was the circular muscularis of the anterior third of the midgut of *Blattella* and of *Periplaneta* (Plate 1, Fig 3). The region was distinctly limited and the reaction was much stronger than elsewhere in the gut. Posteriorly the positive reaction ceased without any change in the histological appearance of the muscles following normal staining procedures. Only the circular muscles, not the longitudinal muscles in contact with them, gave the reaction. It is interesting to note the positive muscles occurred in the same region as the nerves from the stomodeal nervous system, which innervate only the anterior end of the midgut. In the *Tenebrio* adult the intercryptal epithelium and the cells of the crypts themselves gave a faintly positive reaction. In *Tineola* larvae some columnar cells of the epithelium were faintly positive, while in the *Pieris* larva the nuclei but not the cytoplasm of the same cells also gave a positive reaction. In *Lucilia* larvae the midgut (Plate 1, Fig. 4) cells gave a strong positive reaction on their lumen border and the chromatin of the polytene nuclei were also intensely positive (cf. Krugelis 1945). In the pupa (Plate 1, Fig. 5) the reaction at first was confined to the periphery of the cell but appeared to spread through the foamy cytoplasm as the cells prepared to undergo the changes of metamorphosis. The midgut of the adult *Lucilia* was negative.

Hindgut. The epithelium of the hindgut may either be positive or negative for alkaline phosphatase, depending on the species. A negative reaction was observed in the adult *Tenebrio,* and the larvae of *Tineola* and *Lucilia,* while a positive reaction was found in *Ctenolepisma, Blattella, Locusta,* and the larva of *Tenebrio.* In *Blattella* and *Tenebrio* some cells of both the large and small intestines gave a strong reaction on the inner cell border, with a less marked reaction in the nuclei (Plate 3, Fig. 13). In the *Locusta* hindgut, which has a thick chitinous intima, the entire cytoplasm of the epithelial cells was positive, but the muscularis contained no alkaline phosphatase.

In the rectum of *Blattella* (Plate 2, Fig. 7) the "rectal glands" were negative, but the thin epithelium connecting the "glands" was strongly positive. This positive region includes the epithelium up to the chitinous ring which surrounds each rectal gland. The concentration of the enzyme in the cells referred to by Wigglesworth (1933) as "vestigial" suggests that these cells do, in fact, serve a function other than the mere joining of the "rectal glands." It is possible that it is these, rather than the "gland" cells which absorb water from the faecal pellet, as it is formed.

The alkaline phosphatase in the rectal glands of *Locusta* presented a very unusual appearance since the enzyme was confined to the region of the intracellular tracheoles characteristic of some Locustidae. These tracheoles arise from tracheal end cells, the presence of which was observed by Tietz (1923) in *Dissosteira* and by Chauvin (1941) in *Schistocerca,* but which have never been adequately described. The rectum of *Locusta,* like that of most insects, is well tracheated. Large trunks pass through the muscularis and penetrate between

the cells of the epidermal layer of the rectal glands. At this point there is a nucleated tracheal end cell and the trachea gives off a number of tracheoles which become intracellular and run back towards the muscularis. The tracheoles are arranged like the ribs of an umbrella and these and the tracheal end cell both gave a positive reaction for alkaline phosphatase (Plate 3, Fig. 14). This was the only case observed in which the enzyme was associated with the respiratory tissue.

The rectum in the larva of *Tenebrio,* the larva of *Anthrenus,* and the adult honey bee gave no positive reaction.

The rectal papillae of *Lucilia* contained a high concentration of alkaline phosphatase mostly confined to the periphery of the large papillar cells (Plate 1, Fig. 6). The intracellular tracheae (Graham-Smith 1934), unlike those of the *Locusta* rectal gland, gave no positive reaction for alkaline phosphatase.

(iv) *Malpighian Tubules.*—Intracellular granules of phosphate occurred in the malpighian tubules of a number of insects including *Blattella* (Plate 3, Fig. 15), *Periplaneta* (Plate 2, Fig. 8), *Locusta,* adult *Tenebrio, Apis* workers, and *Lucilia* larvae, pupae, and adults. In the larvae and pupae of *Lucilia* there were granules in the tubule lumen as well as in the cytoplasm. The cytoplasm may also give a positive reaction for alkaline phosphatase, as in some regions of the tubules of *Blattella* (Plate 3, Fig. 15), and as reported by Bradfield (1946) in *Cossus.* More frequently only the striated border of the tubules was positive. In *Tineola* the striated border was positive in those regions of the tubules which envelop the rectum, but was less so in other regions, while in the worker honey bee the tubules were positive in some regions and negative in others. In *Lucilia* adults the striated border was positive over the greater part of the tubule (Plate 1, Fig. 6).

(v) *Fat Body.*—In most species studied the fat body contained no alkaline phosphatase. In some lobes of this tissue in *Blattella* and *Periplaneta* (Plate 2, Fig. 8) there were definite positive areas, usually on the periphery in the former species, but mostly contiguous with the bacteroid cells in *Periplaneta.* In *Locusta* the cytoplasm of the fat body was positive and the nuclei strongly positive (Plate 2, Fig. 9). In no other species examined did the fat body contain large amounts of alkaline phosphatase. Only the nuclei stain in *Pieris* larvae and in the larvae and pupae of *Lucilia.* In pupae of the latter species some of the globules of the fat body cytoplasm gave a faint reaction.

(vi) *Respiratory Tissue.*—The trachea or tracheal epithelium was negative in all insects studied. Some nuclei may give a positive reaction as in *Pieris.* The positive intracellular tracheolar system in *Locusta* rectal glands has already been described.

(vii) *Muscle Tissue.*—Muscles in almost all species gave no reaction for alkaline phosphatase. A few, as in the thorax of *Ctenolepisma* and *Lucilia* and some circular muscles of the *Pieris* hindgut, may be faintly positive, but are weakly so in comparison with other tissues of these species.

This makes all the more interesting those cases in which alkaline phosphatase is abundant in muscles, namely, the circular muscles of the anterior end of the midgut of *Blattella* and *Periplaneta* (Plate 1, Fig. 3), some spiral muscles of *Blattella* malpighian tubules (Plate 3, Fig. 15), and a small group of muscles of the female genitalia of *Blattella*.

(viii) *Nervous Tissue.*—Generally the nervous tissue is strongly positive for alkaline phosphatase. For example, sense organs of the leg and pharynx (Plate 1, Fig. 1) of *Ctenolepisma* were positive, as was the cytoplasm of the cuticular sense organs of *Locusta*. The brain was positive in all insects studied and in most species the ganglia and ventral nerve cord were rich in the enzyme. Plate 2, Figure 12, shows a region of the brain of *Locusta*. The nuclei and optic fibre tracts are positive. The ventral nerve cord of *Locusta* was an exception and gave no reaction. The nerve cell bodies of the central nervous system and fibre tracts were the only positive regions in the embryo of *Periplaneta*. The neurilemma was negative.

(ix) *Glands (except Accessory Sex Glands).*—Bradfield (1946) reports a positive reaction in the spinning glands of the moth, *Cossus cossus*. This has been confirmed in the silk glands of *Pieris* and *Tineola*. The salivary glands in *Ctenolepisma* and *Blattella* (Plate 2, Fig. 11) contained some positive regions as did the pygidial glands of the male *Blattella*. The enzyme in *Blattella* salivary glands was present in both the peripheral cells and the granular cells, but was especially strong in all nuclei and in the intralobular ducts.

The corpora allata and cardiaca were negative in all species studied. However, Arvy and Gabe (1947) record a faint positive reaction in the corpus allatum of *Chironomus*. The properties of the phosphatases in *Drosophila* salivary glands have been investigated by Doyle (1947).

(x) *Oenocytes and Pericardial Nephrocytes.*—The oenocytes in most species are free from alkaline phosphatase, except for the nuclei which may show slight positive reactions (*Locusta, Tenebrio, Lucilia*). In the larva of *Pieris* the reaction was strongly positive (Plate 3, Fig. 16). A rather unusual cytological distribution was evident in this case. The peripheral cytoplasm and the irregularly-shaped nuclei had a high concentration of enzyme but surrounding each nucleus there was a clear area frequently almost devoid of enzyme. Pericardial nephrocytes were negative in all species studied.

(xi) *Sex Organs.*—The spermatocytes may be faintly positive in *Periplaneta*. Other gonadal tissue of this species and male gonadal tissue of all other species were negative. Male accessory glands gave a positive reaction in *Blattella* (Plate 2, Fig. 10), *Locusta,* and *Lucilia*. Female accessory glands were positive in *Blattella* but have not been studied in other species.

IV. Effect of Ascorbic Acid on Alkaline Phosphatase

There are many reports of the effect of scurvy on alkaline phosphatase in vertebrates. Thus, for example, in a survey of 241 cases of scurvy in man, Dogramaci (1946) found serum alkaline phosphatase to be abnormally low, and

Bourne (1943*b*) concluded from his study of calcification in guinea pigs that ascorbic acid may act in the formation or stabilization of alkaline phosphatase. But he found (1943*a*), as did Harrer and King (1941), that kidney alkaline phosphatase was only slightly changed in the scorbutic guinea pig. Since the effects of feeding the ascorbic acid antagonist, *d*-glucoascorbic acid, to *Blattella* have recently been investigated (Day 1949), it seemed worthwhile to study these insects for any changes in alkaline phosphatase. The results showed that the positive reaction of the circular muscles at the anterior end of the midgut was slightly reduced. But the enzyme was apparently unchanged in the epithelium of the rectal glands, in the nervous system, and the spiral muscles of some malpighian tubules. In addition there were deposits of phosphate at the distal end of many of the epithelial cells of the large intestine. These occur to some extent in normal *Blattella* and are probably the blue-staining granules reported previously in these cells (Day 1949). In short, the diet containing 10 per cent. *d*-glucoascorbic acid had only a very slight effect on the histochemically detectable alkaline phosphatase.

V. Discussion

(*a*) *Correlation between Histochemical and Chemical Data*

The phosphatases of insects have been studied chemically by Nakamura (1940), Drilhon (1943), and Drilhon and Busnel (1945). The first author found alkaline phosphatase in the silk gland of *Bombyx*, while the later authors found in a number of insects from various orders that the foregut contained a low concentration of alkaline phosphatase and the midgut a stronger concentration, whereas in the hindgut the enzyme was lacking. While they found high concentrations of phosphatase in the malpighian tubules of all species, it was acid rather than alkaline phosphatase and so would not show up in the method used in the present study. The histological results agree with the distribution indicated by chemical methods as far as they go.

(*b*) *Distribution of Enzyme in Tissues of Vertebrates and of Insects*

The majority of vertebrate tissues in one species or another have been found to contain phosphatases (Gomori 1941). They occur in so many locations that their functions must be many and varied. Moog, in her excellent review (1946), has considered phosphatases in relation to calcification, in transport mechanisms, and in relation to growth and differentiation. But even these broad categories do not include the functions of all phosphatases, as for example, the muscle phosphatase of Knoevenagel (1940), that found in the present study, and the phosphatases of the central nervous system (see, for example, Carandante 1941). It will be instructive to compare the distribution in insects with that in vertebrates. So far as calcification is concerned there is no comparision in insects, the place of bony structures being taken by cuticular exoskeleton and apodemes, few of which are associated with alkaline phosphatase. But there are many examples in which the distribution of the enzymes is compatible with a possible

function in transport mechanisms. Bradfield's report (1946) may be considered in this category, and so may the midgut, malpighian tubule, "rectal gland," and male accessory gland phosphatases of this paper.

We have no data on the presence of alkaline phosphatase in insect embryonic tissue, except its observation in the nervous system of *Periplaneta* embryos.

Two interesting points remain to be considered. The first is the wide occurrence of the enzyme in the epithelium of the pharynx. To this organ is usually attributed no function other than the transport of food from the buccal cavity into the crop. Moreover, the epithelium is overlaid by a chitinous intima, sometimes of considerable thickness. When the cytoplasm is continued into this intima as in sense organs, it still gives the positive reaction. The function of the enzyme in this epithelium is an interesting problem and the extent to which this region is capable of absorption requires study. Perhaps the presence of the enzyme can be correlated with the activity of this portion of the gut.

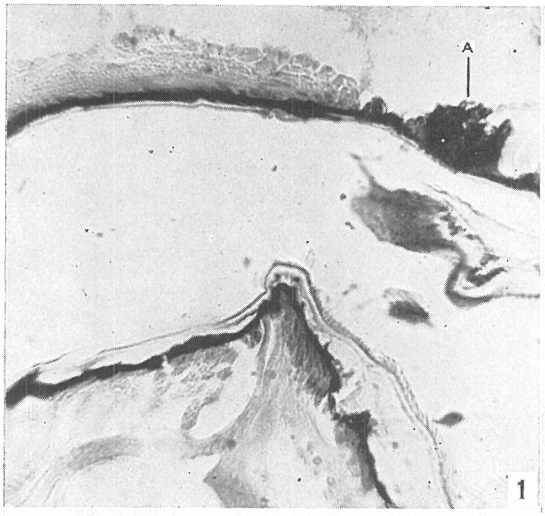
The second point of interest is the degenerating midgut epithelium in the *Lucilia* pupa. The enzyme appears to increase with progressive vacuolization of the cells as though it were playing a part in their destruction. No function for the degenerating midgut cells is known at this stage. In vertebrates the intestinal mucosa is always rich in alkaline phosphatase. The hypothesis has arisen that the enzyme may be involved in the transport of glucose and other molecules through the gut wall. If this is so in vertebrates, some other mechanism must exist in insects, for the enzyme is not conspicuous in the midgut of most species, which region is generally considered to be of considerable importance in absorption. In *Blattella,* when a positive reaction occurs, it is in the golgi zone (cf. Emmel 1945), presumably more concerned with secretion than absorption. The striated border of some tissues is positive, of others negative.

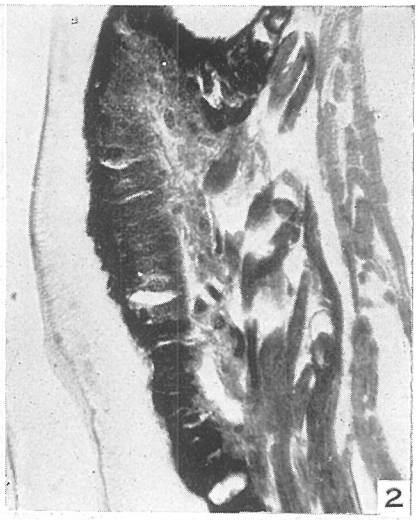
Finally, mention should be made of those tissues in the species examined in which alkaline phosphatase could not be detected despite careful study. These include dermal tissue, pericardial nephrocytes, heart, corpora allata, corpora cardiaca, and imaginal discs (the latter were studied only in *Lucilia*). Two other tissues, fat body and oenocytes, were negative in every species examined except one, and in each exception the tissue was strongly positive. A great deal more will have to be learned of the functions of these organs before an explanation can be advanced.

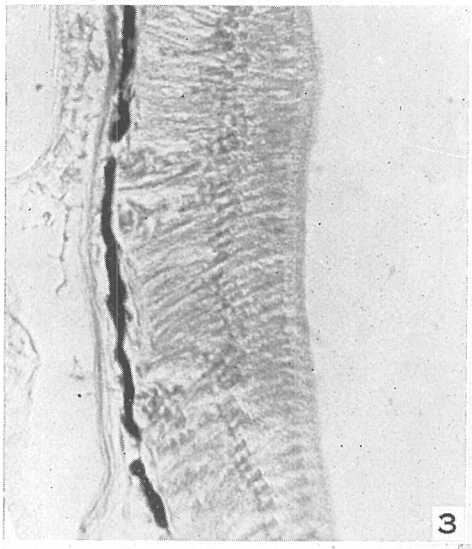
(*c*) *Distribution of Inorganic Phosphate*

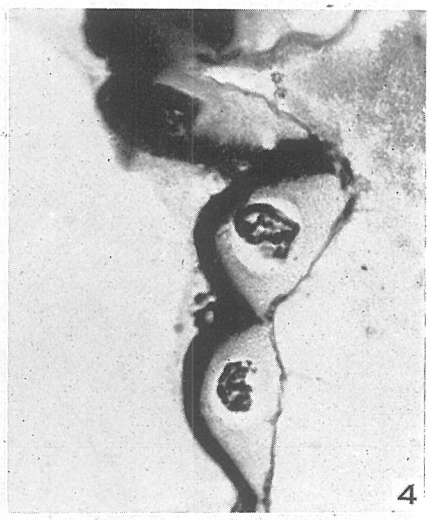
Inorganic phosphate deposits are readily observed both in slides stained for alkaline phosphatase and in the control slides. It is interesting to note that considerable deposits are found in some insect tissues. The accumulation in the lumen of the malpighian tubules of *Lucilia* and in the cytoplasm of the malpighian tubules of a number of species, has already been mentioned.

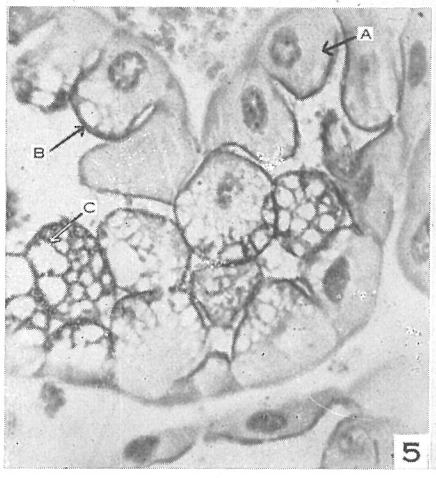
The hindgut of *Blattella* normally contains a little phosphate (Plate 3, Fig. 18), but the deposits increase when the insects are fed *d*-glucoascorbic acid or inorganic phosphate. In *Ctenolepisma,* however, the hindgut normally contains

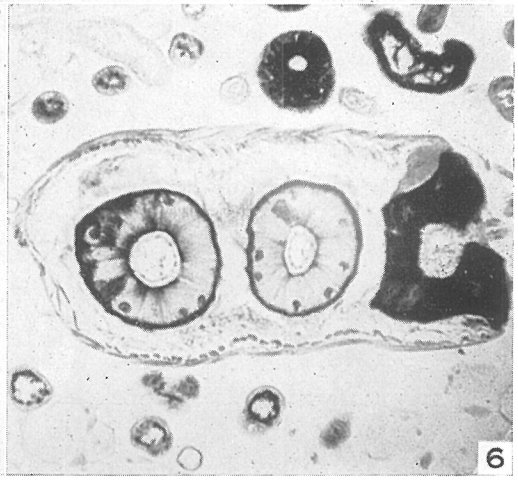




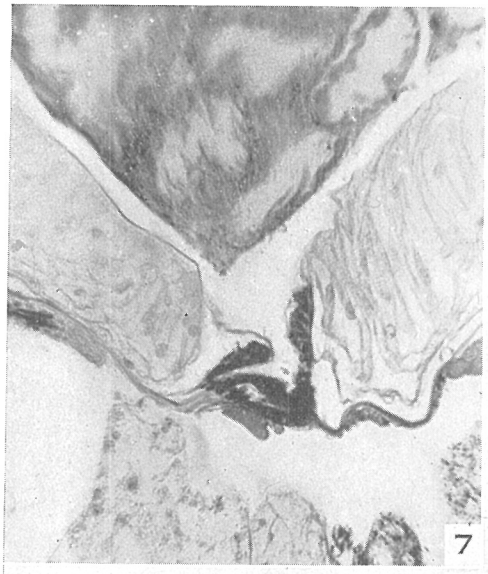








DAY.— THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN INSECTS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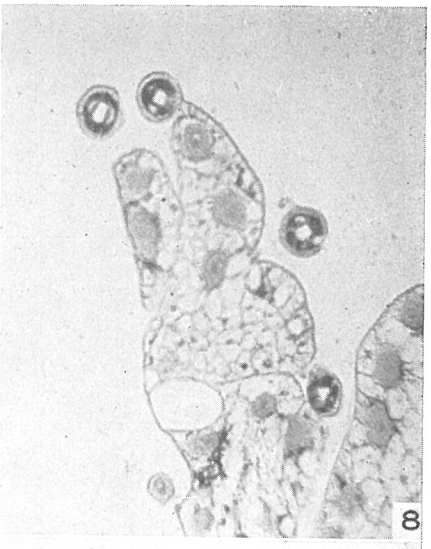
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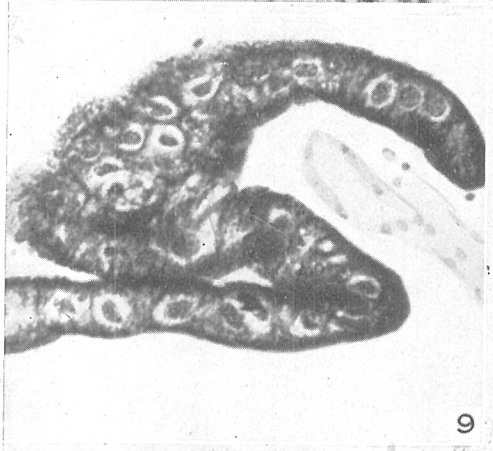
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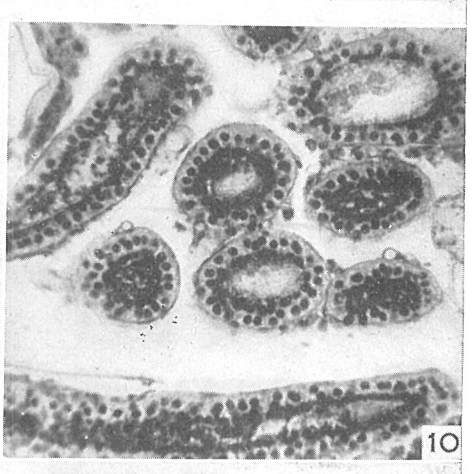
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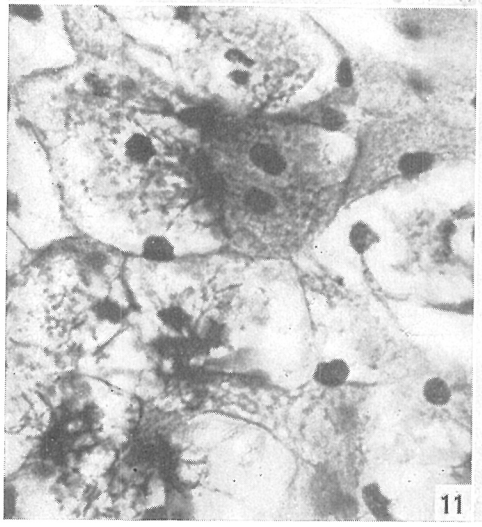
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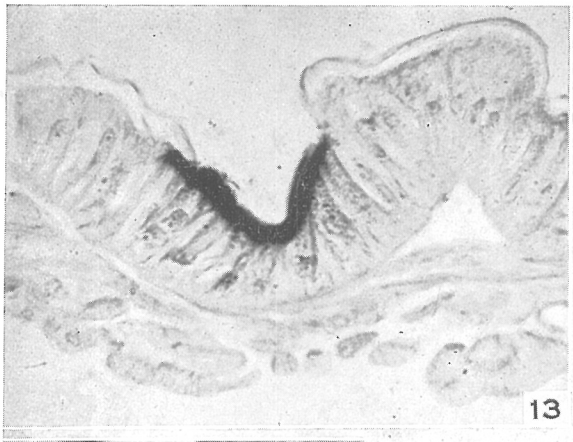
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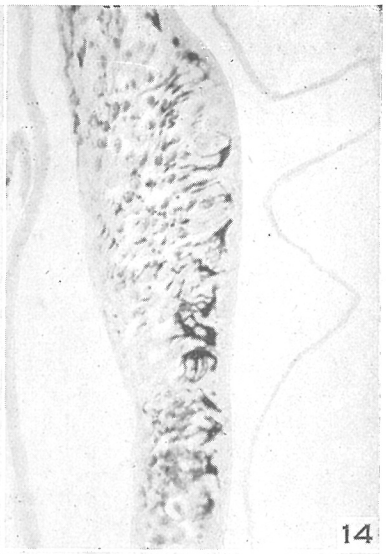
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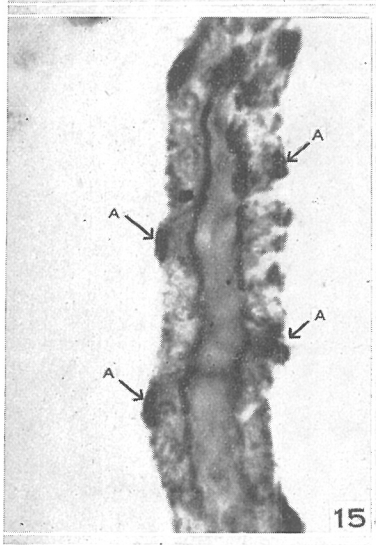

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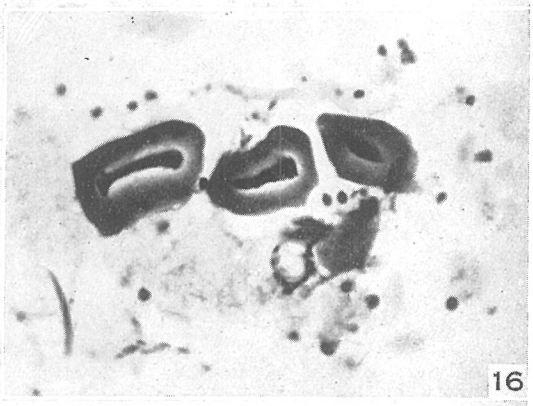
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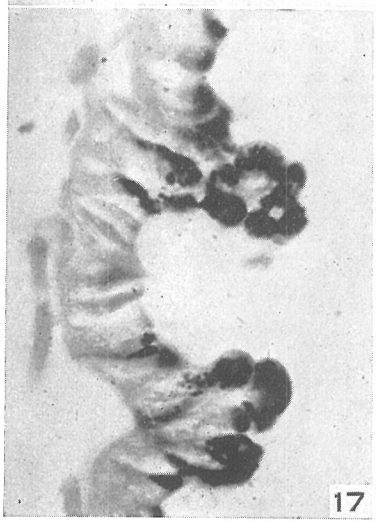
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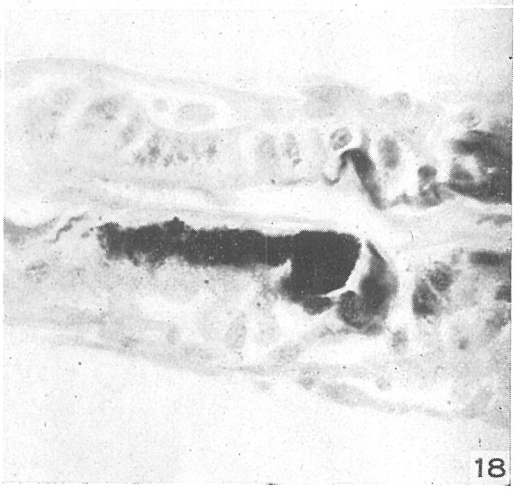
18

DAY.— THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN INSECTS

granules of phosphate. Hoskins and Harrison (1934) recognized that the contents of the honey bee midgut were unusual in containing a large amount of phosphate (0.046M), but found that the excreta contained very little. They considered that absorption occurred in the hindgut. However, the histochemical method demonstrates very considerable deposits in the cells of the midgut (Plate 3, Fig. 17) where it occurs particularly in the apical regions of the epithelial cells at the apex of the folds. Koehler (1920) considered that the midgut cells contained "lime." It is interesting that the granules normally present in *Ctenolepisma, Apis,* and those which appeared in the hindgut of *Blattella* are all very similar in size, but those found in the *Lucilia* malpighian tubules are much larger.

VI. Acknowledgments

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EXPLANATION OF PLATES 1-3

PLATE 1

A Zeiss IBSO attachment was used for the photomicrographs taken under oil immersion. For lower powers a sliding copying attachment for the Leica camera was employed. Section 10 microns, stained for alkaline phosphatase by Gomori technique. Various magnifications.

Fig. 1.—*Ctenolepisma*, L.S. pharynx. Buccal cavity to the right. Note entire pharyngeal epithelium is positive, especially sense organ (A) with positive cytoplasm extending through chitinous intima, on dorsal side at the buccal end. x 160.

Fig. 2.—*Locusta*, L.S. pharynx. Positive epithelium beneath thick chitinous intima. x 220.

Fig. 3.—*Periplaneta*, L.S. midgut. Epithelium, longitudinal muscles, trachea negative. Only circular muscles are positive. x 380.

Fig. 4.—*Lucilia* larva, midgut epithelium. Note positive region of cells towards lumen and positive polytene chromosomes. x 220.

Fig. 5.—*Lucilia* pupa, midgut cells showing increase in phosphatase activity with increasing vacuolization (degeneration) of midgut cells (A to C). x 140.

Fig. 6.—*Lucilia* adult, T.S. abdomen. Rectal papillae show phosphatase on inner and outer cell borders. Malphigian tubules and male accessory glands also positive. x 140.

PLATE 2

Details of photomicrographs as in Plate 1.

Fig. 7.—*Blattella*, T.S. rectum. Only cells giving positive reaction are those of the thin epithelium between the rectal pads. x 140.

Fig. 8.—*Periplaneta*, fat body. Positive regions mainly contiguous with bacteroid containing cells. Malphigian tubules contain granules of phosphate. x 140.

Fig. 9.—*Locusta*, fat body. The cytoplasm of the fat body is positive, the nuclei are strongly positive. x 380.

Fig. 10.—*Blattella*, male accessory glands. Nuclei strongly positive, distal cell borders positive. Surrounding fat body negative. x 140.

Fig. 11.—*Blattella,* salivary gland. Nuclei strongly positive, cytoplasm around central ductules positive. x 620.

Fig. 12.—*Locusta,* brain optic lobes. Nuclei positive, many nerve fibres positive, but not in all regions of the brain. x 380.

PLATE 3

Details of photomicrographs as in Plate 1.

Fig. 13.—*Blattella,* T.S. hindgut – large intestine. Positive zone on periphery of some epithelial cells only. Nuclei in this region also positive. x 380.

Fig. 14.—*Locusta,* L.S. rectum showing unusual appearance of positive tracheal end cells and intracellular tracheoles. x 140.

Fig. 15.—*Blattella,* L.S. portion of malpighian tubule. Spiral muscle in section postive (A). The cytoplasm contains alkaline phosphatase and also granules of inorganic phosphate. x 380.

Fig. 16.—*Pieris,* oenocytes, cytoplasm intensely positive. Nucleus also positive, and nuclei of surrounding fat body similarly positive. x 620.

Fig. 17.—*Apis,* T.S. midgut showing inorganic phosphate granules especially in apical regions of epithelium. x 380.

Fig. 18.—*Blattella,* L.S. hindgut – small intestine, inorganic phosphate granules in distal regions of epithelium. x 380.