STUDIES ON PHOSPHATASES AND LIPASES IN CERTAIN TURBELLARIA

By

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INTRODUCTION

Although a good deal of work has been done on the taxonomy and morphology of the Turbellaria particularly in Europe, comparatively little is known of their physiology. Two major studies on physiology were those of Westblad (1923), on digestion and excretion in triclads and rhabdocoeles, and Willier, et al (1925), on intracellular digestion in the triclads. Each of these works has provided an excellent basis for further studies. In the former a controversy in regard to digestion is raised. St. Hilaire and Metchnikoff on the one hand, believed digestion to be intracellular, and Arnold and Löhner on the other, hold that digestion is chiefly extracellular, with the pharynx acting as the source of enzymes. Of course, the work of Westblad and several studies since that time (1908-1922) have shown that digestion occurs in both an intracellular and an extracellular manner. In both papers, Willier's in particular, the mechanics of feeding, including the phagocytic action of the amoeboid cells, is described. Subsequent spherule formation, absorption, and assimilation into the parenchyma, are also described.

Child and Watanabe (1935) and Rulon (1937) also worked on the physiology of flatworms, but primarily on the factors of significance in regeneration.

Bahrs (1929 and 1931) and Wulsen and Bahrs (1931 and 1935) made a series of nutritional studies on Planaria which have proved useful in connection with the present work.

Turbellaria are for the most part rather small and not readily studied by many of the usual physiological techniques. This may be one of the reasons for the gaps which exist in our knowledge of their physiology. The relatively new histochemical techniques seemed to offer a means of attacking some of these problems. Studies were therefore undertaken on alkaline phosphatase, acid phosphatase, and lipase in several Turbellaria using histochemical methods. Later fats were also investigated to determine whether they were present, and if so whether there was any correlation between their distribution and that of the three enzymes, at least two of which might be expected to act upon them.

The problem was further extended to study a possible correlation between nutrition and the presence and distribution of the enzymes, and to some degree of the fats. This was done because of the activating affect which food or some of its constituents might have on enzymes and their precursors, and because of the fat composition of some food or the conversion of food into fatty substances after absorption.

Histochemical studies have been conducted on many vertebrate tissues, and on some invertebrates, but not on the Turbellaria. Up to 1923 workers interested in enzymes were limited to biochemical methods. These were good for quantitative analyses, but left much to be desired in locating the sites of enzyme activity.

Robison (1923) devised the first histochemical technique for alkaline phosphatase. By a silver-phosphate reaction he showed alkaline phosphatase to be intensely active in areas of bone formation.

This method was quite popular through the late nineteen thirties. In 1939 Gomori, University of Chicago, and Takamatsu, Japan, independently described an improved technique, using the calcium-cobalt-sulfide reaction, which renders the region of activity black. This method has now been used with most of the animal phyla and some plants.

The significance of phosphatase has caused much controversy. Moog (1945) published a detailed survey of work on phosphatases. Danielli (1953) in his Critical Approach to Cytochemistry supports the validity of the Gomori method, provided it is used properly. Both Moog and Danielli attribute a hydrolytic rather than a synthesizing function to alkaline phosphatase. However, Danielli does feel that there is a good deal of evidence pointing to its functioning as a phosphokinase in energy metabolism.

The present study is primarily a qualitative approach, but I have attempted to evaluate the results in a quasi-quantitative manner by using a scale of numbers from 0 to 3 to indicate the intensity of stain. Admittedly this is a subjective evaluation and certainly not an accurate measurement of the activity of the enzymes. However, it has the virtue of at least giving some indication of the degree of activity, and both Gomori and Danielli feel that there is some justification for a quasi-quantitative interpretation.

MATERIALS AND METHODS

Five species representing three orders of freshwater Turbellaria were subjected to tests for alkaline phosphatase, acid phosphatase, and lipase, using the techniques described by Gomori (1952) and for fat, using the McManus (1946) technique.

All specimens were obtained on or near the University of Florida Campus: Stenostomum tenuicaudatum (rhabdocoele) came from a campus sink near 13th Street; Geocentrophora applanata (alloeocoele) from the filters of the experimental sewage disposal plant on campus; Dugesia tigrina (triclad) from a campus sink near the Dairy Laboratory and from Prisser's Pond near 10th Avenue; Curtisia foremani (triclad) from a small creek underpassing Road #26, 3.5 miles northwest of the campus; Bipalium kewense (land triclad) under moist logs in surrounding woodlands.

Worms were either fixed immediately after capture or were starved for definite periods of time before fixation. The purpose of this variation in treatment was to determine whether there was any correlation between length of time since feeding and the amount of enzyme activity.

Preliminary studies were made on rates of digestion and nutritive value of different types of food and on the relationship between length of time since feeding and regenerative ability (using Dugesia tigrina as the experimental animal). Worms thrived on earthworm tissue and could be kept in a good healthy state for as long a period of time as desired on this diet. A 2% solution of

peptone apparently lacked some essential food element, for after a short initial period of growth animals fed on this artificial food stopped growing and within two weeks all were dead. Therefore, earthworm tissue was used as the chief food material for this triclad in the subsequent work on enzymes and fat. Various other foods were occasionally used, however, including liver, fish brain, egg yolk, and fish blood.

In order to obtain some idea of how long after feeding it would take for the gastrovascular sac to be emptied of food, specimens of D. tigrina were fed bits of earthworm tissue until they would eat no more, and were then macerated, some at 10 minutes, others at 30 minutes, and still others at 1, 2, 3, 4, or 5 hours after feeding. The amoeboid cells were well rounded and distended with food after 30 minutes, but a good deal of tissue was still present in the gastrovascular cavity. It was not until about five hours after feeding that the food seemed to be well removed from the digestive cavity.

Child (1935), Hyman (1925), and Rulon (1940) reported experiments in which they demonstrated the relationship between metabolic activity and regeneration in Euplanaria dorotocephala in connection with the axial gradient theory. It seemed possible that regenerative ability might serve as an indication of the metabolic state in the worms. To test this idea a series of worms were cut in half transversely through the base of the pharynx at various intervals after feeding. The percentage of successfully regenerating pieces proved to be definitely higher in specimens which were cut

2, 3, or 4 days after feeding than in those in which a shorter or longer period of time had elapsed since feeding. Further experiments demonstrated that worms cut in half three days after feeding had a higher percentage of successful regeneration than any other group. Since totipotent cells of the parenchyma provide regenerative tissue to wounded areas, this suggests that about three days are required after the ingestion of food by the worm for these parenchymatous tissues to reach their optimum peak of metabolic activity. As will be pointed out later, the work on enzymes produced further evidence for this idea.

For starvation, worms were kept in filtered habitat water in an air conditioned laboratory at 20°- 22°C. Aeration was provided for those taken from streams (Curtisia foremani) or from the sewage filters (Geocentrophora applanata). Those from still water (Dugesia tigrina and Stenostomum tenuicaudatum) were maintained in large open-mouthed jars and fingerbowls, respectively. Throughout this report the number associated with a worm indicates the number of days since the last feeding, i.e., a 10-day worm is one which has not been fed for 10 days.

For either phosphatase or lipase tests, animals in water were placed for thirty minutes in a refrigerator which was set at a temperature of 5°C. They were then fixed in acetone at the same temperature. The Gomori technique followed. The results yielded by this technique are mainly qualitative, but a quasi-quantitative interpretation can be made by a study of the relative intensity of

the stain. Both evaluations are attempted in this paper. An arbitrary and necessarily subjective scale was set up for recording the observations as follows: 0 = no visible activity; 1 = low activity; 2 = medium activity; 3 = high or intense activity.

For fat determinations, animals were fixed in Baker's fluid at room temperature (McManus method modified by fixing 24 hours instead of one week). The routine McManus technique followed.

The paraffin method was used exclusively with the oven maintained at a temperature of 55°C., during embedding.

Animals were serially sectioned at from 8 to 12 micra, but usually at 10 micra. A Spencer rotary microtome with a single-edged razor blade was used.

Tissue mounts were made on regular microscope slides and the various immersion solutions were in coplin jars.

For studies on enzymes the tissue was incubated at 37°C. in a thermostatically controlled oven. All slides received the same treatment except that those used as controls had the substrate omitted. Control tissue and experimental tissue were taken from the same specimen using alternating ribbons of sectioned material.

Occasionally the acid phosphate mixtures were centrifuged to spin down lead phosphate resulting from a reaction between lead nitrate and free phosphate ions. The supernatant was then used as the substrate mixture.

Slides were studied with a compound microscope. The results were recorded in terms of stain intensity, as described in an earlier

paragraph.

Alkaline phosphatase tests were made on 100 Dugesia tigrina, 29 Curtisia foremani, 2 Bipalium kewense, 28 Geocentrophora applanata, and 44 Stenostomum tenuicaudatum.

Acid phosphatase tests were made on 32 Dugesia tigrina, 6 Curtisia foremani, 2 Bipalium kewense, 12 Geocentrophora applanata, and 38 Stenostomum tenuicaudatum.

Tests for lipase were made on 33 Dugesia tigrina, 17 Curtisia foremani, 1 Bipalium kewense, 5 Geocentrophora applanata, and 7 Stenostomum tenuicaudatum.

Determinations for fatty substances were made on 25 Dugesia tigrina, 5 Curtisia foremani, 1 Bipalium kewense, 4 Geocentrophora applanata, and 11 Stenostomum tenuicaudatum.

Photomicrographs were made with a Spencer camera apparatus.

RESULTS

I. ENZYMES

A. Alkaline Phosphatase

Evidence of the activity of alkaline phosphatase was found in abundance, especially in the aquatic triclads. It was not limited to one or only a few loci, but was rather widespread, occurring in most tissues and organs of the triclads *Dugesia tigrina* and *Curtisia foremani*. The intensity and distribution seemed to be correlated with recentness of feeding or state of starvation.

The types of food administered did not appear to produce significant effects on the distribution and intensity of activity. Certain exceptions will be indicated in the discussion of the respective tissues and organs.

Data are presented on the following page (Table I) in the form of mean intensities, taken from the original interpretations which were recorded by a system of arbitrary numbers. Thus, from Table I an overall picture can be obtained, showing a wide distribution of rather intense activity in the aquatic triclads, and the effects of feeding and starving upon the activity of the enzyme. At the same time, it can be seen that the land triclad exhibited some activity, less widely distributed, and similarly for the allococoels and rhabdocoels. These will all be discussed separately later on, and in more detail.

TABLE 1. MEANS OF ESTIMATED INTENSITIES OF ALKALINE PHOSPHATASE ACTIVITY

ANIMAL	Days Starved	Site and Intensity (1 = low; 2 = medium; 3 = high) of Activity										
		Phx	Gut	Par*	T	O	RA	V	NT	ES	LNG	Epid**
Dugesia tigrina (100)	0	1.99	1.78	.88	2.12	1.66	--	--	1.16	1.18	.80	.44
	1	2.44	1.44	.92	3.00	1.00	--	--	.94	.44	1.56	.81
	2	3.00	1.63	1.02	3.00	--	--	--	1.58	.75	2.00	.79
	3	1.77	.94	.93	2.66	--	--	--	.69	.63	.81	.72
	4	2.31	1.58	1.35	3.00	3.00	--	--	1.89	1.44	1.56	1.34
	5	2.50	1.00	1.39	2.25	0.0	--	--	1.69	1.63	1.88	.76
	6	2.69	1.00	1.24	3.00	--	--	--	1.83	2.92	2.33	.63
	7	3.00	.57	1.42	3.00	--	--	--	2.13	1.75	2.38	.50
	8	3.00	0.0	.73	--	--	--	--	.80	.80	.80	.30
	9	2.50	1.00	.90	--	--	--	--	1.50	2.00	2.50	.50
	10	2.00	1.07	.98	--	--	--	--	1.71	2.00	1.43	1.29
	21	2.67	.25	.84	1.00	1.00	--	--	2.00	3.00	.75	1.88
	60	2.53	--	.61	--	--	--	--	3.00	0.0	0.0	1.00
Curtisia foremani (29)	0	1.33	2.01	1.63	.33	1.00	--	--	1.00	1.33	.83	.75
	1	2.33	3.00	2.50	--	--	2.00	--	1.50	2.50	1.50	2.00
	2	1.83	2.25	1.50	2.00	--	1.00	--	1.00	1.50	1.00	1.70
	3	2.25	1.60	2.05	--	--	2.50	--	1.50	2.25	1.00	1.00
	4	2.17	2.00	1.80	3.00	--	2.00	1.00	2.00	2.75	1.00	1.50
	5	3.00	2.62	1.40	2.00	--	2.50	2.00	1.25	1.75	1.25	.50
	6	3.00	1.00	2.10	--	--	--	--	2.50	2.50	1.50	.50
	7	1.00	1.50	.80	--	--	--	--	2.00	1.00	1.00	.50
	8	2.33	2.00	1.20	--	--	0.0	--	2.00	1.00	2.00	.50
	10	.66	0.0	1.70	--	--	--	--	0.0	.40	0.0	2.30
Bipalium kewense (2) Section A	0	--	1.00	2.00	--	--	--	--	--	--	--	2.00
Section B	0	0.0	2.00	1.00	--	--	--	--	--	--	--	2.00
Section C	0	--	1.00	0.0	--	--	--	--	--	--	--	2.00
Geocentrophora applanata (28)	0	2.00	3.00	2.50	--	2.50	--	--	--	--	--	2.50
Stenostomum tenuicaudatum (44)	0	2.00	3.00	2.00	--	--	--	--	--	--	--	3.00

* Includes mesenchyme, muscles, adenal rhabdites, glands, and basal membrane.

** Refer to Page 66 for the key to abbreviations.

1. Dugesia tigrina

This species was perhaps the best one of the five which were studied, in that it was readily fed and starved, with the use of a variety of foods, and provided good, consistent results. As pointed out earlier, there was a great deal of variation in the activity of the enzyme in the various tissues and organs, so they will be listed individually.

Tissues and Organs

a. Pharynx (See Pl.II, Graph 1).

This organ exhibited more evidence of activity of alkaline phosphatase than any other one tissue or organ, and this was true for all stages of nutrition of the worms from well fed to starved. Indeed, for thoroughly starved animals, the pharynx was often the sole site of activity. Such activity was, however, limited almost entirely to the musculature, and with very few exceptions, was uniformly distributed through the three different types of muscles (Pl.III, fig.1). Although some activity was evident in the pharynx at all times, there was a decline immediately after feeding, and eight specimens showed no activity (Pl.III, fig.5). Another similar decline was seen at three days, and still another at 10 days after feeding. Peaks of activity were noted at 2 and 7-8 days after feeding. Another, but less marked rise, occurred on the 21st day and persisted through 60 days after feeding (Pl.II. Graph 1).

b. Gut (See Pl.II, Graph 2).

(1) Amoeboid Cells.- Alkaline phosphatase activity was very pronounced in the amoeboid cells shortly after a feeding. Activity was especially conspicuous in spherules, which appeared in many stages of formation (Pl.III, figs. 3 and 4). In some instances where a 3-5 hour time lag had not been allowed between feeding and fixation, there was little or no visible activity.

As progressively starved animals were investigated, a marked decrease to a complete absence of visible activity was noted on the 8th day. Then a slight recurrence was observed on the 9th day, but there was no apparent activity by the 21st day. By the 60th day there were not even any amoeboid cells present (Pl.IV, fig.20).

(2) Wall. The activity of alkaline phosphatase in the gut wall seemed very closely parallel to that seen in the protruding amoeboid cells. Increased activity (medium) was evident at 4 days after feeding. Another peak (low) was seen at 9 days, and some evidence of activity was still present at 21 days.

c. Parenchyma (mesenchymatous tissue) (See Pl.II, Graph 3).

The location of enzyme activity was somewhat dispersed in the parenchyma, producing a pepper-splashed effect (Pl.IV, fig.7). In liver-fed worms the peak of activity in the parenchyma occurred at a different time from that at which the peak was found in earthworm-fed specimens. During the first three days after feeding the activity was much higher in liver-fed individuals. On the 8th day there was a decline in enzyme activity in liver-fed animals and

simultaneously a sharp increase in activity in earthworm-fed specimens.

d. Dorso-Ventral Musculature

In the dorso-ventral musculature, as in the surrounding parenchyma, the earthworm-fed animals exhibited enzyme activity relatively early (1-3 days), but there was a rapid decline so that by six days there was little or no evidence of activity. Liver-fed specimens on the other hand, showed a sharp increase in activity at five days.

e. Subepithelial Glands

These small structures generally exhibited medium activity so far as alkaline phosphatase was concerned. The intensity became high with prolonged starvation, however. (Pl.IV, fig.14).

f. Gonads

(1) Ovaries.- There was usually low to high activity in the ovaries (only 8 of the observed specimens had ovaries). They stained more intensely in the wall and in the centers of the eggs. Some variation was seen, and the greatest activity occurred 4 days after feeding.

(2) Testes.- Much evidence of alkaline phosphatase activity was seen in the testes. It occurred in the primordial spermatocytes as well as in the spermatids. No variation in intensity occurred. If the organ was present, it always showed a high level of activity.

g. Adenal Rhabdites

There was nearly always some evidence of alkaline phosphatase in these parenchymal structures, but the intensity was only low to medium. The medium state was observed only on the 5th and 9th days of starvation (Pl.IV, fig.17).

h. Basal Membrane

A rather intense stain sometimes accumulated along this membrane peripheral to the parenchyma (Pl.IV, fig.7). A sharp peak of intense activity occurred on the 7th day after feeding in earthworm-fed specimens, followed by a sharp decline so that there was no visible activity on the 8th day. No evidence of alkaline phosphatase was found in any stage of starvation from 8 days on.

i. Epidermal Rhabdites

For the first nine days after feeding activity in the epidermal rhabdites was very slight except on the 4th day (Pl.IV, fig.13). Beginning with the 10th day it increased steadily until it became very high on the 21st day.

j. Epithelial Cells

A small peak of activity in the epidermal cells occurred at 4 days, and there was a still larger upsurge at 10 days. It then remained fairly constant, even in animals starved 60 days.

k. Excretory System

Visible activity remained low in the excretory system for the

first few days after feeding. However, two sharp rises occurred, one on the 6th and the other on the 21st day. This was followed by a gradual decline so that there was no evidence of activity by the 60th day in starved animals.

l. Lateral Mucal (Glands)

A great deal of activity was localized in these structures adjacent to the slime tracts. Peaks of intensity were found on the 2nd, 6-7th (Pl.IV, fig.15), and 9th days, with a decline to the point where there was no visible activity on the 60th day of starvation.

m. Storage Structures

Although these parenchymal glands, or structures, showed intense activity in a number of specimens (Pl.IV, figs.14,19), the overall picture was one of low activity. Further, there was a decline in activity in these glands at the time when the pharynx, lateral mucal glands, and epidermis were showing an increase.

n. Nervous System

There was little or no evidence of alkaline phosphatase in the brain itself, but the ramifying nerves were stained, especially in the cephalic part and adjacent areas. The intensity ranged from low to high, the latter being reached only in those animals which had been starved for 60 days.

2. _Curtisia foremani_

This triclad showed abundant evidence of alkaline phosphatase in most tissues and organs.

A correlation between the length of time since feeding and the distribution and intensity of activity of the enzyme was apparent, but variations in diet appeared to have no effect on the activity of the enzyme.

In general, the intensity of activity increased just after feeding (1-5 days) then waned so that there was little or no visible activity by the 10th day. In some tissues this situation was reversed, however, and so it is necessary to list the results for each organ or tissue.

Tissues and Organs

a. Pharynx

The intensity ranged from low to high in all muscles of the pharynx. Its distribution was quite uniform, except in one group. In specimens fixed 2-4 days after feeding the activity in the circular muscles surpassed that in all other parts of the pharynx (Pl.IV, fig.9). A marked rise in activity throughout the entire pharynx occurred 5-6 days after feeding. This was followed by a general decline so that by the 10th day of starvation activity was quite low. Occasionally there was high activity in the proximal portion of the pharynx with little or none visible in the distal portion.

b. Gut

(1) Amoeboid Cells and (2) Gut Wall.- The activity was so

similar in these two parts of the gut that they may be described together (Pl.IV, fig.5). Only one marked exception occurred. On the 6th day of starvation, when there was no apparent activity in the amoeboid cells, activity of medium intensity was evident in the wall.

In general three peaks of activity were evident in the gut. They occurred on the 1st, 5th, and 8th days, but each was progressively less than the preceding, and there was a gradual decline so that there was no visible activity by the 10th day after feeding.

c. Parenchyma

The parenchyma was characterized by a heavy, somewhat diffuse type of enzyme activity (Pl.IV, Fig.10). Two peaks of intensity appeared, one on the 1st day and the other on the 6th. This was followed by a sharp decline on the 7th day. But even on the 10th day after feeding medium activity was still evident.

d. Dorso-Ventral Musculature

These body muscles generally showed intense activity. This was not the situation immediately after feeding, but a marked rise occurred on the 1st day, followed by a decline in intensity on the 2nd day. Another rise was evident from 3-6 days, followed by a second decline from 7-8 days, and by the 10th day of starvation there was no visible sign of alkaline phosphatase.

e. Subepithelial Glands

Low to medium activity occurred in these structures. It was highest in the 3-4 day and 9-10 day specimens, with a low mark reached in the 5-8 day individuals.

f. Reproductive Organs

(1) Gonads

(a) Ovaries.- These sex organs were observed in six specimens only, all of which happened to be fixed immediately after feeding. Slight evidence of alkaline phosphatase was seen in the ovaries.

(b) Testes.- Much variation was seen in the alkaline phosphatase of these sex organs at the different levels of starvation. They were generally found to have enzyme activity of medium to high intensity (Pl.IV, fig.4), except after 8-10 days when they showed no sign of activity.

(2) Copulatory Apparatus

Enzyme activity observed in the copulatory apparatus ranged from medium or higher intensity, in 0-4 day specimens (Pl.IV, fig.10), to no visible evidence of the enzyme in 8-10 day individuals.

(3) Vitellaria

These yolk glands were found in only six specimens, all of which happened to be in the 4 and 5-day groups. These structures exhibited activity of low intensity 4 days after feeding, followed by a slight increase to medium intensity on the 5th day.

g. Adenal Rhabdites

The activity of the enzyme in these structures ranged from low to just above medium intensity. Three peaks were reached in 1, 4, and 10-day animals. Two low states occurred, at 2-3 days and 5-8 days.

h. Basal Membrane

In this membrane, which bounds the parenchyma, activity of alkaline phosphatase occurred in 2, 6, and 10-day animals. A low state was reached in 4-5 day animals, and no evidence of activity was observed after 7-8 days of starvation.

i. Epidermal Rhabdites

The epidermal rhabdites exhibited alkaline phosphatase activity of low intensity soon (3 hours) after feeding. A similar intensity was observed also after 5-8 days. Two peaks of high intensity occurred at 2 (Pl.IV, fig.6) and 10 days.

j. Epithelial Cells

The activity was much less intense in this tissue than in the rhabdites which it encloses. However, a strikingly similar periodic variation occurred, with the intensity of activity in the two components rising and falling at the same periods.

k. Excretory System

Low to above medium activity occurred in the protonephridial tubes. Peaks in intensity were found at 1, 4, and 6 days of starvation, with rather insignificant declines between, at 2 and 5 days. However, greater declines did occur in 7-8 day and 10-day specimens.

l. Lateral Mucal (Glands)

In these structures adjacent to the slime tracts activity of low intensity was noted in 1-7 day specimens. Eight-day individuals

showed medium intensity, and a very sharp decline was observed after 10 days of starvation.

m. *Storage Structures*

No such parenchymal structures were found in *C. foremani*.

n. *Nervous System*

The peripheral nerves usually showed low to medium activity of alkaline phosphatase. Intensity became progressively higher from 1-6 days. Then a slight decline occurred (7-8 days), followed by a rapid decline so that there was no visible activity by 10 days of starvation.

3. *Bipalium kewense*

This large terrestrial triclad showed less evidence of activity on the part of alkaline phosphatase than either of the two aquatic forms studied. Not only was the activity of the enzyme less intense but it was more limited in distribution.

No attempt was made to feed or starve this animal for a number of reasons. First, it is so large in size (5-20 cm.) that a histological study of serial sections would be an extensive undertaking. In addition it is a very difficult animal to feed and to rear in the laboratory. So the results reported here were obtained from tests on two of these animals, and were undertaken chiefly to determine the presence or absence of the enzyme.

The pharynx was apparently devoid of enzyme activity.

Activity in the gut was most intense in the mid-section (B).

In the anterior section (A) the intensity was about the same as in the posterior section (C). Some unabsorbed food was present in the lumen, indicating that the animals had fed shortly before fixation. The structures protruding into the lumen from the gut wall do not have the appearance of the amoeboid cells seen in aquatic forms, but look more like villi, or appear to be arranged in whorls. They showed medium-high activity (Pl.III, figs.8 and 10).

In the parenchymatous tissues such as mesenchyme cells, muscles (few), adenal rhabdites, subepithelial glands, and rhabdite tracts and fibers, a definite gradient in activity was apparent. The anterior, middle, and posterior sections of the body showed intensities of 2, 1, and 0, respectively.

The epidermis showed medium activity, but it was localized in the rhabdites, pear-shaped glands, and fibrous structures covering the ventral groove. No gradation in activity was observed here, except possibly in the ventral groove structure, which began with medium intensity and was lower from the pharynx posteriorly.

No other evidence of alkaline phosphatase was observed.

4. Geocentrophora applanata

This alloeocoele exhibited a heavy concentration of alkaline phosphatase activity but with a limited distribution. Activity was confined to the gut, parenchyma (very thin tissue between the gut and epidermis), ovaries (ova and follicle cells as described by Jones, 1931), and epidermis, but the intensity was always medium to high (Pl.II, figs.12,13,14, and 16). The slender, longitudinal

and transverse muscle fibers of the pharynx exhibited medium to high activity, but the remainder of this organ gave a negative picture (Pl.II, figs.12 and 16).

These animals were difficult to work with because of their small size. Since tarving reduces the size still more no attempt was made to test starved specimens.

5. Stenostomum tenuicaudatum

The pattern of activity exhibited by the rhabdocoele Stenostomum tenuicaudatum was similar to that described for Geocentrophora applanata. Activity was high in the gut, medium in the parenchyma, and high in the epidermis (Pl.III, fig.18). There was no apparent activity in the pharynx, but some (medium) was observed in the fine muscle fibers running along the pharynx.

All specimens examined had been freshly fed. Although various diets were tried, no significant differences in enzyme activity were apparent.

B. Acid Phosphatase

As Table 2 will show, acid phosphatase was found to occur in many of the same organs and tissues in which alkaline phosphatase occurred. However, quite a different pattern of intensity was noted, especially indicating a greater concentration of acid phosphatase in peripheral parts of the body.

[illegible]

MEANS OF ESTIMATED INTENSITIES OF ACID PHOSPHATASE ACTIVITY

Animal		Days Starved	Intensity (1 = low 2 = medium; 3 = high) Phx	Gut	Par*	DMG	Epid	Pb**
Dugesia tigrina (32)		0	1.50	1.00	1.40	1.50	1.00	1.50
		1	0.0	.50	.40	0.0	1.00	0.0
		2	.20	.80	.75	.75	1.75	.40
		3	0.0	0.0	.75	0.0	1.50	0.0
		4	0.0	1.00	1.25	2.00	2.50	0.0
		5	0.0	1.50	1.50	2.00	2.50	0.0
		6	.88	1.17	1.85	1.00	1.00	.83
		7	1.00	1.33	1.25	1.00	1.75	0.0
		8	1.33	0.0	1.50	3.00	1.00	2.00
		9	0.0	1.50	1.50	0.0	1.50	0.0
		11	0.0	1.75	1.31	0.0	1.35	0.0
Curtisia foremani (6)		0	0.0	1.50	1.70	2.00	3.00	0.0
		2	0.0	1.00	1.50	.50	1.88	0.0
		4	0.0	.38	1.81	1.00	1.80	0.0
	Section							
Bipalium kewense (2)	A	0	0.0	0.0	0.0	—	2.00	0.0
	B	0	0.0	0.0	0.0	—	3.00	0.0
	C	0	0.0	0.0	0.0	—	3.00	0.0
Geocentrophora applanata (12)		0	0.0	0.0	0.0	—	0.0	0.0
Stenostomum tenuicaudatum (32)		0	0.0	0.0	0.0	—	0.0	0.0

* Includes mesenchyme, muscles, adenal rhabdites, glands, and basal membrane.

** Refer to Page 64 for the key to abbreviations.

A substrate of sodium glycerophosphate yielded negative results, but when adenosine triphosphate was used positive results were obtained. As is well known various activators greatly enhance phosphatase activity. Two different activating substances were tried. Ascorbic acid was introduced into both of the substrates, and manganese sulfate was also used. Neither caused any change with glycerophosphate, while both enhanced the activity with ATP. Manganese sulfate was the better of the two, but some exceptions will be indicated.

Since variation in distribution and intensity of activity so characteristic of alkaline phosphatase was also evident with acid phosphatase, it seems desirable to list the individual tissues and [illegible]

1. Dugesia tigrina

Of the three types of food (earthworm, liver, and egg yolk) given to specimens preparatory to a study of acid phosphatase, only the first two will be described in a comparative manner, since egg yolk was fed to a relatively small group of worms which were all fixed at 2 and 3 days, and which showed little or no evidence of acid phosphatase.

Tissues and Organs

a. Pharynx

In liver-fed specimens acid phosphatase appeared to be at a high level in the pharynx immediately after feeding (Pl.V, fig.1),

and after 6, 7, and 8 days of starving. No significant indication of activity involving acid phosphatase was apparent in those specimens fed earthworm tissue and fixed from 1 through 11 days after feeding.

b. Gut

(1) Amoeboid Cells.- In the amoeboid cells where $MnSO_4$ was used as the activator earthworm-fed specimens showed results which were practically identical to those seen in specimens which had been fed liver. However, one group of animals which were starved 7-11 days after eating earthworm tissue and were then subjected to ATP activated by ascorbic acid showed a higher level of acid phosphatase in the amoeboid cells than any other group (Pl.V, fig.7).

(2) Wall.- When the substrate was activated by $MnSO_4$ liver-fed animals showed much higher activity in the gut wall than earthworm-fed specimens, up to 6 days after feeding. However, when animals had been starved longer than 6 days earthworm-fed specimens in which the substrate was activated by ascorbic acid showed a higher level of activity.

c. Parenchyma

High activity was apparent in the parenchyma only in animals which were fed liver and in which the activator was $MnSO_4$ (Pl.V, fig.6). Earthworm-fed specimens showed only medium to low activity regardless of the activator used.

d. Dorso-Ventral Musculature

High to medium activity was evident in the dorso-ventral musculature only in specimens which had been fed liver, and in which the substrate had been activated by $MnSO_4$. No evidence of activity was apparent in other worms.

e. Subepithelial Glands

The subepithelial glands stained intensely in specimens fixed immediately after a liver feeding, provided the activator used with the substrate was $MnSO_4$. The intensity remained about the same with animals starved up to 6 days. It then gradually diminished and was entirely gone by the 8th day. Earthworm-fed specimens, whose tissues were subjected to a similar substrate, but activated by ascorbic acid, showed activity of medium intensity persisting through 11 days.

f. Gonads

There was no visible evidence of acid phosphatase in the gonads.

g. Adenal Rhabdites

In those specimens which fed on earthworms the adenal rhabdites always showed medium to high activity (Pl.V, fig.7). The two activators produced no significantly different results. Liver-fed animals exhibited little or no apparent activity, regardless of activator used.

h. Basal Membrane

No activity was apparent in the basal membrane.

i. Epidermal Rhabdites

The epidermal rhabdites in specimens which were fed liver showed high activity immediately after feeding when ascorbic acid was used to activate the enzyme. This dropped to low intensity by the end of the first day of starving. Where $MnSO_4$ was used as the activator liver-fed specimens showed no activity until the 7th day when it appeared low. By the 8th day this had increased to medium activity. In earthworm-fed specimens where $MnSO_4$ was used as the activator 1-day specimens exhibited medium intensity. This rose to high during the second day and remained so through the 5th day. Ascorbic acid was used as the activator for specimens fed on earthworms and starved 6 to 11 days. The level of activity was high for the 6th through the 9th day, but dropped off somewhat during the 10th and 11th days.

j. Epithelial Cells

Low to medium activity was evident in the epithelial cells of animals which had been fed earthworms. It seemed to make no difference which activator was used.

k. Excretory System

There was no apparent activity in the protonephridial structures.

l. Lateral Mucal Glands

High activity was found in the lateral mucal glands only when the specimens had eaten liver and the activator used was $MnSO_4$. This was observed in animals fixed immediately after feeding (Pl.V, fig.1) and at 6 (Pl.V, fig.6), 7, and 8 days, the only time intervals represented in this series. At 2, 3, and 4 days medium activity occurred in earthworm-fed animals, but here, too, $MnSO_4$ was used to activate the enzyme. In every instance where ascorbic acid was used as an activator no activity was evident.

m. Nervous System

All nerve tissue was devoid of stain.

n. Pharyngeal Epithelium

High activity was found in the inner and outer epithelium of the pharynx in animals which had been fed liver, provided $MnSO_4$ was introduced into the substrate (Pl.V, fig.1). On the 2nd day after an earthworm feeding there was one instance of low activity. Here, too, the activator for the enzyme was $MnSO_4$. Where ascorbic acid was used as an activator no activity was observed.

2. Curtisia foremani

Several preliminary tests indicated that acid phosphatase was difficult to detect in this triclad. Therefore, a lengthly procedure of feeding and starving was avoided.

Six specimens were tested on the routine starving procedure.

These were divided into three lots. Two specimens each of the "habitat-fed," 2-day, and 4-day categories were subjected to the test for the enzyme. Brief mention will be made of some significant activity which was observed. Adenosine triphosphate, with $MnSO_4$ added, was the only substrate used.

Tissues and Organs

a. Pharynx

No activity was determinable in the pharynx.

b. Gut

Only very low activity was found in the amoeboid cells and gut wall. Some in the former persisted through 4 days, but in the latter for only 2 days.

c. Parenchyma

Low to medium activity was evident in the parenchyma through 4 days.

d. Dorso-Ventral Musculature

Very low activity was seen in animals fixed immediately after feeding. No activity was apparent in these structures in starved animals.

e. Subepithelial Glands

In the subepithelial glands low to medium intensity was found in "habitat-fed" individuals, gradually increasing to medium-high intensity with starvation.

f. Gonads

No activity was observed in the gonads.

g. Adenal Rhabdites

High activity was evident in the adenal rhabdites through all stages of feeding and starving (Pl.V, fig.4).

h. Basal Membrane

In the basal membrane activity gradually decreased to low as starvation progressed.

i. Epidermal Rhabdites

High activity was present in the epidermal rhabdites at all observed stages (Pl.V, fig.4).

j. Epithelium

In the epithelium the activity appeared very pronounced immediately after feeding, but in 2 and 4-day animals it decreased to low intensity.

k. Excretory System

No activity was apparent in the excretory system.

l. Lateral Mucal Glands

In the lateral mucal glands the intensity decreased from medium to low as starvation progressed.

m. Nervous System

All nerve tissue was devoid of the stain.

3. *Bipalium kewense*

Only one entire terrestrial triclad and part of another were tested for acid phosphatase, but these provided many slides so that different (alternating) sections could be treated in various ways. Regardless of the length of time of incubation, type of substrate, or the activating substance, only the epidermis and adenal rhabdites showed activity. The rhabdites on the dorsal side were very pronounced due to the intense stain (Pl.V, figs.11,13,and 15). No activity whatsoever was observed when Na-Glycero-phosphate was used as the substrate.

4. *Geocentrophora applanata*

No acid phosphatase was evident in 12 specimens of *Geocentrophora applanata* selected randomly from a group obtained from the habitat and fixed immediately. These were of varying sizes, and appeared to vary also in the recentness of feeding.

5. *Stenostomum tenuicaudatum*

In 28 specimens of this rhabdocoele, fixed under varying conditions, such as directly from the habitat, semi-starvation, or taken from laboratory cultures, no acid phosphatase activity was evident.

C. Lipase

The Gomori technique was used to localize the fat-splitting esterase, lipase. This technique is very similar in principle to the methods used for phosphatases, and the worms were treated in much the same way. This test was far more difficult to carry out than was either of the tests for phosphatase, however, and results were more inconsistent.

Gomori's (1952) technique, using Tween 60, a stearate, and Tween 20, a laurate, as substrates, was tried first, but with negative results. Lillie's (1954) modification of the technique was then tried with both substrates. This also gave negative results. Finally, the first technique proposed by Gomori (1945) was attempted. This yielded some positive results, provided Tween 20 was used as the substrate.

Of the five species studies only three showed any evidence of the enzyme, even with this test. Listed in their respective order of highest to lowest intensity of lipase activity, these were: Dugesia tigrina, Curtisia foremani, and Geocentrophora applanata. Negative results were obtained with Bipalium kewense and Stenostomum [illegible].

Lipase is apparently intracellular, and limited to the phagocytic cells of the gut. It was not found in any other region at any stage of nutrition, and was evident in the gut only up to five days after feeding. Dugesia tigrina and Curtisia foremani were fed and starved just as was done for the phosphatases. The activity of lipase

in the gut was quite pronounced in some individuals immediately after feeding and very inconsistent thereafter. Some differences were noted in the two worms. Dugesia tigrina exhibited more pronounced activity immediately after feeding (Pl.VI, figs.1 and 3), with low activity apparent up to five days after feeding (Pl.VI, fig.5). Curtisia foremani showed only moderate activity after feeding, and no apparent activity thereafter until the fifth day when it became pronounced (Pl.VI, figs.7 and 9). No activity occurred beyond this point of starvation.

No evidence of lipase was observed in Bipalium kewense, the land planarian. However, in this particular specimen, there was no evidence of recent feeding either.

Moderate to very low activity was apparent in the lateral gut regions of Geocentrophora applanata (Pl.VI, fig.10), the alloeocoele, which showed evidence of a recent feeding.

No evidence of activity occurred in Stenostomum tenuicaudatum the rhabdocoele, even though all specimens were taken from a thriving wheat culture of protozoa in which feeding was occurring constantly.

II. FATTY SUBSTANCES

The McManus (1946) technique for the determination of fatty materials, consists of three major steps prior to paraffinization. They are as follows: 1) fixation with Baker's fluid, 2) oxidation with bichromate, and 3) acetone treatment. The second step makes insoluble precipitates of phospholipids, according to Bloor's (1943) interpretation of Ciaccio's original principle. This precipitate then takes a characteristic stain by dissolving the dye in itself. The third step supposedly dissolves out neutral fats. Sudan IV being specific for free substances which may survive the treatment is logically used as a control stain. To detect the presence of combined fatty substances, primarily phospholipids, three stains were used. They were nile blue, Sudan III, and Sudan Black. The general results obtained are listed below (+ = positive and - = negative).

Fatty Substances

Dye Turbellarian	Nile Blue	Sudan III	Sudan IV	Sudan Black
Bipalium kewense	++	+	-	++
Dugesia tigrina	++	++	+	+++
Curtisia foremani		++	-	++
Geocentrophora applanata	++	+	+	++
[illegible] tenuicaudatum	-	-	-	-

Nile blue stains so uniformly throughout the section in many cases that its use as an indicator of fatty substances has been questioned. However, according to Lison (1936), it colors glycerides a metachromatic pink and fatty acids blue, with all intermediate colors occurring in cases of mixtures. These color variations were obtained in most of these worms.

Sudan III had an affinity for globules in the gut, parenchymal depots, adenal and epidermal rhabdites, and epithelial cells in general. In controls which had been purposely subjected to fat solvents in an attempt to remove fatty material the rhabdites stained more conspicuously than in the experimental animals. This indicates that the rhabdites contain some fatty substance which may be too complex to be dissolved by ordinary fat solvents. This may be a lipoprotein.

Sudan Black was used successfully by Bullock (1949) in determining sites of phospholipids in Acanthocephala. It is probably by far the best stain for accuracy, in that it has little or no affinity for non-lipoid structures.

One or another, or all of the dyes used showed evidence of fatty substances in four of the species studied. In order to indicate differences observed each species will be described separately.

A. Dugesia tigrina

If the opinion of Lison (1936) is accepted as to the meaning of the colors obtained with nile blue, then in Dugesia tigrina glycerides, fatty acids, and phospholipids, were present in the gut cells, parenchyma, and epithelium, and in minute amounts in the pharynx, of

specimens which were recently fed (See Pl. VII). Sudan Black stained the ganglionic tissue adjacent to the eyes to a medium intensity, indicating the presence of phospholipids. In specimens which had been starved for 15 days the phospholipid occurred in greater abundance, either deep in the gut wall or in the adjacent parenchyma. Heavy staining in the rhabdites and lateral nasal glands occurred only when Sudan III was the dye used.

B. Curtisia foremani

The distribution of fatty substances in Curtisia foremani was quite limited. In general it was found only in the gut cells and wall, but in one specimen the rhabdites and wall of the copulatory apparatus were stained, while in another fat bodies were very conspicuous in the parenchyma (Pl.VIII, fig.3). Both of these specimens were stained with Sudan III.

C. Bipalium kewense

The land planarian, Bipalium kewense, showed a wide distribution of fatty substances, especially with nile blue, Sudan black, and Sudan III, but little with Sudan IV. The whorls of phagocytic cells lining the gut were very conspicuously stained at their basal attachments (Pl.VIII, fig.7), and the general appearance of the gut suggested that this specimen had undergone some starvation. The parenchyma was peppered with violet dots (nuclei), all surrounded with cytoplasm of a metachromatic pink, when nile blue was the stain used (Pl.VIII, fig.8). The epidermal rhabdites stained medium pink

with Sudan III, while glandular ducts which extend from the parenchyma out to the epidermis were dark blue after the use of nile blue.

D. Geocentrophora applanata

All stains showed Geocentrophora applanata, the alloeocoele, to have fats. Nile blue and Sudan black stained very intensely, with Sudan IV only moderately, and Sudan III producing a uniform pink colour throughout. Stained globules were very characteristic of the gut cells, parenchyma, and ovaries (Pl.VIII, figs.5 and 6).

E. Stenostomum tentuicaudatum

The rhabdocoele, Stenostomum tentuicaudatum, showed no evidence of fat with any of the stains.

The significance of the results which have been obtained in this work may be discussed under two major headings: first, how these results fit in with previous knowledge or theories as to the function of the enzymes studied and, second, how these results add to our understanding of the physiological processes of these worms.

The relationship between nutrition and these enzymes as observed in this study not only demonstrated the presence and distribution of the phosphatases and lipase per se, but also showed their location with respect to each other at various stages of nutrition. For example, alkaline phosphatase and lipase were both strongly evident in the amoeboid cells and gut wall during the first five days after feeding. Thereafter, no lipase was evident in any part of the body, but the activity of alkaline phosphatase continued gradually moving outward toward the periphery of the worm, as though functioning in the transport of food across the cell membranes encountered through the parenchyma. This continued until all peripheral structures were reached, including the muscles, and even the pharynx. Meanwhile, the activity of acid phosphatase was almost entirely limited to the peripheral parts of the body, such as the outer parenchyma and epidermis. It occurred in gut cells, epithelial cells, and muscles, to a marked degree, only during extensive starvation.

The study of fatty substances further bears out these patterns of activity on the part of the enzymes, any one of which could

possibly function in the hydrolysis of fatty substances at one stage or another. That is, assuming that the specificities which have been proposed for the fat stains used are correct, the simpler fats were evident in the gut, where lipase was also confined, while combined fats (mainly phospholipids) were evident in the peripheral parts of the body such as the parenchyma and rhabdites, coinciding with the loci of alkaline phosphatase and acid phosphatase.

This suggests then, a very intimate role on the part of lipase in the initial splitting of fats and of alkaline phosphatase in the subsequent distribution of their products for metabolism. Of course, a simultaneous carbohydrate metabolism would be occurring, as was demonstrated by Willier, Hyman, and Rifenburgh (1925) in Planaria dorotocephala, which might account for an even greater proportion of the activity of alkaline phosphatase.

It appears then that so far as digestion is concerned lipase for fats and alkaline phosphatase for carbohydrates play an active role in hydrolysis for absorption. Alkaline phosphatase apparently also plays an important role in phosphokinesis for transport, and here acid phosphatase may also enter the picture. In the discussion which follows the various proposals which have been made as to the functions of these enzymes will be considered in the light of the results obtained in these worms.

I. ALKALINE PHOSPHATASE

Robison (1923) did the first histochemical work with alkaline phosphatase. He found it to be very active in regions of calcification of cartilage to primary bone. Bourne (1943) also found alkaline phosphatase active in bone cavity healing. And Lorch (1949) found it to be associated with the calcification of fish scales. While these results indicate an important role for alkaline phosphatase in animals which have bones or scales, they have no significance so far as flatworms are concerned. However, alkaline phosphatase apparently has many other functions besides bone formation.

A. Regenerative Roles

That alkaline phosphatase is very active along chromosomes during mitosis and meiosis, especially when the chromosomes are contracting has been shown by Willmer (1942) and Krugelis (1942) working independently with regenerating liver cells, tumors, and embryonic cells. Danielli (1953) observed similar phenomena in amphibia. Mugard (1953) found that in various protozoa phosphatase is a common nuclear constituent, along with DNA. Danielli (1953) found it to be especially abundant in the nucleolus, with the Feulgen reaction giving a positive test for DNA in the surrounding chromosomes of the Walker rat sarcoma. Fell and Danielli (1943) applied chemical warfare agents to rat skin, and found abundant alkaline phosphatase activity in the healing areas. Moog (1944) found waves of formation of alkaline phosphatase at different

stages in the development of the chick embryo. Lorch (1949) observed a similar phenomenon in dogfish and trout, as did Yao (1950) in larvae of *Drosophila*.

The high regenerative power of flatworms is well known but the factors which contribute to it are still under a good deal of experimentation. One thing which is generally agreed upon, however, is that totipotent cells of the parenchyma play a major role in regeneration. The evidence of alkaline phosphatase throughout the parenchyma at times of morphological changes, such as during the alternate appearance and disappearance of various structures correlated with feeding and starving, is suggestive of a function in regeneration. Since, basically, regeneration is a process similar to embryonic growth, carcinogenic growth, or any other type of mitotic activity, this appears to lend support to the idea that alkaline phosphatase plays an important role in the growth and reproduction of cells.

B. Digestive Role

Many papers have appeared in the literature indicating that alkaline phosphatase plays a role in digestion. Danielli (1953) pointed out the work of Martin and Jacoby (1949) which was designed to test the tendency for diffusion of phosphatase. Since they found little diffusion in most tissues, but a rapid rate of diffusion when intestinal tissue was used, Danielli immediately attributed a digestive action to phosphatase. Muzard (1953) subjected *Ochryoglena*, a ciliate, to feeding and starvation. He found that

no phosphatase was evident in starved animals, but that after the ingestion of food phosphatase appeared in the vicinity of the food vacuoles, within seconds after the formation of the vacuoles. Shortly afterward it appeared in the macronucleus and micronucleus and throughout the cytoplasm. Cora (1947) demonstrated the role of intestinal phosphatase in the absorption of neutral fats in the mouse. Emmel (1945) showed the important role of the enzyme in phosphorylation during the breakdown of carbohydrates and fats and the passage of these substances through the intestinal epithelium.

As has been pointed out, alkaline phosphatase activity was high in the amoeboid cells of the gut of these flatworms within thirty minutes to three hours after feeding. It remained high so long as there was food in the gut cavity or spherules in the amoeboid cells, and gradually declined as the digestive action of the cells was completed. The movement of the digested or partly digested food through the gut wall and out into the parenchyma was accompanied by the appearance of high alkaline phosphatase activity in these areas, but in starved animals there was little or no activity in any part of the gut. Therefore, at least in the gut, the activity of the enzyme is correlated with the presence of food materials, indicating that it may play a role in digestion.

The somewhat longer delay in the appearance of the enzyme after feeding in the flatworms, as compared with the condition in the ciliate, may be due to a difference in membrance structure, but in both cases the delay can probably be attributed to the necessity

for the activation of proenzymes.

C. Transporting Role

The phosphatases have been considered by many workers to function in the transport of metabolites from one locus to another in the body. Axelrod (1948) and Meyerhoff and Green (1950) independently demonstrated that phosphatases may act as phosphokinases in catalyzing the transfer of a phosphate residue from one organic molecule to another. This would seem to be a somewhat general role, in that it would be an essential one in the carrying out of most of the other proposed functions.

The situation which has been observed in the flatworms, particularly in the complex aquatic triclads which have extensive parenchyma, offers striking circumstantial evidence for a role in transporting materials through membranes on the part of alkaline phosphatase. One of the most interesting phenomena which has been observed in this study is the manner in which the movement of digested or partly digested food through the body of the worm is accompanied by the appearance of alkaline phosphatase. Thus during the first five days after feeding, while food is still present in the gut or the amoeboid cells, alkaline phosphatase activity is high in this region, but during this same period it is gradually building up in the gut wall. During the next three days, when the food is disappearing from the gut cavity and the spherules in the amoeboid cells have also largely gone, there is a steady decline in the activity of the enzyme in the amoeboid cells and an increase

in the gut wall and parenchyma. Shortly afterward this activity in the gut wall begins to decline, but there is an increase in activity in the parenchyma and the same wave-like process continues on out to the epidermis. Finally in animals starved 10 to 60 days activity is reduced to a minimum throughout the animal. Some increases do occur during continued starvation, but there is no "wave-like" phenomenon associated with its reappearance. This is probably correlated with autolysis, by means of which some of the protoplasm of the body is sacrificed to keep the essential vital activities going. Since this autolysis probably occurs in all or nearly all of the tissues more or less simultaneously, rather than progressively, any transport which is involved would probably be of a purely "local" nature and would not involve any prolonged or continuous transport or movement of materials in one direction as occurs when the food moves outward from the gut. Therefore, any build up in alkaline phosphatase activity would be expected to occur more or less simultaneously in the various regions of the body and this is actually the case.

The slowness of the movement of digested or partially digested food outward toward the periphery of the body may well be correlated with the lack of a circulatory system in these animals. Presumably much of the movement is accomplished by transport through membranes from one cell to another. It would be interesting to investigate the Nemertinea which are in many respects closely related to the Turbellaria but in which a circulatory system is present, to see whether this "wave-like" movement is as pronounced in this group.

D. Secretory Role

As early as 1936 a role in secretion was postulated for alkaline phosphatase, when Verzar (according to Danielli, 1953) suggested that the secretion of substances might be facilitated by phosphorylation in the presence of phosphatases and a source of phosphate. This idea was supported when Wilbrandt (according to Danielli, 1953) inhibited secretions simply by poisoning the enzymes. But perhaps the work of Gomori (1939) and that of Takamatsu (1939) showing the presence of alkaline phosphatase in the brush borders of the glucose-secreting kidney tubule cells, and the work of Danielli and Lorch, as related by Danielli (1953), on the secreting cells of aglomerular fishes, have done even more to demonstrate the role of alkaline phosphatase in secretion. J.R.G. Bradfield (1946) also showed the secretory cells of milk glands to be a site of activity. The fact that intense activity was observed in glandular structures of these flatworms must support the idea that alkaline phosphatase plays an important role in secretion.

E. Excretory Role

Danielli (1943) demonstrated that alkaline phosphatase played a role in the secretion of glucose from the kidney. Glucose passes out easily, and if phosphate ions are outside to combine with it to make glucose-phosphate, the glucose cannot get back inside readily. Likewise, phosphorylation on the inside of membranes would form a balance. Danielli (1953) found that the

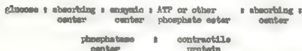
proximal tubules of rat kidneys have intense enzyme activity. The brush border cells show the greatest evidence of activity, indicating a role in reabsorption. Pantin and Danielli (1950) found that the nephridia of a terrestrial triclad had alkaline phosphatase, and in the same year, Bradfield and Yao independently observed activity of the enzyme in the Malpighian tubules of a tick, and in insect larvae. In addition Kugler and Birkner (1948) demonstrated the presence of the enzyme in the green gland of crayfishes.

Alkaline phosphatase activity was pronounced in the protonephridia of the two aquatic triclads. In fact, these tubules stood out because of it, and could be traced for about one-half of the length of the worm, that is, from the anterior end back to just behind the pharynx, because of the dense precipitate accumulated about them. The stain was heavier at the anterior end and steadily decreased posteriorly, until it could no longer be detected.

This activity could be associated with any combination of a number of possible roles of the enzyme. It appears that dephosphorylation would release materials for discharge, and would also aid in the energy metabolism necessary to carry out the mechanics of discharge. If this were followed by phosphorylation on the other side of the membrane, then the substance would be excreted. However, these processes very likely occur repeatedly along tubules as excretion and resorption are accomplished.

F. Energy Relationships

It is obvious that in any of the activities outlined above energy is constantly needed. Some rather recent work has been done which seems pertinent. Goldacre (1950) theorized that secretion is facilitated by a folding and unfolding of protein molecules. Osterhout (1951) said that it takes place by ionic actions within cell membranes. Then Danielli (1953) hypothesized a combination of both of these proposed mechanisms, plus an assist from phosphatase. He attributed a hydrolytic and/or a transporting (phosphokinase) role to alkaline phosphatase. In such an arrangement the phosphatase is an enzymic center through which the energy of adenosine triphosphate is transferred to the contractile protein. The following diagramatic scheme was proposed by Danielli (1953).



This would help to explain the contraction of chromosomes while undergoing mitosis and meiosis, and would seem to be applicable to any contractile tissue.

The dorso-ventral muscles play an important part in the body movements of the flatworm. The animals are usually rather sluggish for some time after feeding, but resume activity as their food supply is exhausted. The rise and fall of enzyme activity is closely correlated with the increase and decrease in the activity of these

muscles. The pharynx begins to show evidence of increased enzyme activity at the same time, and this coincides with its renewal of probing operations in search of food. The enzyme activity in the pharynx increases until it is quite high and continues so until the physical motion of the pharynx subsides. Probably much of the enzyme activity throughout the animal could also be attributed to the energy metabolism associated with secretive and absorptive action, except in the pharynx, where supposedly no absorption takes place.

II. ACID PHOSPHATASE

As Gomori (1951) has indicated, the phosphatases which are functional in an acid medium (p^H of 5.0 to 6.5) are much more difficult to demonstrate than those operating in an alkaline range. Several factors may contribute to this difficulty. First, the optimal p^H range is more limited. Second, the tissue as a whole is more likely to be alkaline, thereby leaving only certain minute cellular parts to be acidic. Thirdly, the acid phosphatases seem to require some very specific activator(s), and this even appears to vary from one organ to another, as though there were a series of slightly different enzymes.

Yao (1950) found evidence of both alkaline and acid phosphatase in Drosophila melanogaster during embryonic development. Frequently they occurred simultaneously in the same cells, but usually alkaline phosphatase originated in the periphery and appeared to work inward as differentiation and morphogenesis proceeded. After a time there was a marked decline, but some activity remained in the gut epithelia, salivary glands, and Malpighian tubules. On the other hand, acid phosphatase occurred mainly in the ovary, nuclei, egg follicles, yolk, and testes, with that in the yolk being the most active and that in the nuclei next. Also, there was some evidence of the enzyme from cleavage up to the time of hatching, and a more constant level of activity on through life than was the case with alkaline phosphatase. That is, the sharp increases and decreases in activity, which are so characteristic

of alkaline phosphatase, are not as obvious with the acid phosphatases.

In general the above statements are true for the Turbellaria also. Although the activity of alkaline phosphatase is far more conspicuous in nearly all of the structures from the gut outward through the gut wall and into the parenchyma, following a feeding, and up to approximately eight days of starvation, acid phosphatase is always evident in small amounts and has a very similar distribution. However, upon extensive starvation, a very interesting thing happens. As the activity of alkaline phosphatase decreases, that of acid phosphatase increases, which seems to indicate a change in pH in the animal. It is known that these animals digest their own less essential organs and tissues for the benefit of the more essential ones. How does such a decomposition occur? It seems reasonable to assume that this is an autolytic process, which would be facilitated by the cathepsins. The acid phosphatases function in this favorable pH, at least temporarily, since most digestion, just as in the lowest protozoa and in the highest vertebrates, begins in an acid medium. This is then followed by an increase in the alkaline enzyme activity, as the acid phase is completed.

If this idea should be correct, and the alternating pattern of activity shows a good deal of evidence for it, then the two phosphatases or groups of phosphatases functioning in the wide pH range of 5.0 to 9.5 would seem to be very important factors in the life processes of these worms. And since such high powers of regeneration exist in these animals there is an almost constant process of

differentiation and morphogenesis taking place, a process which has been shown by several workers to be promoted by the phosphatases.

This does not offer any explanation for the region of more persistent and intense acid phosphatase activity, which is the epidermis and its rhabdites. Since the activity of alkaline phosphatase was apparently less in these structures it may be indicative of a rather constant acid pH in the epidermis. Such acidity might be the effect of the rhabdites, or it might be the result of the final breakdown of food.

III. LIPASE

According to Gomori (1945, 1946, and 1952) lipase is present in almost every organ of the vertebrate body, but pathological and tumorous conditions seemed to reduce lipase activity. Lipase can be demonstrated by its action upon several Tweens. The Tweens are palmitic, stearic, or lauric esters of sorbitan or mannitan.

Lipase was evident in Turbellaria only in the gut, and there only until the fifth day of starvation. This indicates that it is probably functioning as a catalyst in the hydrolysis of fats engulfed by the amoeboid cells, after which the fatty acids would be absorbed by the parenchyma and converted into combined forms or into simple carbohydrates for storage or energy metabolism.

It is interesting that only the more complex aquatic forms of Turbellaria showed any marked evidence of lipase. The rhabdocoele showed no evidence of this enzyme.

Since only the Tween with the laurate ester yielded positive results, it seems probable that there is only one very weak lipase in the worms, which would make them very selective so far as fats are concerned.

IV. FATTY SUBSTANCES

The question of whether these flatworms had fats and any lipolytic enzyme with which such fats might be split or synthesized in the worm, caused four men (St.Hilaire, Arnold, Metchnikoff, and Löhner) to make a series of studies on the triclad *Dendrocoelum lacteum* from 1908 to 1920 (Westblad,1923). After first thinking an extracellular enzyme for fat-splitting did occur and was secreted upon food as it came through the pharynx and passed the flask cells located near the entry into the gut, they were finally in general agreement that no lipolytic enzyme was present in these animals. These conclusions were reached by a series of observations on ingested fats, in vivo, using vital stains. Sudan III and nile blue were also used to some extent on preserved material. These studies showed that fatty substances entered the amoeboid cells and were unchanged as they began a mechanical circulation through the parenchyma still within amoeboid-like cells. They were gradually changed only in an incidental way, as they came in contact with other body fluids, but were never stored as fat.

Improved techniques made it possible to demonstrate fatty substances not only as they entered the pharynx and passed on into the lumen of the gut and the amoeboid cells but also throughout the parenchyma. In fact, assuming that the specificities of the staining reactions of certain fat stains as outlined by Lison (1936), Bloor (1943), and McManus (1946) are correct, even phospholipids and other combined forms of fats may be demonstrated. Also since lipase

was localized histochemically in the gut cells in the more complex worms, it would appear that these cells are the site of lipolytic activity. Subsequent action by phosphatases might result in the production of phospholipids. If these were circulated through the body their ultimate dephosphorylation would release phosphate radicals as well as lipids.

The amount and distribution of fatty substances also appears to be correlated with the increasing complexity of the worms, perhaps even more strikingly so than was the case with lipase. No evidence of fat was seen in the rhabdocoels. Only a moderate amount was evident in the alloeocoels. It was much more pronounced in the two aquatic triclads, with a good deal of evidence of combined forms of fatty substances present in the parenchyma, and even in the rhabdites of the epidermis. The land triclad, although showing no evidence of lipase, did stain very intensely with all of the fat stains used, indicating that more of the simple fatty acids and glycerides were present in the parenchyma. Actually, it seems probable that some lipase was present in the land planarian, although it was not revealed by the substrate used.

The results obtained in this study add to our knowledge of the physiology of these worms in several ways. The presence and locations of the enzymes and their relationships to each other are pointed out for the first time. In addition, the relationship between the activity of the enzymes and nutrition aids in explaining the process of digestion and assimilation. The wide distribution

and high level of activity of the phosphatases, particularly alkaline phosphatase, indicate that it is certainly one of the most important enzyme systems in these worms, and may be the basic system in their metabolism. The fact that lipase appears only in the more complex of the forms studied, and even then only in the early stages of food digestion, may well indicate that it is a new enzyme system for these worms. In other words, it is possible that in the Turbellaria a major advance in physiology is accomplished through the appearance of a new enzyme system in more complex forms. This enables those forms to utilize fatty substances in foods which lower forms in the same class may not be able to do, but fat metabolism appears to be at a low level of development even in more complex worms. Numerous workers, including, as previously mentioned, St.Hilaire, Arnold, Metchnikoff, and Löhner (according to Westblad,1923), have indicated that fat and/or lipase were absent in the Turbellaria. The results obtained in this work indicate that this may be true for some Turbellaria, but is definitely not true of all. This study also supports the view that the process of digestion is relatively slow in the Turbellaria. It takes only three to five hours after a meal has been eaten by these worms for the gut cavity to be emptied, but an additional four to five days are required for the spherules in the food vacuoles of the amoeboid cells to undergo their many alterations and finally disappear. The relatively long period of time required for digestion may be another indication that the enzyme systems of these worms are simple and consist of relatively

few enzymes.

Florkin (1949) points out the primitiveness of the intracellular type of digestion found in the invertebrates, and the acquisition of extracellular enzyme systems in the higher forms as being an evolutionary advancement. It is known that the food vacuoles in even the lowest protozoa show evidence of hydrolytic enzymes capable of altering foods containing proteins, fats, and carbohydrates. This intracellular type of digestion continues through the proifera. In the coelenterates, on the other hand, there is an initial phase of extracellular digestion in the gastrovascular cavity, followed by a completion of the digestive process within the individual cells. The free-living flatworms accomplish digestion in much the same manner, in that extracellular enzymes are secreted onto the solid food as it is drawn into the enteron by the suction of the pharynx, after which the particles are engulfed by the phagocytic cells for intracellular digestion. The major role of the phosphatases throughout the metabolic processes in all of these worms has been previously mentioned. The fact that lipase is limited to more complex forms may be indicative of an evolutionary beginning of a second system of extracellular digestion, namely, that of lipolytic activity in the lumen.

This work has not included any study of proteolytic enzymes. In view of the fact that the worms studied are largely carnivorous they are undoubtedly equipped with proteolytic enzymes. In all probability these split the protein molecules into amino acids, which, after a process of deamination, can then be acted on by the phosphatases.

SUMMARY

1) Following the technique of Gomori histochemical tests were used to demonstrate the presence and loci of activity of the enzymes alkaline phosphatase, acid phosphatase, and lipase in five species, representing three orders of Turbellaria. Tests for fatty substances were made with the McManus technique.

2) All five of the species studied (Dugesia tigrina, Curtisia foremani, Bipalium kewense, Geocentrophora applanata, and Stenostomum tenuicaudatum) showed evidence of alkaline phosphatase. Only the first three (all triclads) gave positive tests for acid phosphatase activity, while only the first two of these (aquatic triclads) and Geocentrophora applanata (alloeocoels) showed evidence of lipase.

3) Evidence of fatty substances was found in all of the forms studied except the rhabdocoels S. tenuicaudatum, which also gave negative results for lipase. This worm was the smallest and least complex of those studied.

4) It was found that the patterns of distribution of the enzymes within the tissues varied according to the recentness of feeding. The food appeared to be an activator for the enzyme in the gut amoeboid cells. Likewise, as

nutrients moved through the gut wall and on through the parenchyma, waves of enzyme (alkaline phosphatase) activity were evident.

5) Alkaline phosphatase was the most widely distributed and most active of the enzymes studied. In _S. tenuicaudatum_ its activity was confined mostly to the gut, but in the other four forms it was found throughout the body.

6) Acid phosphatase activity was apparent mainly at times of starvation, when alkaline phosphatase was less evident. This suggests that it may replace alkaline phosphatase during periods of autolysis.

7) Lipase activity occurred in the gut only, and only during the first five days after feeding. During the same period and afterwards alkaline phosphatase was very active in the same (gut) region, and extended into the parenchyma.

8) Special roles on the part of the phosphatases in the activities of regeneration, digestion, transporting, secretion, excretion, and energy metabolism are suggested. These are in substantial agreement with the findings of various investigators who worked with other organisms.

9) The significance of this work in interpreting the

physiological activity of these worms is pointed out. And a possible evolutionary sequence for several major enzyme systems is suggested.

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KEY TO ABBREVIATIONS FOR FIGURES AND TABLES

AC amoeboid cells of the gut
AR adenal rhabdites
BM basal membrane
C circular muscles of the pharynx
CS creeping [illegible]
DVM dorso-ventral muscles
E egg
EG epidermal glands
Epid epidermis
ER epidermal rhabdites
ES excretory system (protonephridia and tubules)
FC follicle cells
Gds gonads
L longitudinal muscles of the pharynx
LMG lateral mucal (glands)
NT nervous tissue
O ovary
Par parenchyma
Phx pharnyx
PE pharynx epithelium
R radial muscles of the pharynx
RA reproductive apparatus
RC rhabdite cells
RD rhabdite ducts
SEG subepithelial glands
ST slime tract
T testes
TC totipotent cells
V vitellaria
W wall of the gut

PLATE I

DIAGRAMMATIC TRANSVERSE SECTIONS OF THE TURBELLARIA WHICH WERE USED IN THIS STUDY

These diagrams will serve as a key for the identification of the various tissues, organs and structures in the figures shown in the remaining plates.

Fig. 1. A diagram of the aquatic triclads Dugesia tigrina and Curtisia foremani sectioned transversely through the distal part of the pharynx, thus showing the two posterior branches of the gut on each side of the pharynx. The parenchyma and the various structures included within it are noted peripheral to the gut and pharynx.

Fig. 2. A diagram of the terrestrial triclad Bipalium kewense sectioned transversely through the two branches of the gut posterior to the pharynx. Note the large amount of parenchymatous tissue, the totipotent cells, and the thick epidermis.

Fig. 3a. This is a diagram of the alloeocoele Geocentrophora applanata as it appears when sectioned through the pharynx near the anterior end.

Fig. 3b. This is a diagram of Geocentrophora applanata as it appears when sectioned transversely through the gut and ovary near the posterior end.

Fig. 4a. This is a diagram of the rhabdocoele Stenostomum tenuicaudatum cut transversely through the simple pharynx in the anterior end, showing the surrounding parenchyma.

Fig. 4b. This diagram of Stenostomum tenuicaudatum, as it appears when sectioned transversely through the posterior region, shows only parenchyma and the epidermis surrounding the gut.

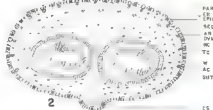
PLATE I

DUGESIA TIGRINA

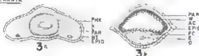
AND

CURTISIA FOREMAN

B.PALIUM KEWENSE



GEOCENTROPHORA APPLANATA



STENOSTOMUM TENU CAUDATUM

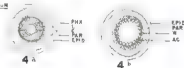


PLATE II

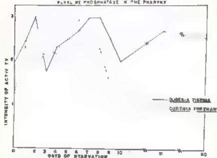
Graph 1. Alkaline phosphatase in the pharynx of the two aquatic triclads Dugesia tigrina and Curtisia foremani, showing some enzyme activity in this structure at all times, but with the peaks occurring at times when the pharynx is being used most.

Graph 2. Alkaline phosphatase in the gut of the two aquatic triclads listed above. Note the highest peaks of enzyme activity immediately after and one day after feeding. Then a general decline in activity is noted as the animals are starved. Some small peaks occurring during starvation were attributed to an effect on nutrients from autolysis.

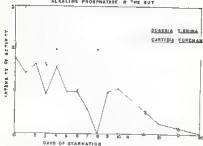
Graph 3. Alkaline phosphatase in the parenchyma of the same triclads. Parenchyma here includes the parenchyma proper and the several structures located within it, such as the dorso-ventral muscles, gonads, glands, protonephridia, and the adenal rhabdites. Note the similarity of the C. foremani curve to that for the gut in Graph 2, while that for D. tigrina shows an early lag, followed by a general increase at the time when activity in the gut has decreased. Digestion appears to proceed more rapidly in the former worm than in the latter.

PLATE II

Graph 1



Graph 2



Graph 3

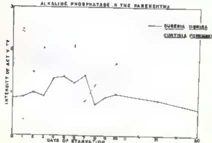


PLATE III

ALKALINE PHOSPHATASE ACTIVITY IN SPECIMENS FIXED SOON AFTER FEEDING

Fig. 1. Dugesia tigrina. Note the intense activity in the pharynx (central structure) and in the gut cells.

Fig. 2. Control for No. 1. The pharynx and gut are pale.

Figs. 3 and 4. Anterior and posterior branches of the gut of D. tigrina.

Fig. 5. Section through the distal end of the pharynx, which was apparently devoid of enzyme activity, while the two gut branches are intensely stained.

Fig. 6. Control for No. 5.

Fig. 7. Longitudinal section, showing the intense activity along the circular muscles of the pharynx, and in the gut cells just dorsal to the pharynx of C. foremani.

Fig. 8. Bipalium kewense. The gut, subepithelial glands, and rhabdites show activity.

Fig. 9. Control for No. 8.

Fig. 10. Bipalium kewense, with activity in the amoeboid cells.

Fig. 11. Control for No. 10.

Figs. 12, 13, and 14. Geocentrophora applanata, anterior to posterior transverse sections. The activity is confined mainly to the gut, and glands.

Fig. 15. Control for 12, 13 and 14, and for 16.

Fig. 16. Geocentrophora applanata, sectioned through the posterior gut region. Note the intense alkaline phosphatase activity in the gut cells, and in the follicle cells of the ovaries.

Fig. 17. Geocentrophora applanata, sectioned longitudinally, with activity in the gut, ovaries, and pharyngeal fibers.

Fig. 18. Stenostomum tenuicaudatum. Note the intense activity in the gut region.

Fig. 19. Control for 18. Some preformed phosphate is thus evident in the epidermis.

PLATE III

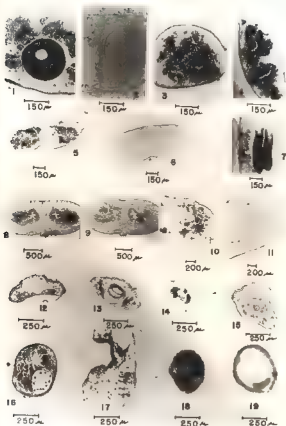


PLATE IV

ALKALINE PHOSPHATASE ACTIVITY IN STARVED SPECIMENS

Fig. 1. Section through the gut of Dugesia tigrina one day after feeding. Note the location of activity now.

Fig. 2. Control for No. 1. No phosphate is evident.

Fig. 3. The lateral region of a D. tigrina 2 days after a feeding. Alkaline phosphatase has become active in the protonephridia and tubules.

Fig. 4. Testis of Curtisia foremani 1 day after feeding.

Fig. 5. Section showing amoeboid cells and protonephridial tubule of C. foremani, 2 days after feeding.

Fig. 6. Copulatory apparatus of C. foremani, 3 days after feeding. Note high activity in the sperms in the penis.

Fig. 7. D. tigrina 4 days after feeding. Note intense activity in the parenchyma and basal membrane.

Fig. 8. Control for No. 7.

Fig. 9. C. foremani 4 days after feeding. Note intense activity in the inner circular muscles of the pharynx, and the two excretory tubules just latero-ventrally.

Fig. 10. C. foremani 4 days after feeding. Intense activity is seen in the parenchyma and around the copulatory apparatus.

Fig. 11. D. tigrina 5 days after feeding. The pharynx and dorsoventral muscles exhibit alkaline phosphatase activity.

Fig. 12. Control for No. 11.

Fig. 13. Protonephridia and lateral mucal glands of D. tigrina after 4 days of starvation.

Fig. 14. D. tigrina 6 days after feeding. Compare with Fig. 4 of Plate III. The gut is now almost devoid of activity.

Fig. 15. The same as Fig. 14, but including the pharynx.

PLATE IV--Continued

Fig. 16. Longitudinal section through the pharynx of C. foremani 1 day after feeding. Note the intense alkaline phosphatase activity in both the inner and outer circular muscles.

Fig. 17. Note the somewhat narrowed limits of activity in the protonephridial system of this 9-day D. tigrina as compared to the 4-day one in Fig. 13. Note the intense activity in the rhabdites nearby.

Fig. 18. Control for No. 17.

Fig. 19. In this 10-day specimen of D. tigrina some activity is evident in the pharynx, but the main concentration appears to be within peripheral structures of the parenchyma, which has been quite characteristic of this species during extensive starvation.

Fig. 20. In this worm, D. tigrina, which has been starved for 60 days, the activity of alkaline phosphatase has disappeared from practically all structures except for an apparent accumulation in the pharynx.

PLATE IV

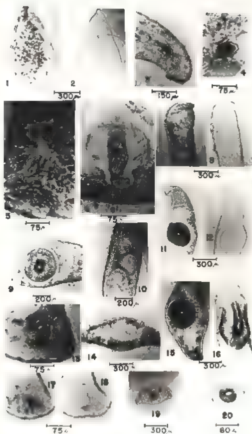


PLATE V

ACID PHOSPHATASE ACTIVITY IN FED AND STARVED SPECIMENS

Fig. 1. Dugesia tigrina soon (3 hrs.) after feeding. Note the apparent lack of activity in the gut, while the periphery and pharynx are intensely stained.

Fig. 2. Curtisia foremani treated in the same way, thus showing specific differences for acid phosphatase. Some activity is evident in the epidermis and gut.

Fig. 3. Control for No. 2.

Fig. 4. Curtisia foremani starved 4 days. The epidermal and adenal rhabdites are very intensely stained.

Fig. 5. Control for No. 4.

Fig. 6. D. tigrina 6 days after a liver feeding. The parenchyma is uniformly stained, indicating high activity.

Fig. 7. D. tigrina 7 days after eating egg yolk. Low activity remains in the gut, and some in the epidermal region.

Fig. 8. Control for No. 7.

Fig. 9. Bipalium kewense, showing low activity in the rhabdites, and no evidence of activity in the pharynx.

Fig. 10. Control for No. 9.

Fig. 11. Anterior section of Bipalium kewense. Note the high activity which is evident in the epidermal and adenal rhabdites.

Fig. 12. Control section for No. 11.

Fig. 13. A posterior section of B. kewense. Activity is very pronounced in the epidermal and adenal rhabdites.

Fig. 14. Control section for Nos. 13 and 15.

Fig. 15. The adenal rhabdites show even more pronounced acid phosphatase activity in this most posterior section of Bipalium kewense.

PLATE V

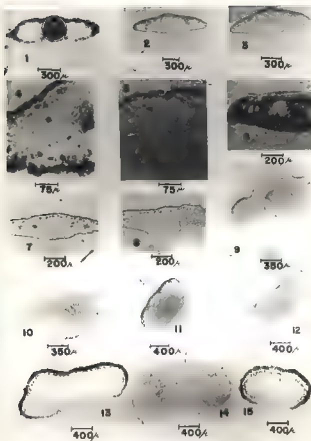


PLATE VI

LIPASE ACTIVITY IN FED AND STARVED SPECIMENS

Fig. 1. Dugesia tigrina soon (3 hrs.) after feeding. Note the lack of activity in the distal part of the pharynx, while there is high activity noted in the amoeboid cells of each of the two branches of gut.

Fig. 2. This is a more anterior section, showing less activity of lipase near the base of the pharynx in the gut, of Curtisia foremani, 5 days after feeding.

Fig. 3. In this anterior gut of D. tigrina, also fed recently, high activity is evident in the gut cells.

Fig. 4. Control section, especially for No. 3. Some preformed soaps are evident in the periphery.

Fig. 5. Dugesia tigrina 3 days after feeding. Note the spotty distribution of lipase activity in the gut.

Fig. 6. Control for No. 5.

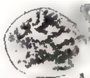
Fig. 7. Curtisia foremani 5 days after feeding. This section through the anterior branch of the gut shows high lipase activity in the amoeboid cells.

Fig. 8. Control section for No. 7, also for No. 9.

Fig. 9. Another specimen of Curtisia foremani, treated in the same manner as that shown in Fig. 7. Note the high activity of lipase in the gut cells of the two posterior branches, lateral to the pharynx.

Fig. 10. Geocentrophora applanata sectioned through the gut and ovaries. High lipase activity is evident in the lateral margins of the gut and in the nurse cells of the ovaries. No control section could be photographed, since the tissue was too light to produce any contrast.

PLATE VI



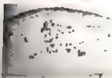


PLATE VII

PHOTOMICROGRAPHS OF SECTIONS STAINED FOR FATTY SUBSTANCES

Fig. 1. D. _tigrina_, soon after feeding. Sudan black stained heavily in the amoeboid cells of the gut.

Fig. 2. This is a section of D. _tigrina_, treated as above, showing the intense Sudan black stain in and around the amoeboid cells.

Fig. 3. D. _tigrina_, soon after feeding, and stained with Sudan III. Note the medium (pink) stain in the amoeboid cells of the gut, and in the rhabdites.

Fig. 4. The same as Fig. 1, but through the anterior branch of the gut.

Fig. 5. D. _tigrina_, soon after feeding, with Sudan III staining very intensely in the amoeboid cells of the gut (as globules) and in the adenal and epidermal rhabdites.

Fig. 6. The same as in Fig. 5, but through the pharynx also. Note a ring of stained substances within the pharynx, among the radial muscles, between the [illegible] circular and outer longitudinal muscle regions.

Fig. 7. This is another section very similar to Fig. 5, and the higher magnification makes the stained pod-like globules more apparent.

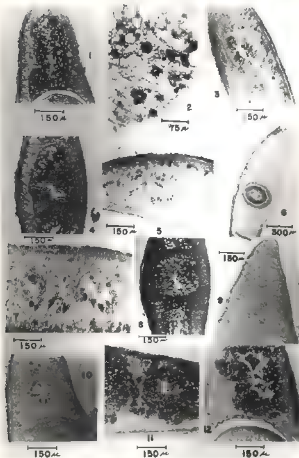
Fig. 8. D. _tigrina_, 15 days after feeding. Compare with Fig. 4. Apparently some fat is still remaining in the gut, but more is now evident in the parenchyma and peripheral regions.

Fig. 9. Control for No. 8. This worm was treated with fat solvents during fixation.

Fig. 10. Control for section stained with Sudan III. Note that rhabdites and some gut materials are still stainable.

Fig. 11. _Curtisia foremani_, soon after feeding. The gut and large bodies in the parenchyma stained a deep pink with Sudan III.

Fig. 12. This section through the mid-pharynx area of C. _foremani_, recently fed, shows evidence of fat in the gut.


1
150 µ
2
75 µ
3
50 µ
4
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6
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7
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8
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150 µ
11
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12
150 µ

PLATE VIII

PHOTOMICROGRAPHS OF SECTIONS STAINED FOR FATTY SUBSTANCES

Fig. 1. Curtisia foremani, soon after feeding. This shows food particles still in the pharynx. Note that Sudan black stained these particles as well as the material already in the gut with similar intensity.

Fig. 2. A section very similar to Fig. 1, but stained with Sudan III. Globules are apparent in the food particles entering by way of the pharynx, as well as in the gut.

Fig. 3. C. foremani, starved for an unknown period. The parenchyma stained a uniform pink with Sudan III, and what appeared to be fat bodies became a bright red.

Fig. 4. Control for Fig. 3. The fat bodies have almost entirely dissolved away, but epidermal rhabdites are still prominent.

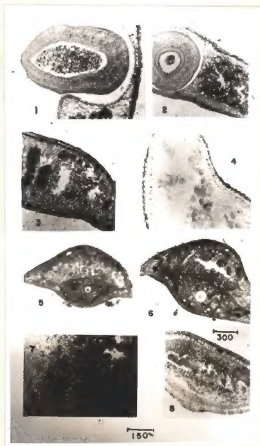
Fig. 5. Geocentrophora applanata, stained with nile blue. The ovaries are uniformly stained, and some stain is seen in the gut and parenchyma.

Fig. 6. With Sudan black, G. applanata stained much more intensely through the same structures observed in the section shown as Fig. 5, probably indicating that there are more phospholipids than fatty acids and glycerides.

Fig. 7. Bipalium kewense, stained with nile blue, showing evidence of fatty acids and glycerides (?).

Fig. 8. The same as in Fig. 7, only under lower magnification to show the overall picture. The gut and the parenchyma toward the dorsal side are stained very heavily with nile blue.

PLATE VIII



BIOGRAPHICAL SKETCH

Paul James Osborne was born in Blackwater, Virginia on December 29, 1921. He received his public school training in the Blackwater schools and received a Teachers' Certificate in 1941 from Lincoln Memorial University, Harrogate, Tennessee.

He taught for four years, 1941-42 and 1945-48, in the Lee County, Virginia, schools. From 1942 to 1945 he was in the U. S. Army, serving chiefly in a clerical capacity.

In June, 1948, he entered the University of Virginia. He received his B. A. in 1950 and M. A. in 1951.

Following a year as research assistant in the Biochemistry Department of the University of Virginia he entered the University of Florida in 1952. He served as a teaching assistant in C-61 and C-62 from 1952 to 1954, held graduate assistantships for the summers of 1953 and 1954, and a fellowship during 1954-55.

He was married to the former Addie Mae Bledsoe of Blackwater, Virginia, in 1942. They have two sons, Paul Douglas Osborne and Dennis Kay Osborne.

He is a member of the Phi Sigma Biological Society, the Association of Southeastern Biologists, and an associate member of the Society of the Sigma Xi.

He has accepted an appointment as Associate Professor of Biology at Lynchburg College, Lynchburg, Virginia, beginning in September, 1955.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 13, 1955

C. F. Byers
Dean, College of Arts and Sciences

Dean, Graduate School

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