Appl. Ent. Zool. **3**(3): 130—138 (1968)

Physiological Studies on the Digestion of Coccinellid Beetles (Coleoptera : Coccinellidae), with Special Reference to their Food Habits

Hironori SAKURAI[1]

Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, Japan

(Received August 28, 1967)

Comparative studies on the physiological natures of the digestion between entomophagous and phytophagous coccinellid beetles were made, using adults of four entomophagous species of *Coccinella septempunctata bruckii* MULSANT, *Harmonia axyridis* PALLAS, *Chilocorus kuwanae* SILVESTRI and *Rodolia cardinalis* MULSANT, and two phytophagous ones of *Epilachna vigintioctopunctata* FABRICIUS and *E. vigintioctomaculata* MOTSCHULSKY. The ratio of total alimentary canal length to the body length in phytophagous coccinellids was about twice as large as that of entomophagous ones. The length of each part of alimentary canal was ranked in the same order in both phytophagous and entomophagous groups as follows: midgut>hindgut>foregut. The activity of digestive enzymes, protease, lipase and trehalase in entomophagous insects was much higher than that in phytophagous ones. Optimum pH for carbohydrase and protease ranged between 5~6, which almost coincided with the pH of digestive juice in the alimentary canal. Experimental results suggested that the physiological differences in digestion between entomophagous and phytophagous forms are attributable to the gradient of natural selection at the development of diversity of food habits.

INTRODUCTION

Coccinellid species are classified into entomophagous, phytophagous and fungiphagous forms by their food habit. The great majority of entomophagous coccinellids are beneficial as natural enemies of pest insects, but some phytophagous coccinellids are injurious and often cause serious damage to crops. In relation to the biological control of insect pests, many studies have been made on the rearing of entomophagous coccinellids (SMIRNOFF, 1958; TANAKA and ITOYAMA, 1959; TANAKA and MAEDA, 1960; HUKUSIMA and SAKURAI, 1963), and in several species it comes near the practical use. Now on this current, it will be important to study the basic nature of the physiology of coccinellids. In this paper the physiological differences of digestion between entomophagous and phytophagous forms were reported and discussed in relation to their food habits.

[1] Present address: Laboratory of Public Health, Wakayama Medical College, Wakayama, Japan.

MATERIALS AND METHODS

Six species of Coccinellidae used for experiments were aphidphagous *Coccinella septempunctata bruckii* MULSANT and *Harmonia axyridis* PALLAS, coccoidophagous *Rodolia cardinalis* MULSANT and *Chilocorus kuwanae* SILVESTRI, and phytophagous *Epilachna vigintioctopunctata* FABRICIUS and *E. vigintioctomaculata* MOTSCHULSKY. They were bred on natural diets from egg-hatching in the rearing room and adults only were used for experiments.

Measurement of the alimentary canal length: After measurement of the body length, alimentary canal was dissected out in 0.9% saline solution, then the lengths of the fore-, mid- and hindgut were measured under a binocular microscope.

Determination of the pH in the alimentary canal: One-day-starved adults were fed on 5% glucose solution containing pH indicators. After 1 or 2 days, they were dissected, and the pH values in the fore-, mid- and hindgut were determined by the color changes. The pH values were also ascertained with pH testing paper. Bromophenol blue (2.8—4.4), bromocresol green (4.0—5.6), chlorophenol red (4.8—6.4), bromocresol purple (5.2—6.8), methyl red (5.4—7.0), bromothymol blue (6.2—7.8) and phenol red (6.8—8.2) were used as pH indicators in glucose solution.

Analytical methods of the digestive enzyme: A definite number of midguts collected from the one-day-starved adults were homogenized with a POTTER-ELVEHJEM type homogenizer under chilling. The homogenate was diluted with distilled water, then centrifuged at 3,000 rpm. The filtrate of the supernatant through a monolayer gauze was used as the enzyme source for assay.

1) Carbohydrases: Amylase activity was measured by the method of APPLEBAUM et al. (1961). The reaction mixture consisting of 0.5 ml of 2% soluble starch as the substrate, 0.5 ml enzyme solution and 1 ml buffer solution, was incubated for 20 min at 30°C. After the reaction, hydrolyzed maltose was determined colorimetrically with dinitrosalicylic acid reagent (BERNFERD, 1955). Buffer solutions were 1/10 M acetate buffer in acid range, 1/10 M phosphate buffer in neutral range and 1/10 M glycine buffer in alkaline range. The protein content in the enzyme solution was determined colorimetrically with FOLIN reagent by the method of LOWRY et al. (1951).

The activity of maltase and invertase was measured chromatographically by the method of KRISHNA (1958). The reaction mixture consisted of the substrate of 0.1 ml of 5% maltose or sucrose solution, 0.1 ml of 0.02 M phosphate-citrate buffer and 0.1 ml enzyme solution in which the protein concentration was made equall among each coccinellid species. After 10 hr of incubation, the reaction was stopped by adding 0.01 ml of 0.1 N NaOH. For detecting the hydrolyzed or unhydrolyzed sugar after the reaction, incubation mixture was subjected to paper chromatography, employing n-butanol-acetic acid-water (4 : 1 : 5, v/v) as a solvent and benzidine trichloroacetic acid as a color reagent (BACON and EDELMAN, 1951). The portion of hydrolyzed or unhydrolyzed sugar on the nonsprayed chromatogram was eluted with water. Percentages of hydrolyzed maltose or sucrose were calculated from the sugar content determinded with anthron method (MOKRASCH, 1954).

Trehalase activity was measured using the reaction mixture system of SAITO

(1960) as in the following: 0.5 ml of 0.02 M trehalose, 0.5 ml enzyme solution and 1 ml of 0.02 M phosphate-citrate buffer (pH 5.6), were incubated at 30°C. After the reaction, the rate of glucose formation was determined colorimetrically with dinitrosalicylic acid reagent.

2) Protease: Protease activity was measured by the method of YOSHIHARA (1961). The reaction mixture was made to contain 1 ml of 2% casein as the substrate, 2 ml of 0.1 M citrate-phosphate buffer and 1 ml enzyme solution. After the incubation for 1 hr at 30°C, the reaction was stopped by adding 2 ml of 0.4 M trichloroacetic acid. Amino acids freed from the substrate were obtained by filtering the reaction mixture, then these contents were measured colorimetrically with three-fold diluted FOLIN reagent (LOWRY et al., 1951) at 660 mμ.

3) Lipase: Lipase activity was determined manometrically by MARTIN and PEERS method (1953). The reaction flasks contained 0.5 ml of each substrate (4% v/v tributyrin, triacetin, tween 20, tween 60 or tween 80 in 0.0148 M $NaHCO_3$) in the side arm, and 1.5 ml distilled water in the main chamber. The flasks and manometers were gassed for 3 min with a mixture of 95% N_2 and 5% CO_2, then the reaction was started with tipping the substrate in the side arm. The reading of the manometer was taken at regular intervals for 1 hr at 30°C.

RESULTS

The length of alimentary canal

The alimentary canal length and body length of five coccinellids were presented in Table 1. The midgut proved to be the longest part in canal in all the species examined, irrespective of feeding habits. However, the ratio of alimentary

Table 1. ALIMENTARY CANAL LENGTH AND BODY LENGTH IN FIVE COCCINELLID SPECIES

Insect species	Sex	No. of insects	Body length (A) mm	Length of the alimentary canal				
				Foregut mm	Midgut mm	Hindgut mm	Total(B) mm	B/A
Entomophagous								
Coccinella septempunctata	♀	15	8.0±0.8	2.0±0.4	5.9±0.9	4.4±0.3	12.3±1.2	1.54
	♂	10	8.5±0.4	2.0±0.2	6.9±0.7	4.8±0.3	13.5±1.0	1.59
Rodolia cardinalis	♀	8	3.3±0.2	0.8±0.1	3.0±0.3	1.7±0.2	5.6±0.7	1.70
	♂	4	3.5±0.3	0.9±0.2	3.1±0.1	1.9±0.1	5.9±0.1	1.69
Chilocorus kuwanae	♀	6	3.5±0.4	0.7±0.1	2.6±0.3	2.6±0.1	6.0±0.4	1.71
	♂	4	3.8±0.1	0.9±0.2	3.2±0.3	2.5±0.2	6.7±0.6	1.76
Phytophagous								
Epilachna vigintioctopunctata	♀	8	6.7±0.7	1.2±0.1	18.1±2.9	5.8±0.4	25.0±3.4	3.73
	♂	17	6.9±0.5	1.2±0.3	18.3±2.7	6.0±0.3	25.4±3.1	3.68
E. vigintioctomaculata	♀	5	7.7±0.4	1.8±0.2	16.8±2.7	6.1±0.5	24.7±2.9	3.21
	♂	6	7.5±0.6	1.5±0.3	16.8±2.2	6.4±0.5	24.5±2.6	3.27

a) Mean ± S. D.

canal length to body length in two phytophagous species was about twice as large as that in three entomophagous species, i.e. 3.7 in *E. vigintioctopunctata,* 3.2 in *E. vigintioctomaculata,* 1.5—1.6 in *C. septempunctata,* 1.7 in *R. cardinalis* and 1.7—1.8 in *C. kuwanae.* Between male and female, clear difference in the length and/or the ratio of alimentary canal to body length was not observed.

The pH of alimentary canal

As shown in Table 2, pH values of alimentary canal in six species ranged from 4.6 to 6.4. In *C. septempunctata, H. axyridis, E. vigintioctopunctata* and *E. vigintioctomaculata,* pH values of mid- and hindgut were slightly higher than those of foregut. The pH values of whole guts of coccoidophagous *R. cardinalis* and *C. kuwanae* were nearly the same as four other coccinellid species.

Table 2. pH of the Alimentary Canal in Adults of Six Coccinellid Species

Insect species	Foregut	Midgut	Hindgut
Coccinella septempunctata	5.0—5.8	5.4—6.0	5.0—6.0
Harmonia axyridis	4.8—5.6	5.2—5.8	4.8—5.6
Epilachna vigintioctopunctata	5.0—5.8	5.4—6.2	5.2—6.3
E. vigintioctomaculata	5.0—5.8	5.6—6.4	5.5—6.4
Rodolia cardinalis		4.6—5.4[a]	
Chilocorus kuwanae		4.8—5.2[a]	

a Values measured in whole gut homogenate

The activity of digestive enzymes

Amylase activities in various pH of *E. vigintioctopunctata* and *E. vigintioctomaculata* (phytophagous) were slightly higher than those of *C. septempunctata* and *H. axyridis* (entomophagous), though the difference was not significant (Fig. 1).

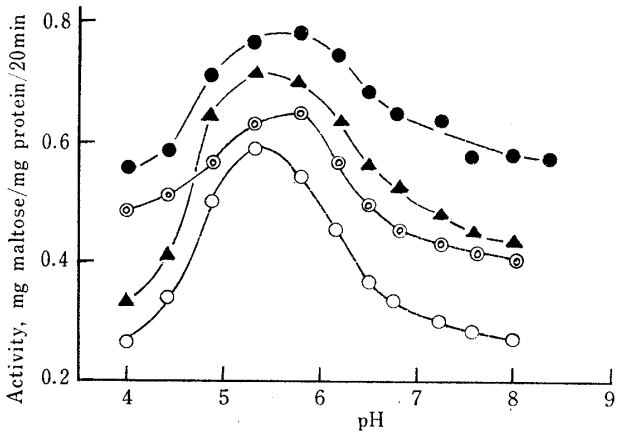

Fig. 1. Amylase activity of four species of Coccinellidae adults in different pH. ○, *Coccinella septempunctata*; ◎, *Harmonia axyridis*; ●, *Epilachna vigintioctopunctata*; ▲, *E. vigintioctomaculata.*

The activity of invertase was a little higher than that of maltase in *C. septempunctata*, *R. cardinalis*, *E. vigintioctopunctata* and *E. vigintioctomaculata* (Fig. 2). There was no significant difference in invertase and maltase activity among these coccinellid species. Optimum pH for amylase, invertase and maltase activity was found to be 5—6, and almost the same with that of their alimentary canal.

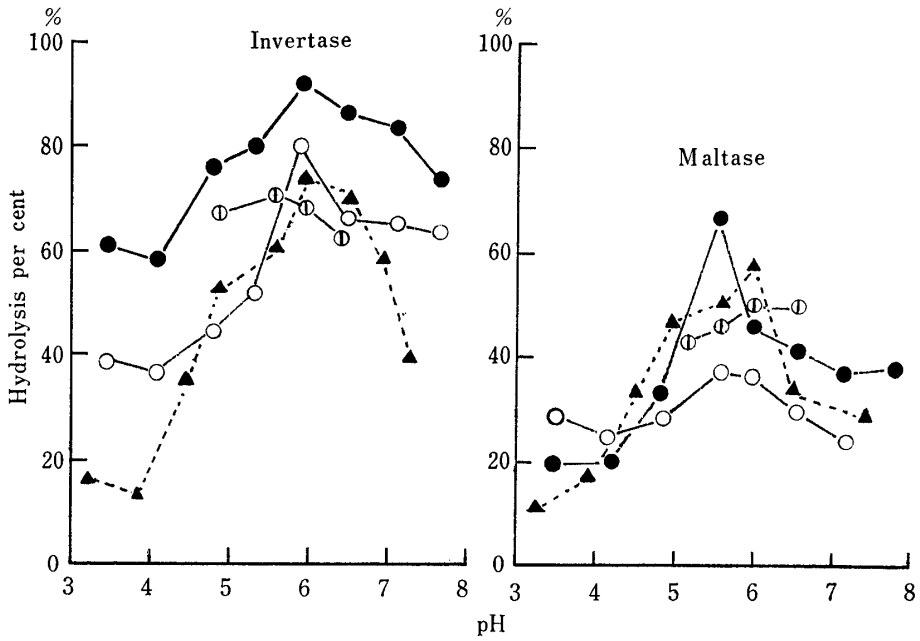

Fig. 2. Invertase and maltase activity of four species of Coccinellidae adults in different pH. ○, *Coccinella septempunctata*; ⦶, *Rodolia cardinalis*; ●, *Epilachna vigintioctopunctata*; ▲, *E. vigintioctomaculata*.

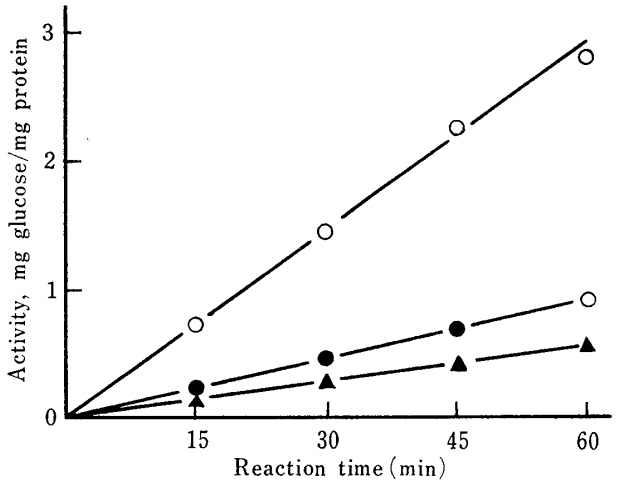

Fig. 3. Trehalase activity of three species of Coccinellidae adults. ○, *Coccinella septempunctata*; ●, *Epilachna vigintioctopunctata*; ▲, *E. vigintioctomaculata*.

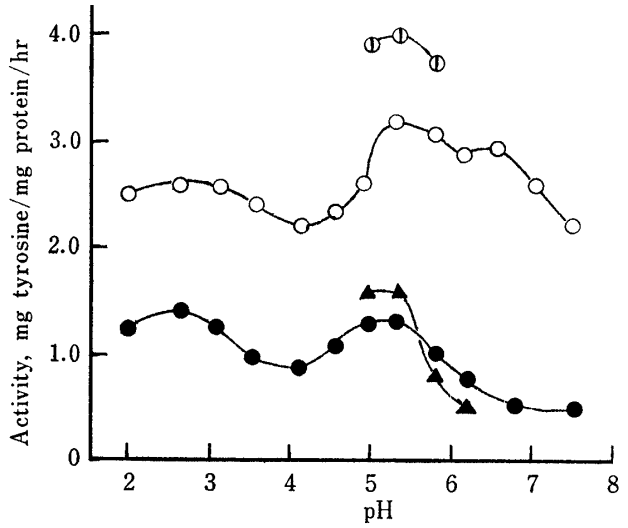

Fig. 4. Protease activity of four species of Coccinellidae adults in different pH. ○, *Coccinella septempunctata*; ⓘ, *Rodolia cardinalis*; ●, *Epilachna vigintioctopunctata*; ▲, *E. vigintioctomaculata*.

Trehalase activities in *C. septempuncta, E. vigintioctopunctata* and *E. vigintioctomaculata* were shown in Fig. 3, apparently indicating that the activity of the first one, entomophagous, was much higher than those of other two phytophagous species.

Protease activities in *C. septempunctata, R. cardinalis, E. vigintioctopunctata* and *E. vigintioctomaculata* were indicated in Fig. 4 showing two optimum pH ranges of 2—3 and 5—6 for activities in *C. septempunctata* and *E. vigintioctopunctata*. The activity in pH 5—6 seems to be attributable to trypsin action and that in pH 2—3 to pepsin action. Trypsin like activity in two entomophagous species was much higher than those in two phytophagous species.

Table 3. Lipase Activity in Adult of Three Coccinellid Species

Insect spceies	Substrates					
	Tributyrin	Triacetin	Methyl butyrate	Tween 20	Tween 60	Tween 80
Coccinella septempunctata	1.12	0.85	0.16	0.12	0.06	0
Epilachna vigintioctopunctata	0.13	0.14	0.05	0.06	0.01	0
E. vigintioctomaculata	0.26	0.30	0.06	0.12	0.01	0

Activity unit is mm^3 CO_2/mg protein/hr, and final substrate concentration is 1 % (v/v)

Lipase activities in *C. septempunctata, E. vigintioctopunctata* and *E. vigintioctomaculata* were presented in Table 3. Activity for tributyrin or triacetin was much higher than that for tween 20 or tween 60 throughout three species. No hydrolysis of tween 80 was observed. The activity for tributyrin or triacetin in *C. septempunctata* was much higher than that in other two phytophagous species,

E. vigintioctopunctata and *E. vigintioctomaculata*.

DISCUSSION

It has been generally accepted that the alimentary canal in phytophagous insects is longer than that in entomophagous insects. The results of coccinellids in the present study also agreed well with the general acceptance, elucidating that the ratio of the total length of alimentary canal to body length in phytophagous species was about twice as large as that in entomophagous ones.

In coleopterous species, the length ratio of fore-, mid- and hindgut shows various patterns. For instance, in both of phytophagous and sarcophagous Scarbaeoidae, midgut develops into conspicuously complicated forms and occupies the largest portion in the canal (UMEYA, 1960; 1964). While, in sarcophagous Caraboidea, foregut in ciccindellids and harbalids, or hindgut in carabids is the largest portion, respectively (HUKUSIMA and MATSUNO, 1962). However, in both of phytophagous and entomophagous coccinellids investigated here the midgut was found to be the longest and the most developed part in the canal, suggesting the presence of the most active digestive function in the midgut.

As a rule, the contents of the gut tend to be more alkaline in phytophagous species than in carnivorous ones (WIGGLESWORTH, 1934), but there are many exceptions in phytophagous insects, as pH in *Tribolium* and *Criptolestes* adults was 4.2—5.6 (SINHA, 1959), and pH of digestive fluid of *Schistocerca gregaria* adult was 5.7 in foregut, 6.2—7.1 in midgut, and 7.0 in hindgut (EVANS and PAYNE, 1964). The pH in both entomophagous and phytophagous coccinellids is nearly the same value as 4.5—6.5, and pH in foregut is a little lower than that in midgut and hindgut. This pH values coincided with pH of three coccinellid species measured by HEIMPEL (1960). Weak acid or middle pH range in the alimentary canal of phytophagous coccinellids is recorded as the exceptional example among phytophagous insects.

Optimum pH range of 5—6 for amylase activity in midgut in four species of coccinellid adults obtained here was nearly the same as that in many species reported hitherto, e.g. *Blattella germanica* adult (WIGGLESWORTH, 1927), *Anax parthenope* adult and *Cybister japonicus* larvae (SHINODA, 1930), *Bombyx mori* larvae (HORIE, 1959), *Tenebrio molitor* larvae (APPLEBAUM et al., 1961), *Tribolium castaneum* larvae and adult (KRISHNA and SAXENA, 1962), and *Schistocerca gregaria* adult (EVANS and PAYNE, 1964). This pH range corresponded with optimum pH for invertase and maltase in Coccinellidae. Considering the pH in the gut of coccinellids, carbohydrates will be digested in maximum efficiency in the midgut under the pH of 5—6. In regard to pepsin action in proteases in insects, GREENBERG and PARETSKY (1955) showed two ranges of optimum pH in acid and alkaline ranges for protease activity in early stage of housefly. Also in coccinellids such pepsin like and trypsin like activity were found.

The activity of protease, lipase and trehalase, respectively, was much higher in entomophagous coccinellids than in phytophagous ones, hence this tendency may be correlated to their food habits. As regard to the relationship between food habits and the function of alimentary canal, UMEYA (1961) stated that the

pattern of amino acid composition in the midgut of lamella beetles was parallel to the grouping followed by their food habits. Therefore, the higher activities in protease, lipase and trehalase in entomophagous coccinellids rather than in phytophagous ones may be understood by taking account of their food habits, since the aphids and coccoids, the victims of entomophagous coccinellids contain much higher proteins, lipids and trehalose as their tissue components.

According to KOYAMA (1950) that phytophagous coccinellids evolutionally developed from entomophagous species, the differences in the length of alimentary canal and the activity of digestive enzymes between phytophagous and entomophagous species may be caused by the natural selection by the food habits at the differentiation from carnivorous habits. The evolution problem in insect species is a very interesting subject, but the approach to the resolution is on the complicated and difficult way. For this study, coccinellids will be the most proper and suitable materials, so further experiments should be performed.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Prof. K. IYATOMI and Assoc. Prof. T. SAITO for their instructions and criticisms. The author also wishes to express his gratitude to Dr. K. KANEHISA for his valuable advices, and Mr. H. HONDA for his help in collecting experimental insects.

REFERENCES

APPLEBAUM, S. W., M. JANKOVIĆ and Y. BIRK (1961) Studies on the midgut amylase activity of *Tenebrio molitor* L. larvae. *J. Insect Physiol.* **7** : 100–108.

BACON, J. S. D. and J. EDELMAN (1951) The carbohydrate of the jersalem artichoke and other compositae. *Biochem. J.* **48** : 114–126.

BERNFERD, P. (1955) Amylases, α and β. *Methods in Enzymology* (Ed. by S.P. COLOWICK and N. O. KAPLAN) **1** : 149–158. Academic Press, New York.

EVANS, W. A. L. and P. W. PAYNE (1964) Carbohydrases of the alimentary tract of the desert locust *Schistocerca gregaria* FORSK *J. Insect Physiol.* **10** : 657–674.

GREENBERG, B. and D. PARETSKY (1955) Proteolytic enzymes in the house fly, *Musca domestica* (L). *Ann. ent. Soc. Amer.* **48** : 46–50.

HEIMPEL, A. M. (1960) The application of pH determinations to insect pathology. *Proc. ent. Soc. Ont.* **91** : 52–76.

HORIE, Y. (1959) Physiological studies on the alimentary canal of the silkworm, *Bombyx mori*. II. Carbohydrases in the digestive fluid and in the midgut tissue. *Bull. sericul. Exp. Stn* **15** : 365–382.

HUKUSIMA, S. and T. MATSUNO (1962) Comparative studies on the length of alimentary canal in adult Caraboidea (Coleoptera). *Kansai Byotyugai Kenkyu Kaiho* **4** : 6–11.

HUKUSIMA, S. and H. SAKURAI (1963) Studies on the physiological difference between artificially and naturally reared entomophagous Coccinellid beetles. *J. Tokai Branch ent. Soc. Japan* **15** : 12–21.

KOYAMA, N. (1950) Studies on the rearing and growth with its own eggs of *Epilachna vigintioctomaculata M. Kontu* **18** : 140–142.

KRISHNA, S. S. (1958) Further studies on digestion of food in the gut of *Trogoderma* larvae. I. Digestive enzymes–carbohydrases. *Physiol. Zoöl.* **31** : 316–323.

KRISHNA, S. S. and K. N. SAXENA (1962) Digestion and absorption of food in *Tribolium castaneum* (HERBST). *Physiol. Zoöl.* **35** : 66-78.

LOWRY, O. H., N. J. ROSENBROUGH, A. L. FARR and R. J. RANDAL (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **139** : 265-275.

MARTIN, H. F. and F. G. PEERS (1953) Oat lipase. *Biochem. J.* **55** : 523-526.

MOKRASCH, L. C. (1954) Analysis of hexose phosphates and sugar mixture with anthrone reagent. *J. biol. Chem.* **208** : 55-59.

SAITO, S. (1960) Trehalase of the silkworm, *Bombyx mori*. Purification and properties of the enzyme. *Jap. J. Biochem.* **48** : 101-109.

SINHA, R. N. (1959) The hydrogen-ion concentration in the alimentary canal of beetles infesting stored grain and grain products. *Ann. ent. Soc. Amer.* **52** : 763-765.

SHINODA, O. (1930) Contributions to the knowledge of intestinal secretion in insects. IV. Comparison of the pH optima of the digestive enzymes from different groups of insects. *Kyoto Imp. Univ. Anniversary* Vol. *Dedicated to Masumi Chikashige,* p. 9-24.

SMIRNOFF, W. A. (1958) An artificial diet for rearing Coccinellid beetles. *Can. Ent.* **90** : 563-565.

Smith, B. C. (1960) A technique for rearing Coccinellid beetles on dry foods, and influenced of various pollens on the development of *Coleomegilla maculata lengi* TIMB (Coleoptera: Coccinellidae). *Can. J. Zool.* **38** : 1047-1049.

TANAKA, M. and K. ITOYAMA (1959) Studies on the artificial rearing of Coccinellid beetles. *Kyushu agric. Res.* **21** : 9-12.

TANAKA, M. and Y. MAEDA (1960) Studies on the method of artificial rearing of prominent species of Coccinellid beetles. *Kyushu agric. Res.* **22** : 77-88

UMEYA, K. (1960) A comparative morphology of the alimentary tract in the adults of lamellicorn beetles (Coleoptera). *Memor. Fac. Agr. Hokkaido Univ.* **3** : 60-96.

UMEYA, K. (1961) Study on the free amino acid composition of midintestine in several species of lamellicorn beetles. *Ent. exp. appl.* **4** : 54-61.

UMEYA, K. (1964) The alimentary canal of *Platycerus delicatals* LEWIS (Coleoptera, Kucanidae). *Kontû,* **32** : 52-54.

WIGGLESWORTH, V. B. (1927) Digestion in the cockroach. II. The digestion of carbohydrates. *Biochem. J.* **21** : 797-811.

WIGGLESWORTH, V. B. (1934) *Insect Physiology,* Methuen and Co., London 42 pp.

WIGGLESWOOTH, V. B. (1935) *The Principles of Insect Physiology.* Methuen and Co., London, 332 pp.

YOSHIHARA, F.(1961) On the protease in the polyhedral virus particles of silkworms. *Science Bull. Fac. Agr. Kyushu Univ.* **18** : 257-262.