BEITRÄGE ZUR ENTOMOLOGIE
11. BAND · NUMMER 1/2 · BERLIN · MÄRZ 1961

Mode of Action of Insecticides

I. Observations on the effect of endrin on blood cell count of some insects

K. N. TREHAN & H. R. PAJNI

Zoology Department, Panjab University
Chandigarh, Punjab, India

Introduction

To determine the mode of action of contact insecticides, their effect was studied on the blood of insects. The present investigations deal with the differences observed in the total number of blood cells in the treated insects but the future studies will be aimed at finding the changes induced in the blood cell picture and in the process of its coagulation.

The blood cell picture in a few insects has already been described both by preparing smears or by making the living studies under the phase contrast microscope (YEAGER, 1945; ARNOLD, 1952; JONES, 1950, 1954, 1957, 1959). TAREEVA & NENJUKOV (1931), PILAT (1935), LEPESME (1937), YEAGER & MUNSON (1942), TOUMANOFF & LAPIED (1950) and ARNOLD (1952) have published their results on the effects of some toxic agents whereas TAYLOR (1935) and JONES & TAUBER (1952, 1954) gave an account of the action of some experimentally induced conditions on the blood cells of different insect species. Some records are also available of the blood cell counts of insects (YEAGER & TAUBER, 1932, 1933; TAUBER & YEAGER, 1934, 1935, 1936; JONES & TAUBER, 1951). Since references on the effect of insecticides and other toxic substances on the number of blood cells are very few an attempt was made to take up this problem and study the effect of contact insecticides on the total blood cell counts in some insects.

SHULL et al. (1932) noted the effect of 34 toxic gases on the blood of *Blatta orientalis* L. and found only a few gases inducing any change in its blood or in blood cell counts. FISHER (1936) reported a significant decrease in the total cell count of this very species when killed with mercuric chloride, white arsenic and sodium fluosilicate and a significant increase with pyridine. YEAGER & MUNSON (1942) also found a fall in the blood cells of southern army worm, *Prodenia eridania* (CRAM.) when treated with mercuric chloride, sodium fluoride, barium fluosilicate and calcium arsenate. JONES & TAUBER (1954) observed the number of haemocytes rising four times the normal number in the meal worm (*Tenebrio molitor* L.) when the insects were killed in nicotine sulphate vapour.

Certain physiological and pathological conditions also have been reported to be associated with low or high total haemolymph cell counts in insects. TAUBER & YEAGER (1934, 1935, 1936) have found a higher number of blood cells in a large number of insects, diseased or in special conditions of oviposition or ecdysis, belonging to both holometabola & hemimetabola groups. Similarly lack of food and moisture is believed to have caused a rapid rise in the blood cells of *Periplaneta americana* L. (TAYLOR, 1935). On the other

DOI: 10.21248/contrib.entomol.11.1-2.1-11

hand, acute bacterial infection (BABERS, 1938) and the advent of pupal stage in holometabolous insects (TAUBER & YEAGER, 1936) have been found to affect a decrease in the number of haemocytes.

Since nothing definite is known about the action of toxic substances in general and the insecticides in particular, on the total haemocytes of insects, a thorough study on the action of some standard insecticides on the blood cell counts of a number of insects was considered very essential. Therefore the present observations were recorded on the effect of endrin on the blood cell counts of cockroach, (*Periplaneta americana* L.), mature caterpillars of 'Kutra' moth, (*Amsacta moorei* BUTLER), silkworms (Japanese breed Taihei × Choan) and black caterpillars on 'Nargis' (*Narcissus poeticus* L.). Efforts were also made to make similar studies on the adults of red cotton bug *Dysdercus cingulatus* F. and the blister beetle *Mylabris phalerata* PALL.

Material and Methods

The silkworms (representing a Japanese breed Taihei × Choan, produced by Katakura, Tokyo, Japan) were reared in the laboratory, from the eggs supplied by the Incharge Government Sericulture Research Station, Sujanpur, Punjab. The red hairy caterpillars of *Amsacta moorei* BUTLER and black caterpillars (sent for identification) of 'Nargis' (*Narcissus poeticus* L.) were collected from the fields of maize and the plants of 'Nargis' respectively. Adult cockroaches were reared in the laboratory from the last instar nymphs collected from damp places near sewerage etc. The culture of red cotton bugs, *Dysdercus cingulatus* F. was maintained in the laboratory and the nymphs and adults were supplied wet cotton seed as food.

For taking the blood, the end of antenna of the cockroaches and red cotton bugs and the end of first proleg of the caterpillars was cut and the body of the insect pressed gently to squeeze the blood drops. A small quantity of blood oozed out from the cut surface and was taken on a clean glass slide. A desired amount of the blood was taken in a pipette with a capacity to dilute the sample 1000 times, and diluted by a special saline solution.[1]) The blood cells were counted with the help of a haemocytometer consisting of two micropipettes, a slide and a small coverslip.

By examining a drop of blood in the counting chamber of the haemocytometer (FISHER, 1935), and not in oil (YEAGER et al., 1932; YEAGER & KNIGHT, 1933), a lot of blood coagulation was noticed and this difficulty could not be removed even by adopting the rapid method of dilution (YEAGER & TAUBER, 1932, 1933) by drawing the blood direct into the diluting fluid. Therefore, as an anti coagulation measure, the insects were exposed to glacial acetic acid vapour, before the extraction of blood (SHULL et al., 1932; SHULL, 1936). A five minute exposure to acetic acid vapour was found sufficient to inhibit the coagulation completely and longer exposures as observed by SHULL & RICE (1933) and FISHER (1935) were not necessary.

For observations on the treated insects, the normal individuals were sprayed with 0.1% endrin and blood taken from the freshly paralysed ones after 4—8 hours of the treatment. The paralysed individuals were also exposed for 5 minutes to acetic acid vapour to check the coagulation of blood.

Results

1. *Periplaneta americana* (L.)

Average number of blood cells in the normal female cockroach was 41830 mm³. with a range of 22750—69125 mm³. In the freshly paralysed

[1]) Containing 0.018 M Na cl, 0.002 M K cl, 0.001 M Ca cl_2, 0.005 percent gentian violet and 0.125 percent acetic acid, and described by TAUBER & YEAGER (1934, 1935).

DOI: 10.21248/contrib.entomol.11.1-2.1-11

insects treated with endrin the number of haemocytes tends to fall considerably and an average of 18000 mm³. has been recorded with a range of 11650—21625 mm³. In fact the number of haemocytes began to decrease in the treated insects even before paralysis. A few observations were also made on the total cell count of cockroaches which were treated with endrin but were not paralysed wherein the average number of cells was found to be 37281 mm³. Thus there seems a clear indication that endrin reduces the number of haemocytes and this fall in number becomes more conspicuous in paralysed insects.

All attempts to get blood from the completely paralysed roaches proved abortive. In most of such cases no blood was obtained at all and in some cases the quantity of blood that oozed out was so small that it could not be used for haemocyte counts.

During the observations on cockroaches it was noticed that a few insects which harboured a large number of bacteria in their blood, showed a considerably low number of blood cells. However, these were not considered in the calculation of the average number of haemocytes.

2. Full grown caterpillars of *Amsacta moorei* Butler

Experiments conducted on these caterpillars have shown the average number of blood cells in the normal individuals to be 27735 mm³. with a range of 20500—33625 mm³. The average number in the freshly paralysed caterpillars, when sprayed with endrin and studied 6—8 hours after the treatment was 16650 mm³. with a range of 12500—22500 mm³. Accordingly here also endrin caused a fall in the number of haemocytes. In the completely paralysed caterpillars which appeared almost dead and were studied after 24 hours of the treatment, the number of blood cells was much more than the number in the normal caterpillars. It may, however, be stated that the amount of blood that oozed out from the completely paralysed individuals was very small as compared to the normal and freshly paralysed caterpillars and this change in their number was probably due to it.

3. Full grown caterpillars of Silk worm (Japanese breed)

The silk worms provide probably the best material for work on the haemocyte counts, because of the presence of a large amount of haemolymph, least blood coagulation and relatively low number of blood cells. The average number of blood cells in the normal and freshly paralysed caterpillars was 8280 mm³. (range 8100—8500 mm³.) and 6870 mm³. (range 5960—7500 mm³.) respectively. Here also the number of blood cells fell in the freshly paralysed caterpillars after treatment with endrin.

4. Full grown caterpillars of 'Nargis' (*Narcissus poeticus* L.)

The average blood cell counts of the normal caterpillars were found to be 10225 mm³. with a very wide range of 2625—20500 mm³., and those

DOI: 10.21248/contrib.entomol.11.1-2.1-11

of the freshly paralysed caterpillars as 17408 mm³. with a range of 8125—28625 mm³. It was observed that there was an increase in the number of blood cells in the treated caterpillars.

5. Red cotton bug, *Dysdercus cingulatus* F.

The average number of haemocytes in the normal females of red cotton bug was only 1417 mm³. Unfortunately the treated insects could not provide the least amount of blood required for dilution (0.1 cu. mm.) and therefore it was not possible to find the number of cells in the treated insects.

Normally the culture of red cotton bugs was reared by feeding them on moistened cotton seed. Some of the haemocytes in their blood were found to be filled with what appeared to be fat granules and numerous free fatty spheres were found floating in the haemolymph. Due to the presence of these inclusions it became very difficult to inhibit the coagulation of blood by acetic acid vapour. For the present study, therefore, the adult bugs were fed on the fresh cotton leaves to check the accumulation of fat in the blood. Fat inclusions have also been observed in the blood cells of southern army worm, *Prodenia eridania* (Cram.) by Munson & Yeager (1944).

Attempts were also made to study the blood cell counts of the beetle *Mylabris phalerata* Pall. but the coagulation of its blood could not be inhibited by even a 30 minutes exposure to acetic acid vapour. It appears that blood of this beetle has an abnormally strong tendency for coagulation.

Although no detailed study of the types of haemocytes has been made but broadly speaking two types of blood cells, of large and small size, are present in the insects examined. It has been noticed that coagulation of the blood is brought about always by the formation of clusters by the small haemocytes. Coagulation of blood seems to involve the haemocytes alone, there being no apparent effect on the haemolymph.

Discussion & Conclusions

There appears to be no unanimity in the procedures adopted for making counts of the blood cells in insects. Therefore, conflicting results in the same or allied species may be only due to different techniques employed. Average cell counts per cubic millimeter for *Blatta orientalis* (L.) as reported by different authors is 31673 (Yeager & Tauber, 1933), 32698 (Tauber & Yeager, 1935), 34635 (Fisher, 1935) and 36173 (Fisher, 1936). Fisher (1935) found that blood cell count from the cockroach, *Blatta orientalis*, when treated with glacial acetic acid vapour to inhibit coagulation, was approximately twice as great as that from cockroaches which were not treated. On the other hand, Yeager & Tauber (1932, 1933) and Tauber & Yeager (1935) showed the average number almost equal to the treated cockroaches of Fisher (1935) by the rapid dilution method without the

DOI: 10.21248/contrib.entomol.11.1-2.1-11

use of acetic acid vapour. Recently JONES (1954) has described the number of blood cells of the mealworm larvae rising when these are heat fixed by keeping at 55°c for a few minutes. He is of the view that in unfixed larvae many haemocytes are held back presumably as a result of non specific adhesion to tissues. His remarks "whether heat fixation preserves the number of haemocytes circulating at the moment of fixing or whether heat drives previously non circulating haemocytes off from tissue surface is an important and critical question that merits further study", could also be applied to the action of acetic acid vapour. But in the light of the observations of YEAGER & TAUBER (1932, 1933) and TAUBER & YEAGER (1935) we are tempted to conclude that a higher average noticed by FISHER (1935) and JONES (1954) by exposure to acetic acid vapour and with heat treatment respectively is perhaps due to the inhibition of coagulation and clump formation of the haemocytes in the blood and not due to the preservation of the circulating haemocytes or setting free of non circulating ones.

Anticoagulation properties of the acetic acid (SHULL et al., (1932) and other fatty acids (SHULL, 1936) are well established. Most of the coagulation was inhibited with an exposure of 10—12 minutes at 30°c (SHULL & RICE, 1933) and with an exposure of 30 minutes (FISHER, 1935). Complete inhibition of coagulation, however, was observed by the latter author to occur when the insects were subjected to acetic acid vapour till death. During the present investigations, however, the insects were exposed to acetic acid vapour for 5 minutes and practically no coagulation occured. But in *Mylabris* coagulation of haemolymph could not be inhibited even by an exposure of 30 minutes. In this connection it may be pointed out that this beetle occasionally resorts, on disturbance, to reflex bleeding. The blood comes out through weak chitinous areas at the joints of antennae and legs. The punctures are later blocked probably with clusters of haemocytes and the beetle has an extraordinary tendency for blood coagulation.

For testing the blood for coagulation, a drop of the diluted blood was examined in the counting chamber of the haemocytometer after FISHER (1935) and was not observed by putting a drop of blood in oil as reported by YEAGER et al. (1932) and YEAGER & KNIGHT (1933). The small and large haemocytes observed in the insects studied are probably the 'chromophils' and 'amaebocytes' respectively as described by TAYLOR (1935) in *Periplaneta americana* and later named as 'cystocytes' and 'plasmatocytes' by JONES (1957). According to our observations only the small haemocytes were found to be associated with the clumps formed due to the coagulation of blood. Cystocytes (coagulocytes) which are responsible for initiating the coagulation of blood are also relatively smaller cells as reported by JONES (1954, 1957, 1959). Coagulation, whenever present, was brought about by a clumping of haemocytes alone without any apparent effect on the haemolymph as also observed by YEAGER et al. (1932). YEAGER & KNIGHT (1933), on the other hand, have divided insects into three tentative groups

DOI: 10.21248/contrib.entomol.11.1-2.1-11

according to their coagulation process, (i) no plasma coagulation and no or only a negligible cell coagulation, (ii) cell coagulation and no or only a negligible plasma coagulation, (iii) marked plasma coagulation and usually, some degree of cell coagulation.

Previous workers who noted the effect of toxic substances on the blood cell counts of insects (SHULL et al., 1932; FISHER, 1936; JONES & TAUBER, 1954) did so by killing the insects in the toxic gas or with the toxic substance and comparing the number of cells with that of the normal insects or those which were killed in acetic acid vapour. But in the present investigations the blood of the treated insects has been studied in the paralysed condition and compared with that of the normal insects. Both the normal and treated insects were exposed to acetic acid vapour to check the coagulation of blood.

SHULL et al. (1932) noted the effect of 34 toxic gases including carbon disulphide, carbon tetrachloride, acetic acid, hydrogen cyanide, xylene, pyridine and naphthalene on the blood of *Blatta orientalis* (L.) and found only a few gases producing some visible effect upon the process of blood coagulation or upon the blood cells or bringing readily visible chemical changes in its blood. They found that cockroaches which were killed with carbon disulphide and pyridine yielded a small quantity of blood, poor in cells in former case and with an apparently normal cell content in the latter. Except for the inhibition of coagulation with acetic acid vapour and appearance of crystals of magnesium ammonium phosphate in the blood of cockroaches which succumbed to ammonia gas, no other gas was found introducing any change. They therefore concluded, "It is probable that lethal concentration of most gaseous compounds do not produce marked visible changes in the blood of the insects."

The present authors have also recorded that the completely paralysed cockroaches and 'Kutra' caterpillars which were almost dead, produced a little amount of blood. It shows that endrin produces changes in the blood similar to those produced by carbon disulphide and pyridine and probably all the three have a similar mode of action. SHULL et al. (1932) did not make actual counts of cells but reported an apparently less number of cells in the cockroaches treated with carbon disulphide. On the contrary, we have observed that the completely paralysed red hairy caterpillars in which the quantity of blood was very small, have a relatively much higher number of haemocytes as compared to the normal caterpillars. Similarly in the case of cockroach also, though the quantity of blood yielded was less than the minimum required for dilution but apparently a drop of blood contained more cells than the normal blood. We feel that the finding of SHULL et al. (1932) was defective regarding the number of cells in cockroaches killed with carbon disulphide, because in the case of pyridine treated cockroaches also they described apparently normal cell content but it was shown later by FISHER (1936) that pyridine caused a significant

DOI: 10.21248/contrib.entomol.11.1-2.1-11

increase in the blood cells in the same species of cockroach and that with carbon disulphide there was no apparent effect on the number of blood cells. SHULL et al. (1932) did not observe any effect of nicotine on the blood of *Blatta orientalis* but JONES & TAUBER (1954) have reported a four times increase in the number of blood cells of meal worms (*Tenebrio molitor* L.) when killed in nicotine sulphate vapour. FISHER (1936) also found that the death of cockroach, *Blatta orientalis*, by white arsenic, mercuric chloride and sodium fluosilicate caused, respectively, significant decreases from the acetic acid average of 36173 to 8179, 7083 and 6778 cells per cu. mm. of blood. Similarly YEAGER & MUNSON (1942) reported a fall in the number of blood cells by a few toxic substances. We have observed that endrin causes a decrease in the blood cells of cockroach, silkworms and 'kutra' caterpillars and an increase in the caterpillars of 'Nargis' plant. It may therefore be said that the action of toxic substances on the blood of insects may be dependent on different species.

Before attributing any cause to the change in number of blood cells, some factors other than the toxic substances, may also be considered responsible for bringing about a change in the cell count. TAUBER & YEAGER (1934, 1935, 1936) found certain physiological or pathological conditions such as parasitism, ecdysis and oviposition, to be associated, perhaps causally, with high total haemolymph cell counts in the insects. They (1935) concluded that "Just how these various conditions are causally related to the associated high count is not clear. It is obvious, however, that an increase in total count may result from either a dehydration of the hemolymph or an increased rate of formation of hemolymph cells, or from both". TAYLOR (1935) reported that lack of food and moisture in *Periplaneta americana* results in rapid rise in the number of amaebocytes but after prolonged abnormal condition their number falls to a few cells. A low cell count has also been found associated with bacterial infection in southern army worm (BABERS, 1938) and by the advent of pupal stage in holometabolous insects (TAUBER & YEAGER, 1936). Fall in the number of cells by bacterial infection has also been observed in *Periplaneta americana* by the present authors. On the other hand, JONES (1954) noticed the total count rising in the heat fixed meal worm larvae of *Tenebrio molitor*. Earlier, JONES & TAUBER (1951) while discussing the cause of the excessively high ("hemocytosis") and low number ("hemocytopenia") to form a wide range of haemocyte count in the normal *Tenebrio molitor*, concluded, "Hemocytosis in the meal worm is believed to be brought about either by mitotic division of hemocytes in the circulating hemolymph or by a flushing out of hemocytes from some fortuitous temporary site of accumulation in the hemocoel, or both. Hemocytopenia in the meal worm appears to be due to a non specific adhesion to tissues."

Mitosis was not observed in the haemocytes of all the insects studied at present although a very rare occurrence of mitosis in the blood cells

DOI: 10.21248/contrib.entomol.11.1-2.1-11

has been reported in *Periplaneta americana* (TAYLOR, 1935) and *P. orientalis* (YEAGER & TAUBER, 1933). The view of the former author that haemocytes are furnished to the blood by some out side tissue or tissues, therefore, finds further support. As a result, the belief of JONES & TAUBER (1951) that haemocytosis is brought about by mitotic division of haemocytes can not be entertained at least in the present case. TAUBER & YEAGER (1935) opined that higher number of haemocytes is brought about either by a dehydration of the haemolymph or by an increased rate of formation of the haemocytes or from both. The present authors agree with them partially and it appears to us that the change in the blood cell counts, whether an increase or decrease from the normal, is brought about only by the alteration in the amount of haemolymph present, since we are considering the number of blood cells present per cubic millimeter of the blood and not the total haemocytes present in an insect. The cell count therefore depends on the quantity of haemolymph present at the moment. Our observations on the completely paralysed cockroaches and *Amsacta* caterpillars which produce a very little amount of blood and show a higher cell count, and similar observations of SHULL et al. (1932) and FISHER (1936) on the pyridine treated cockroaches, *Blatta orientalis*, have convinced us that the higher cell count in such cases is probably due to a decrease in the amount of haemolymph present. In the same way, the low cell count met with in the treated insects in the present investigations and also reported by other workers, is probably due to an increased amount of haemolymph. We believe that toxic substances and perhaps also some physiological conditions bring about some change in the amount of the haemolymph present in the normal insect but may not materially affect the total number of the blood cells. Accordingly, change in the amount of blood may result in changing the number of cells per cubic millimeter. The occurrence of a very low cell count observed by us in the bacteria infested cockroaches and in southern army worm by BABERS (1938) is probably due to some interference of pathological nature. As already said we suppose that higher number of cell counts recorded by using acetic acid vapour (FISHER 1935) and by heat treatment (JONES, 1954) are due to the complete inhibition of coagulation caused by these treatments. But in those cases where both the normal and treated insects are subjected to anti coagulation measures the possibility of setting free of non circulating haemocytes from some temporary site of accumulation or their non specific adhesion to some tissues. (JONES & TAUBER, 1951; JONES, 1954) interferring with blood cell count in the treated or conditioned insect can not be denied altogether.

It is clear that there is a wide difference in the minimum and the maximum number of haemocytes of a range in both the normal and the treated insects. TAUBER & YEAGER (1934) have observed in *Gryllus assimilis pennsylvanicus* BURM. a ratio of 1:10 in the lowest and the highest recorded number of haemocytes and more or less similar ratios have been

DOI: 10.21248/contrib.entomol.11.1-2.1-11

recorded by them (1935) in other insects also. The difference in the number of haemocytes is probably due to the varying amount of haemolymph present in the different insects. But the wide variation in the cell count, according to FISHER (1935), is perhaps largely due to physiological differences in individual insects.

Acknowledgements

The present investigations were carried out under a Scheme financed by the I. C. A. R. whose financial assistance is gratefully acknowledged. The authors are also grateful to the Head of the Zoology Department, Panjab University, for providing the necessary laboratory facilities.

Summary

Preliminary trials were made to determine the effect of endrin on the number of blood cells in cockroaches (*Periplaneta american* L.), red hairy caterpillars of *Amsacta moorei* BUTLER, silkworms (Japanese breed Taihei × Choan) and the caterpillars on 'Nargis' (*Narcissus poeticus* L.). Number of blood cells decreased in the paralysed individuals of cockroaches, red hairy caterpillars and silkworms whereas it increased in the caterpillars of 'Nargis' (*Narcissus poeticus*). Both the normal and treated insects were exposed to the vapours of acetic acid for 5 minutes to avoid immediate coagulation of blood. Results of the previous workers have been discussed and it has been concluded by the authors that the toxic substances probably bring about a change in the amount of haemolymph which results in the rise or fall of the blood cells per cubic millimeter.

Zusammenfassung

Es wurden vorläufige Versuche zur Bestimmung der Einwirkung von Endrin auf die Anzahl der Blutzellen bei Schaben (*Periplaneta americana* L.), Raupen von *Amsacta moorei* BUTLER, Seidenraupen (japanische Zucht Taihei × Choan) und Raupen auf „Nargis" (*Narcissus poeticus* L.) durchgeführt. Die Zahl der Blutzellen verringerte sich in den paralysierten Individuen der Schaben, der Raupen von *Amsacta moorei* und der Seidenraupen, wahrend sie bei den Raupen auf *Narcissus poeticus* anstieg. Behandelte wie auch unbehandelte Insekten wurden 5 Minuten lang den Dämpfen von Essigsäure ausgesetzt, um die Koagulation des Blutes zu verhindern. Die Ergebnisse fruherer Arbeiten werden besprochen. Die Autoren kommen zu dem Schluß, daß die toxischen Substanzen wahrscheinlich eine Veränderung in der Gesamtmenge der Hämolymphe mit sich bringen, die sich als Zu- oder Abnahme der Zahl der Blutzellen pro Kubikmillimeter äußert.

Резюме

Проводились предварительные опыты для определения воздействия эндрина на количество кровяных клеток у тараканов (*Periplaneta americana* L.), гусеницах *Amsacta moorei* BUTLER, шелковичных червей (японский вид Taihei × Choan) и гусениц на *Narcissus poeticus* L. Количество кровяных клеток у парализованных особей тараканов, гусениц *Amsacta moorei* и шелковичных червей уменьшилось, между тем, как оно увеличилось у гусениц *Narcissus poeticus*. Как обработанные, как и необработанные насекомые в течение пяти минут подвергались парам уксусной кислоты для того, чтобы предотвратить коагуляцию крови. Обсуждаются результаты прежних работ. Авторы приходят к выводу, что токсические вещества вероятно вызывают изменение в общем количестве гемолимфы, которое находит свое выражение в увеличении и уменьшении количества кровяных клеток в кубическом миллиметре.

DOI: 10.21248/contrib.entomol.11.1-2.1-11

References

Arnold, J W , Effect of certain fumigants on haemocytes of the Mediterranean flour moth, *Ephestia kuehniella* Zell (*Lepidoptera Pyralididae*) Canad Journ Zool , **30**, 365—374, 1952

—, The haemocytes of the Mediterranean flour moth *Ephestia kuehniella* Zell (*Lepidoptera Pyralididae*) Canad Journ Zool , **30**, 352—364, 1952

Babers, F H , A septicemia of the southern army worm caused by *Bacillus cereus* Ann ent Soc Amer , **31**, 371—373, 1938

Fisher R A , The effect of acetic acid vapour treatment on the blood cell count in the cockroach, *Blatta orientalis* L Ann ent Soc Amer , **28**, 146—153, 1935

—, The effect of a few toxic substances upon the total blood cell count in the cockroach, *Blatta orientalis* Linn Ann ent Soc Amer , **29**, 335—340, 1936

Jones, J C , The normal hemocyte picture of *Tenebrio molitor* L (*Coleoptera*) Iowa State Coll Journ Sci , **24**, 355—361, 1950

—, A study of meal worm hemocytes with phase contrast microscopy Ann ent Soc Amer , **47**, 308—315, 1954

—, A phase contrast study of the blood cells of the adult cockroach, *Periplaneta americana* (L) (Abstract) Anat Recd , **128**, 571, 1957

—, A phase contrast study of the blood cells in *Prodenia* larvae (order *Lepidoptera*) Quart Journ micr Sci , **100**, 17—23, 1959

Jones, J C & Tauber, O E , Normal total hemocyte counts of *Tenebrio molitor* Ann ent Soc Amer , **44**, 539—543, 1951

— Effects of hemorrhage, ligation, dessication, and starvation on hemocytes of meal worm larvae Iowa State Coll Journ Sci , **26**, 371—386, 1952

—, Abnormal hemocytes in meal worms (*Tenebrio molitor* L) Ann ent Soc Amer , **47**, 428—444, 1954

Lepesme, P , L'action externe des arsenicaux sur le criquet pelerin (*Schistocerca gregaria* Forsk) Bull Soc Hist nat Afrigue Nord, **28**, 88—103, 1937

Munson, S C & Yeagar, J F , Fat inclusions in blood cells of the southern army worm, *Prodenia eridania* (Cram) Ann ent Soc Amer , **37**, 396—400, 1944

Pilat, M , The effects of intestinal poisoning on the blood of locusts (*Locusta migratoria*) Bull ent Res , **26**, 283—288, 1935

Shull, W E , Inhibition of coagulation in the blood of insects by the fatty acid vapour treatment Ann ent Soc Amer , **29**, 341—342, 1936

Shull, W E & Rice, P L , A method for temporary inhibition of coagulation in the blood of insects Journ econ Ent , **26**, 1083—1089, 1933

Shull, W E , Riley, M K & Richardson, C H , Some effects of certain toxic gases on the blood of the cockroach, *Periplaneta orientalis* (Linn) Journ econ Ent , **25**, 1070—1072, 1932

Tareeva, A I & Nenjukov, D V , Effect of poisons on normal digestion and the blood of *Calliptamus* Bull Plant Protect , Leningrad, **3**, 39—49, 1931

Tauber, O E & Yeager, J F , On the total blood (hemolymph) cell count of the field cricket, *Gryllus assimilis pennsylvanicus* Burm Iowa State Coll Journ Sci , **9**, 13—24, 1934

— On the total hemolymph (blood) cell counts of insects 1 *Orthoptera, Odonata Hemiptera* & *Homoptera* Ann ent Soc Amer , **28**, 229—240, 1935

—, On the total hemolymph (blood) cell counts of insects II *Neuroptera, Coleoptera, Lepidoptera* and *Hymenoptera* Ann ent Soc Amer , **29**, 112—118, 1936

Taylor, A , Experimentally induced changes in the cell complex of the blood of *Periplaneta americana* (*Blattidae Orthoptera*) Ann ent Soc Amer , **28**, 135—145, 1935

Toumanoff, M C & Lapied, M , Action du dichlorphenyl trichlorethane (DDT) sur les chenilles de las fausse teigne des ruches *Galleria mellonella* L Effet de la tempera ture Action sur le sang Acad Agr France, May 24, 1950 (4 pages)

DOI: 10.21248/contrib.entomol.11.1-2.1-11

YEAGER, J. F., The blood picture of the southern army worm (*Prodenia eridania*). Journ. agr. Res., **71**, 1—40, 1945.

YEAGER, J. F. & KNIGHT, H. H., Microscopic observations on blood coagulation in several different species of insects. Ann. ent. Soc. Amer., **26**, 591—602, 1933.

YEAGER, J. F. & MUNSON, S. C., Changes induced in the blood cells of the southern army worm (*Prodenia eridania*) by administration of poisons. Journ. agr. Res., **64**, 307—332, 1942.

YEAGER, J. F., SHULL, W. E. & FARRAR, M. D., On the coagulation of blood from the cockroach *Periplaneta orientalis* (Linn.) with special reference to blood smears. Iowa State Coll. Journ. Sci., **6**, 325—345, 1932.

YEAGER, J. F. & TAUBER, O. E., Determination of total blood volume in the cockroach, *Periplaneta fuliginosa*, with special reference to method. Ann. ent. Soc. Amer., **25**, 315—327, 1932.

—, On counting mitotically dividing cells in the blood of cockroach, *Periplaneta orientalis* Linn. Proc. Soc. exp. Biol. Med., **30**, 861—863, 1933.

Studies on the Physiology of Digestion in the last Instar Larva of the Rice Moth (Corcyra cephalonica Stainton)

II. Digestive enzymes and effect of starvation on their secretion

(*Lepidoptera*:*Galleriidae*)

P. N. SRIVASTAVA

Department of Zoology, Lucknow University
Lucknow, India[1])

Introduction

SRIVASTAVA (1960) studied the hydrogen-ion concentration of the gut of the larva of *Corcyra cephalonica* STAINTON. The present paper deals with the various digestive enzymes secreted by the alimentary canal of the larva and also the effect of starvation on the secretion of these enzymes.

Material and methods

Larvae were reared in the laboratory as already described (SRIVASTAVA, 1960). Fully developed larvae of the last instar were taken out from the culture for each set of experiment. They were equally divided in two sets. Estimations were made immediately from one set of the larvae, while the larvae of the other set were starved individually for a period of five days in small petri dishes, and their excreta cleaned four times daily in order to avoid its ingestion. No larva died because of this short starvation during the course of experiment.

Homogenate was prepared in ice-cold double distilled water, as glycerol had inhibitory effect on some of these enzymes (SRIVASTAVA, 1959). Ten larvae were taken at a time for the preparation of the homogenate, they were dissected in ice-cold distilled water. Alimentary canal after being cleaned with the adhering tissues was divided into

[1]) Part of the thesis approved for the degree of Doctor of Philosophy of the Lucknow University in 1958.

DOI: 10.21248/contrib.entomol.11.1-2.1-11