HEALING OF GUT WOUNDS IN THE MOSQUITO *AEDES AEGYPTI* (L.) AND THE LEAFHOPPER *OROSIUS ARGENTATUS* (EV.)

By M. F. DAY* and MARGARET J. BENNETTS*

[*Manuscript received May* 1, 1953]

Summary

Healing of wounds made by puncturing the replete midgut of adult female *Aedes aegypti* occurs without the intervention of the haemocytes, which play an important role in all previously described examples of wound healing in insects. An eschar is formed on the cuticle within 24 hr, apparently as the result of haemolymph coagulation, but initial closure of the wound appears to be the result of the activity of the midgut musculature and healing to be due to the regenerative powers of the midgut epithelium. Survival as high as 80 per cent. is attained when the gut of engorged females is punctured. The midgut wound may remain gaping for 24-48 hr, and in one case was unhealed at 54 hr. Ingested mammalian erythrocytes generally do not escape into the mosquito haemocoele, because they are agglutinated in the midgut and are held together by a peritrophic membrane. In adult *Orosius* the midgut epithelial cells are polyploid and regenerate slowly. The early stages of the healing process are therefore performed by haemocytes. These are present in small numbers only, and undergo active mitosis to form the wound tissue. The significance of these observations on some results on the transmission of viruses by mosquitoes and leafhoppers is discussed.

I. INTRODUCTION

There is substantial evidence that plant viruses transmitted by leafhoppers are ingested with the infected host sap, penetrate the midgut, reach the salivary glands through the haemocoele, and are reinjected with the insect's saliva into a second host plant (Storey 1939). Certain strains of *Cicadulina mbila* (Naude), the leafhopper vector of maize streak virus, were incapable of transmitting the virus, but Storey (1933) was able to convert insects of an "inactive" strain into vectors by puncturing the midgut. He therefore suggested that the midgut constituted a barrier to the penetration of the virus. Following this demonstration, the concept of the midgut as a barrier to virus infection was considered also in mosquitoes. Merrill and Ten Broeck (1935) were able to increase the ability of *Aedes aegypti* to transmit eastern equine encephalomyelitis virus by puncturing the midgut of this mosquito. Philip (1948) also studied the effect of gut puncture on the transmission of *Rickettsia burneti* by *Aedes aegypti.*

Despite these studies of virus-vector relationships by operations on the mosquito and leafhopper midgut, no work has been done on the rate of healing of the wounds or, in fact, on any aspect of the healing process in these insects. In the cockroach *Periplaneta,* healing of gut wounds is efficiently performed by the combined action of haemocytes and regeneration of the gut epithelium (Day

* Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

1952). It might be expected that the process described in *Periplaneta* would have general application to other insects. However, preliminary observations on the mosquito and the leafhopper indicated that this was not so. A more detailed study of wound healing in these insects was therefore necessary for better understanding of the use of gut puncturing in the study of virus-vector relationships of *Aedes* and *Orosius*.

II. Materials and Methods

(*a*) Aedes aegypti (*L.*)

A colony of *Aedes aegypti* was maintained at about 27°C and 60-80 per cent. R.H. Adults were fed on a guinea pig, and the midgut was punctured 1-4 hr after feeding.

Using a finely sharpened steel needle, the wounds were made by puncturing the abdominal pleura of engorged females. Preliminary observations confirmed the statement of Merrill and Ten Broeck (1935) that survival of operated females was considerably lower if the midgut was punctured when it contained no blood. Survival in later series approximated 80 per cent., and some adults lived for 3 wk before being fixed for histological examination in the alcoholic modification of Bouin's fluid. No attempt was made to keep the mosquitoes alive longer than 3 wk. Serial sections (10 μ) of 165 specimens were studied following fixation at various times from 1 hr to 14 days after wounding.

(*b*) Orosius argentatus (*Evans*)

Colonies of this leafhopper have been maintained in the glass-house for work on virus transmission (see Helson 1942 for methods). Operations similar to those described by Storey (1933) were performed on 91 adults, using carbon dioxide anaesthesia. Survival approximated 40 per cent., and serial sections were made of 32 specimens fixed from 1 to 12 days after the operation.

III. Observations on Aedes aegypti

Healing of midgut wounds occurs in *A. aegypti* by an efficient but apparently simple mechanism. No tissue other than the simple epithelium and the gut musculature is involved. The first stage of healing is the result of a contraction of the gut musculature in the vicinity of the wound. This normally has the effect of stopping the passage of solid contents of the gut into the haemocoele, and has been observed up to 29 hr, but not at 45 hr, after wounding. In only one case were ingested erythrocytes observed to have passed into the haemocoele. This was in a mosquito operated upon at least an hour after the blood meal, and fixed 2 hr after puncturing. The muscular spasm produces a marked change in the shape of the epithelial cells surrounding the wound (Plate 1, Fig. 1). After feeding, the epithelial cells are flattened, but gradually regain their columnar shape as digestion of the erythrocyte mass proceeds. The muscular spasm in the immediate vicinity of the wound rapidly causes the epithelium to assume a columnar shape and this is retained until the muscles relax.

It seems likely that the ability of the engorged midgut to contract provides a partial explanation of the higher survival rates observed in engorged females than in unfed females. In addition it is true that fewer cells are damaged in puncturing the gut of an engorged female than in performing the same operation on the gut of an unfed mosquito.

Before relaxation of the gut musculature occurs, proliferation of the midgut epithelium begins, and continues for several days until epithelial continuity is established (Plate 1, Fig. 2). Excess proliferation may result in reduction in size of the nearby cells. Recovery from this apparent exhaustion may be incomplete 14 days after wounding. There is little necrosis of the epithelial cells, even those only one or two cells removed from the wound. However, vacuolization may occur in some epithelial cells (see Plate 1, Fig. 3).

No sign of epithelial damage could be detected in complete serial sections of three out of 11 insects fixed 3 days after puncturing, and in eight insects out of 12 fixed 7 days after puncturing. Healing must therefore occur with remarkable completeness, doubtless correlated with the normal regenerative ability of the midgut epithelium.

The healing process just described may be complicated by several factors. For example, midgut contents (partially digested erythrocytes) may protrude into the wound and may delay epithelial regeneration (Plate 1, Fig. 3). In one specimen normal epithelial regeneration was modified by the formation of a plug of relatively unorganized cells that persisted for 7 days (Plate 1, Fig. 4). Healing may be delayed for reasons not understood. For example, Plate 1, Figure 5, shows a gaping wound found 54 hr after the wound had been made. In this specimen the cuticle wound had healed normally, and in two other mosquitoes punctured and fixed at the same time the midgut wound was normally and completely healed.

In only one of the 165 specimens examined were haemocytes involved in the healing process. In this example, groups of haemocytes extended from the cuticle wound to the gut wound, presumably along the path taken by the puncturing needle. They showed no tendency to accumulate at sites of necrosis, as they do in *Periplaneta* (Day 1952).

Methods are not available for measuring the strength of the regenerated tissue in the *Aedes* midgut. Effective repair may be considered complete when the gut is able to withstand the ingestion of a second blood meal. Mosquitoes that were disturbed before ingestion was complete were wounded and then permitted to feed a second time. Two of these took a second blood meal 5 hr after wounding, two more 24 hr after wounding, and one fed 3 days after wounding. When the second blood meal was taken 5 hr after wounding, the erythrocytes escaped into the haemocoele, but this was not observed when the second meal was taken 24 hr after wounding.

Healing of the cuticle wounds likewise proceeded efficiently without the intervention of haemocytes. An eschar was frequently formed of necrotic tissue and presumably also consisted of coagulated haemolymph. Plate 1, Figure 6, illustrates a fairly extensive cuticle wound involving also some necrotic fat-body. Occasionally adhesions between the cuticle and midgut were found, but did not appear to hinder the process of repair.

IV. Observations on Orosius argentatus

In only 25 per cent. of *O. argentatus* operations was the gut punctured. If *C. mbila* anatomy is similar to that of *O. argentatus*, which is probable, it seems likely that in a fairly high percentage of Storey's (1933) operations the gut of the leafhopper was not actually damaged. Serial sections were made of insects 1, 2, 7, 8, and 12 days after the operation. Practically no healing was visible in the 1- and 2-day series, but in the 7- and 12-day series healing was apparent. In all these examples haemocytes were present at the site of the wound and had effectively closed it (Plate 2, Figs. 7 and 8). However, the bulk of the wound tissue was very small by comparison with *Periplaneta* and this was clearly due to the small number of haemocytes available. Haemocytes were never found to be involved in healing of cuticular wounds (Plate 2, Fig. 9). In distinction to the situation in *Periplaneta*, where mitoses were found in only one of over 100 examples of wound tissue studied, mitotic figures were found in every one of six examples of wound tissue in *Orosius*. This is undoubtedly correlated with the observation that haemocytes are too few to cover the wound completely and therefore undergo compensatory hypertrophy after having been incorporated in the wound tissue. A comparison was made of the role of haemocytes in walling off a small pellet of paraffin implanted in the abdomen of *Orosius*. Twenty-four hr after implantation in the haemocoele of *Periplaneta* such a pellet was covered by many layers of haemocytes (Day 1952, Plate 1, Fig. 5), but after a similar period no haemocytes were found surrounding a pellet in *Orosius*. Even 48 hr after implantation only a thin layer of haemocytes surrounded the pellet (Plate 2, Fig. 10).

Changes in the *Orosius* midgut epithelium were more difficult to follow than those of *Aedes*. There was no change in cell shape surrounding the wound shortly after puncture (compare Plate 2, Fig. 11, with Plate 1, Fig. 1), but in later stages of healing the epithelium was obviously involved (Plate 2, Figs. 8 and 12).

V. Discussion

It is clear from the observations described above that wound healing in insects can occur by mechanisms other than that described by previous authors (see Day 1952). In all previous reports the first and most conspicuous changes were produced by the haemocytes. These were responsible for the initial plugging of the wound and the formation of a bulky wound tissue. In *A. aegypti* wound healing can occur entirely without the intervention of haemocytes. Yet the process is as fast and as effective in the mosquito as in *Periplaneta*. In *O. argentatus* the haemocytes are responsible for the greater part of the healing process, but their numbers are inadequate and the process is slow because they must undergo mitosis at the site of the wound.

The eschar formed on the cuticle is the result of the exposure of haemolymph to the air, but the activities of the epithelium alone are sufficient to bring about midgut healing in *A. aegypti.* Haemolymph may play a part but this would not be visible in sections.

The rate of healing in *Aedes* and *Orosius* may be compared with that found in *Periplaneta.* In this cockroach, haemocytes begin to form effective wound tissue about 12 hr after the operation and the wound is generally fairly well covered after 24 hr. Epithelial growth is apparent after 3 days. In *Aedes* the wound is effectively closed after 24 hr, whereas in *Orosius* the wound is often still unhealed after 48 hr. Likewise encapsulation of a paraffin pellet is slower and less complete in *Orosius* than in *Periplaneta.*

The rate of healing is important from the viewpoint of the effect of wounding on the ability of the insects to transmit viruses. The data reported here suggest that exchange of substances may occur between the gut lumen and the haemocoele for over 24 hr without the necessity of penetrating epithelial cell membranes. The wound may be closed earlier than 24 hr or in rare instances may be plugged for more than 7 days with cells differing histologically from the normal midgut epithelium (Plate 1, Fig. 4). It seems likely that these regenerating cells differ in permeability from the normal epithelium, and so may permit the passage of materials to which they are normally impermeable. Once viruses such as eastern equine encephalomyelitis have reached the haemocoele they presumably find tissues satisfactory for their multiplication. The results of Philip (1948) suggest that this is not so for *Rickettsia burneti.* However, Weyer (1950, 1952) reported that several species of *Rickettsia* multiply in insect tissues other than those they normally invade if injected into the haemocoele.

The observations made in the course of this work have provided clear evidence for the existence of a membrane in *Aedes aegypti* surrounding the ingested blood cells. A peritrophic membrane has been generally thought to be absent in adult mosquitoes, although its presence has been claimed by Yaguzhinskaya (1940). Evidence will shortly be presented that the membranes observed in this work are, in fact, true peritrophic membranes (Waterhouse 1953).

VI. Acknowledgments

The authors wish to thank Mr. D. Wilson for his care with the photomicrographs, and Dr. D. F. Waterhouse for discussions concerning the mosquito peritrophic membrane.

VII. References

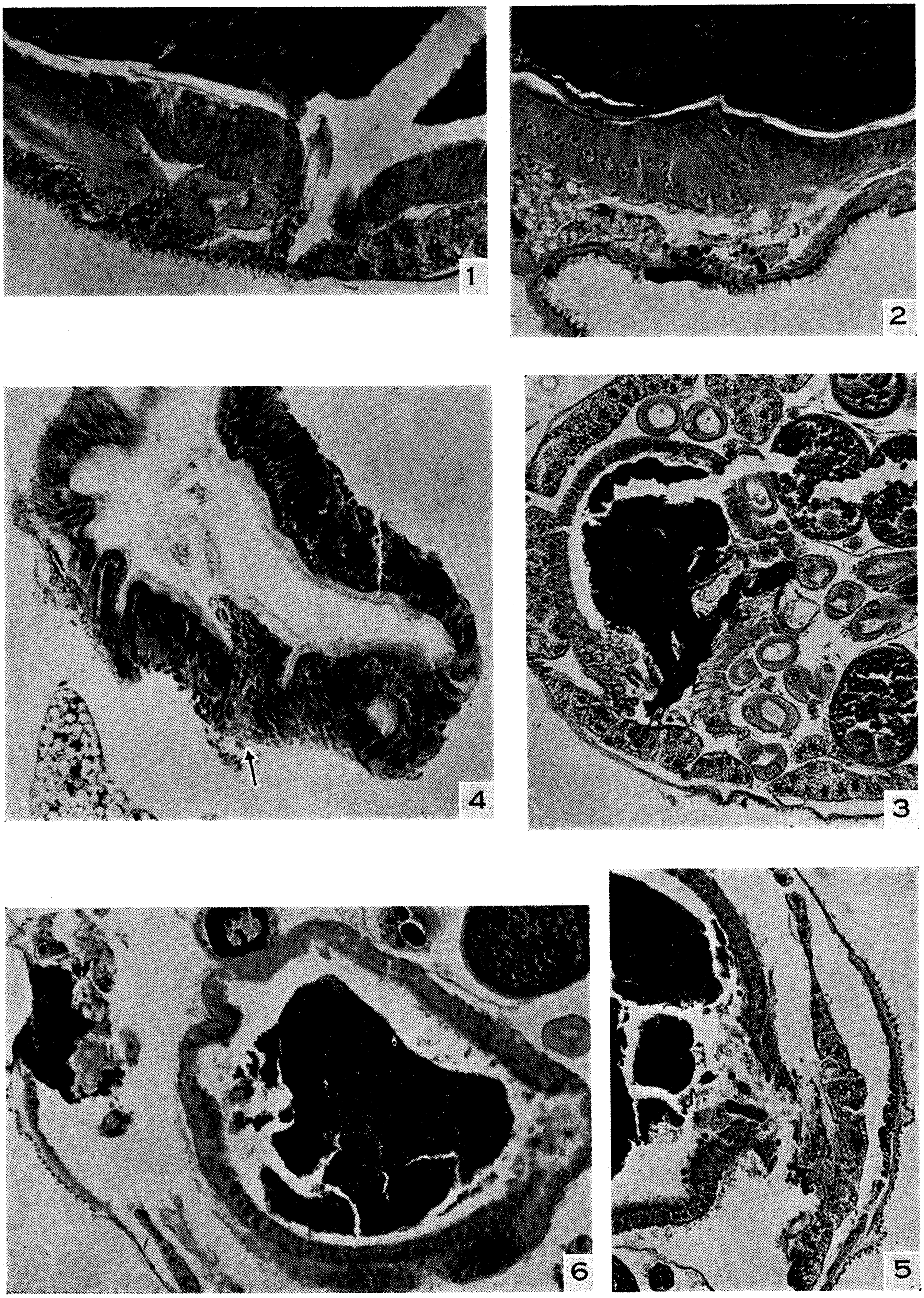
Day, M. F. (1952).—Wound healing in the gut of the cockroach *Periplaneta.* *Aust. J. Sci. Res.* B 5 (2): 282-9.

Helson, G. A. H. (1942).—The leaf hopper *Thamnotettix argentata* Evans, a vector of tobacco yellow dwarf. *J. Coun. Sci. Industr. Res. Aust.* **15** (2): 175-84.

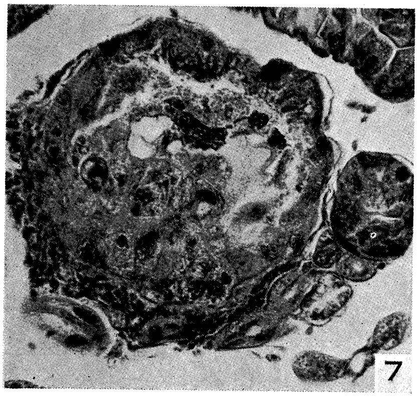
Merrill, M. H., and Ten Broeck, C. (1935).—The transmission of equine encephalomyelitis virus by *Aedes aegypti.* *J. Exp. Med.* **62**: 687-95.

Philip, C. B. (1948).—Observations on experimental Q fever. *J. Parasit.* **34**: 457-64.

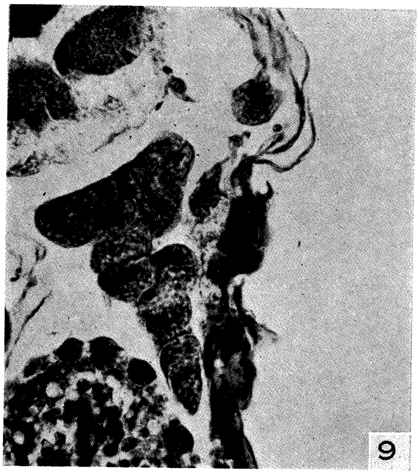
WOUND HEALING IN *AEDES* AND *OROSIUS* GUT

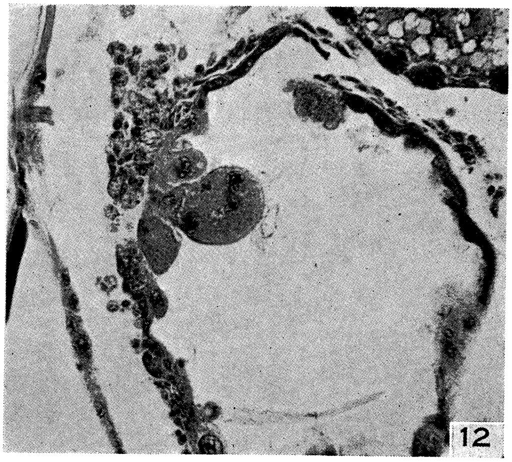


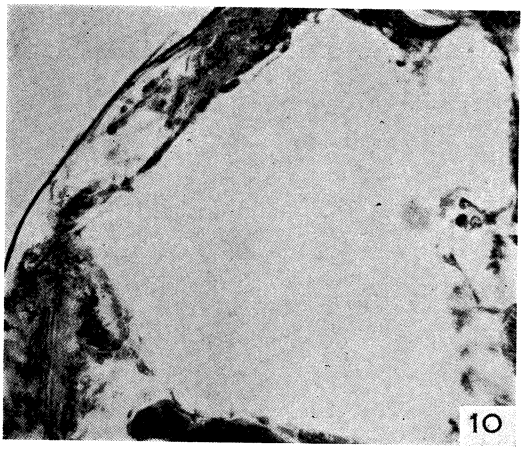
WOUND HEALING IN *AEDES* AND *OROSIUS* GUT

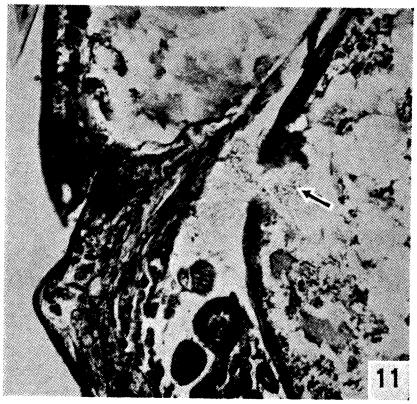

7


8


9


12


10


11

STOREY, H. H. (1933).—Investigations of the mechanism of the transmission of plant viruses by insect vectors. I. *Proc. Roy. Soc.* B **113**: 463-85.

STOREY, H. H. (1939).—Transmission of plant viruses by insects. *Bot. Rev.* **5**: 240-72.

WATERHOUSE, D. F. (1953).—The occurrence and significance of the peritrophic membrane, with special reference to adult Lepidoptera and Diptera. *Aust. J. Zool.* **1** (3): 299.

WEYER, F. (1950).—Die Entwicklung von Rickettsien in den larven von Mehlkäfer (*Tenebrio molitor* L.) *Schweiz. Z. allg. Path.* **13**: 478-86.

WEYER, F. (1952).—Die kunstliche Injektion von Kleiderläusen und Mehlkäferlarven mit *Rickettsia conori* (Zeckenbissfieber). *Acta Trop. Basel* **9** (2): 170-83.

YAGUZHINSKAYA, L. V. (1940).—Présence d'une membrane péritrophique dans l'estomac de la femelle adulte d'*Anopheles maculipennis.* (In Russian.) *Med. Parasit. Moscow* 9: 601-3.

EXPLANATION OF PLATES 1 AND 2

PLATE 1

Photomicrographs of 10-μ sections showing stages in wound healing in midgut of *Aëdes aegypti.*

Fig. 1.—One hr after wounding. Note absence of epithelial regeneration; note differences in shape of epithelial cells surrounding the wound compared with those at the left hand top corner. The latter were typical of the cells in the remainder of the gut.

Fig. 2.—Sixteen hr after wounding. Midgut regeneration has resulted in closure of the wound. The cuticle wound and necrotic fat-body may also be seen.

Fig. 3.—One hr after wounding. The dark, partly digested erythrocytes have been carried by the needle into the wound. Epithelial cells in the vicinity of the wound are markedly vacuolated.

Fig. 4.—Seven days after wounding. Wound plugged by relatively unorganized group of epithelial cells at arrow. Note absence of haemocytes from wound tissue.

Fig. 5.—Fifty-four hr after wounding. Note absence of healing. Compare with Figure 6.

Fig. 6.—Almost complete regeneration of midgut epithelium 54 hr after wounding. Cuticular wound on the left has incorporated necrotic fat-body cells, but erythrocytes are entirely absent.

PLATE 2

Photomicrographs of 10-μ sections showing wound healing in *Orosius argentatus.*

Fig. 7.—Healing of midgut 7 days after wounding. Note that haemocytes have formed a wound tissue, but that necrotic epithelial cells are still present. Mitotic figures in the wound tissue were observed using oil-immersion objective.

Fig. 8.—Healing of midgut 8 days after wounding. Mitoses were present in wound tissue composed of haemocytes. Midgut epithelium more normal than in Figure 7.

Fig. 9.—Cuticular wound 7 days after wounding. Note complete absence of haemocytes. Eschar was intensely fuchsinophilic, and was apparently formed from coagulated haemolymph.

Fig. 10.—Loose wound tissue surrounding paraffin pellet implanted into abdomen for 48 hr. The pellet, now dissolved, occupies the large clear area. The slight role of the haemocytes in encapsulation is to be compared with their abundance in *Periplaneta* (see Day 1952, Plate 1, Fig. 5).

Fig. 11.—Midgut 2 days after wounding, showing absence of healing and flow of material at arrow from lumen into the haemocoele.

Fig. 12.—Midgut 12 days after wounding, showing sparse wound tissue in vicinity of wound. Regenerated epithelium has completely covered the injured midgut.