creases the number of heterosexual contacts that might lead to pair formation, and it promotes spawning synchrony. These findings point to the existence of sexual facilitating pheromones in fishes in addition to those affecting male-female interaction.

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Lysozyme Retention by Cockroach Periplaneta americana L.

Abstract. *Blood from the cockroach* Periplaneta americana *usually does not react with a suspension of* Micrococcus lysodeikticus. *When lysozyme is added to the mixture it causes an immediate reduction in the optical density at 450 nanometers which is soon followed by a strong rise. Blood from roaches injected with lysozyme causes a similar series of changes in the optical density of a suspension of* M. lysodeikticus, *an indication that lysozyme has been retained in the blood. It may persist for many days or weeks.*

The bacterial symbiont of the cockroach (*Periplaneta americana*) is intimately connected with the egg (*1*) and with mycetocytes of the fat body (*2*). This association suggests that it may have an influence on reproduction and growth, and indeed its removal proves to be injurious to these functions (*3*). It is by no means sure, however, whether such injury is due to the loss of the symbionts or to the sterilizing treatment by heat (*4*), antibiotics (*2*, *3*), or lysozyme (*3*).

Use of the first of these two has entailed considerable mortality. However, Malke (*3*) has sterilized the cockroach *Periplaneta americana* with egg-white lysozyme, 0.5 mg of which destroyed most of the bacteroids within a week and eventually completely disintegrated them. Such a dose is not lethal to the insect, and even the injection of 22 successive doses at 3-day intervals is not toxic (*3*). If this is so, it would aid in understanding the physiology of growth and reproduction in this and similar associations.

It might be feasible to detect the enzyme in 20 μl of blood, the volume expected from an individual male. We used egg-white lysozyme which was crystallized three times, dialyzed, and lyophilized; it had an activity of 30,000 sigma units (*5*) per milligram. To reduce coagulation of the blood, we used as diluting fluid 0.2 percent potassium oxalate solution (*6*) in 0.066*M* potassium phosphate buffer (*p*H 6.2) in the ratio of 4 : 1. Twenty microliters of blood were added to a reaction volume of 2.6 ml, except for the experiment shown in Fig. 1*b*, in which 50 μl of normal blood pooled from several insects was used to attain an appropriate optical density (O.D.). This increase in concentration of blood increased its tendency to clot. Reactions were conducted at room temperatures. The addition of 0.1 ml of a solution of lysozyme (0.1 mg/ml) to 2.5 ml of a suspension of *Micrococcus lysodeikticus*, having an $O.D._{450}$ of about 0.6, caused a pronounced and uninterrupted decrease in the absorbance until lysis was completed and the suspension became clear (Fig. 1*a*). The addition of the same amount of lysozyme to 50 μl of normal blood in 2.5 ml of potassium oxalate buffer showed no significant effect (Fig. 1*b*) (the slight decline in absorbance is attributed to developing coagulation and can be considerably reversed by inverting the container).

The addition of 20 μl of normal blood to 2.6 ml of a suspension of *M. lysodeikticus* generally shows no effect (Fig. 1*c*). However, some blood samples do react. This is shown after a delay of from 1 to 2 minutes by a strong rise in absorbance over a period of 6 to 8 minutes, to a value that may be two to three times the original absorbance to complete a sigmoid curve (Fig. 2*a*). On the other hand, the addition of 0.1 ml of lysozyme solution to a mixture of 2.5

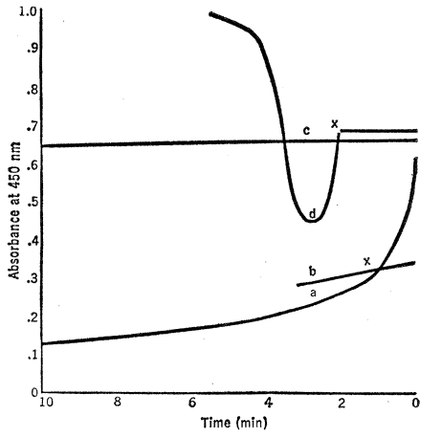

Fig. 1. (*a*) Action of lysozyme on suspension of *Micrococcus lysodeikticus*. (*b*) Action of lysozyme on normal blood. The lysozyme was added to the blood sample at time *X*, and produced no significant change in absorbance. The slight decrease in absorbance was due largely to coagulation of the blood which, in this sample of 50 μl, was greater than in the samples of 15 to 20 μl used in the other experiments. (*c*) Apparent lack of action of normal blood on *M. lysodeikticus*. (*d*) Action of lysozyme on a mixture of normal blood and *M. lysodeikticus*. Lysozyme added at time *X*.

ml of bacterial suspension and 20μl of normal blood caused an immediate and sharp reduction in absorption, followed in about 1 minute by an even greater rise (Fig. 1*d*). This evidently was the type of curve to expect from blood with high lysozyme activity. If the injected lysozyme were uniformly distributed throughout the insect, a 0.5-mg dose in an 800-mg male cockroach would yield approximately 0.06 mg of the enzyme per 100 ml of blood. Twenty microliters of such blood added to 2.6 ml of a suspension of *M. lysodeikticus* would thus

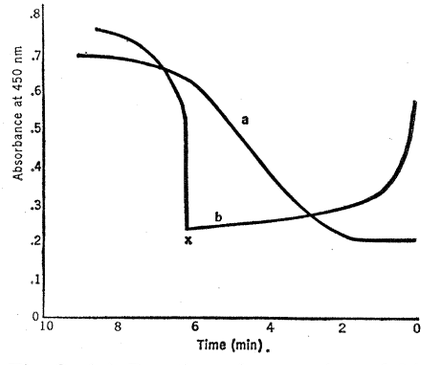

Fig. 2. (*a*) Reaction of suspension of *M. lysodeikticus* and blood from certain normal males. Note delayed rise in absorbance. (*b*) Immediate increase in absorbance and continuing rise after addition, at time *X*, of normal blood to a reacted mixture of lysozyme and *M. lysodeikticus*.

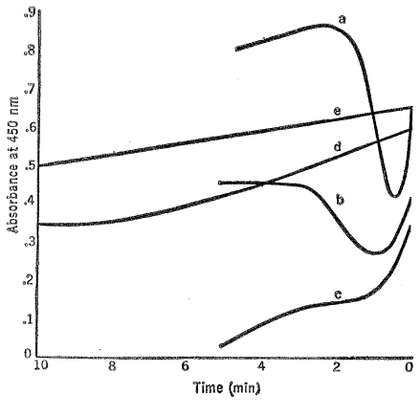

Fig. 3. (*a* to *d*) Action on *M. lysodeikticus* of blood taken from insect injected (*a*) 5 days (compare with Fig. 1*d*), (*b* and *c*) 13 days, and (*d*) 18 days previously with 0.05 ml of 2 percent lysozyme solution. (*e*) Action on *M. lysodeikticus* of blood taken from insect injected 18 days previously with 0.05 ml of 1 percent lysozyme solution.

result in a reaction mixture containing about 0.012 mg of lysozyme, approximating the 0.01 mg of enzyme used in the reference assay (Fig. 1*a*).

Adult male cockroaches were injected with 0.05 ml of 1 or 2 percent lysozyme in 0.5 percent NaCl solution and examined at intervals of several days. Blood (15 to 20 μl) was rapidly withdrawn from the chilled insect and mixed with 2.6 ml of bacterial suspension; the mixture was analyzed in a Beckman linear recording spectrophotometer at a wavelength of 450 nm. The reaction obtained with blood withdrawn 5 days after injection of 2 percent lysozyme (Fig. 3*a*) duplicates in all essentials that of lysozyme in a mixture of normal blood and micrococci (Fig. 1*d*). Blood withdrawn 13 days after injection of 2 percent lysozyme also reproduces this curve (Fig. 3*b*), although indications of lower lysozyme concentration are seen in this and still more in another specimen (Fig. 3*c*). Activity is still evident 18 days after injection (Fig. 3*d*), even after injection of 0.05 ml of only 1 percent lysozyme (Fig. 3*e*). It may then be concluded that the indicated activity is due to the presence of residual lysozyme.

The addition of 20 μl of normal blood to a reacted mixture of lysozyme and *M. lysodeikticus* results in an immediate and sharp increase in absorbance, which continues as a gradually rising curve (Fig. 2*b*). In view of this and of the sharp increase that follows the initial reduction in absorbance in a suspension of the bacteria to which has been added either normal blood and then lysozyme (Fig. 1*d*), or blood from a cockroach injected with lysozyme (Fig. 3*a*), it appears that lysis of the bacterial cell wall enables the lysed bacteria to react with the blood to produce highly absorbing products. Thus, lysis must first begin; but since the change in absorbance does not equal that produced by lysozyme acting on the bacteria alone, it seems that the bacterial products are already reacting with the blood before lysis is completed. It is therefore not feasible to assay the lysozyme concentration in the presence of blood. The foregoing phenomenon may explain the reaction of certain normal bloods with the bacteria (Fig. 2*a*). The blood of *P. americana* is weak in native lysozyme and *p*H 3.5 is the optimum for activity of this enzyme (*7*). With a low concentration and less than optimum *p*H, lysozyme could be conceived as having a weakly lytic action on the bacteria, which is made all the more cryptic by the competing absorbance of the developing reaction between bacterial cell products and blood; hence the lack of a decline in absorbance and the delayed increase. We have no proof, however, that the observed reaction is the consequence of lysozyme action.

It is interesting that the injected lysozyme should persist in the insect for a considerable time, for notwithstanding Malke's view (*3*) that the egg-white lysozyme used was identical with the insect's lysozyme and therefore incapable of causing a foreign body reaction, Powning and Irzykiewicz (*7*) have shown that they differ. The observed evidence of its toxicity in the cockroach (*8*) is probably due to both its difference and its persistence.

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23 September 1968

Preferred Centripetal Conduction of Dendritic Spikes in Alligator Purkinje Cells

Abstract. *Dendritic action potentials in alligator Purkinje cells tend to have a unidirectional preference which favors centripetal over centrifugal propagation. This unidirectional tendency funnels the peripherally evoked dendritic spikes into the lower dendrites and soma of these cells, and it allows the peripheral dendritic branches to operate to a certain extent as partially independent functional units.*

Alligator Purkinje cell dendrites have been shown to generate action potentials near their end terminals (*1*). These action potentials seem to be conducted toward the cell's soma with an average velocity of 30 cm/sec, the conduction velocity being smaller at the periphery (10 cm/sec) than at the main dendritic branches (40 cm/sec). The results to be reported here suggest that the conduction of these spikes is preferentially centripetal although centrifugal dendritic invasion can also occur.

Alligators (*Caiman sclerops*) were anesthetized with pentobarbital and then immobilized with gallamine (50 mg/kg intraperitoneally) and artificially ventilated. The surface of the cerebellar cortex was stimulated electrically by means of a local electrode which activated a small bundle or "beam" of parallel fibers in the molecular layer (*1*, *2*). Purkinje cells were also activated antidromically through a bipolar electrode (WM) in the white matter near the cerebellar peduncle. The field potentials generated by local (Loc) or white matter (WM) stimulation were recorded by an array of three or four micropipettes filled with 4*M* NaCl and which had an average d-c resistance of 1 to 2 megohm. The micropipettes were placed in a carrier which allowed their tips to be positioned at 200 μ from each other laterally and in the same vertical plane with the height of each electrode adjusted so that they entered into simultaneous contact with the cerebellar surface. This particular interelectrode distance was chosen because the lateral spread of the Purkinje cell dendrite is in the vicinity of 250 to 300 μ, and so the two electrodes would be within one Purkinje cell spread from each other. Each microelectrode was connected to a separate field-effect transistor amplifier whose output was displayed on an eight beam dual-gun oscilloscope.

Field potentials evoked by parallel