seen in adult cattle and frequently terminates fatally. Symptoms of the disease are elevated body temperature, anemia, anorexia, atony of the rumen, accelerated respiration and pulse, pale mucous membranes, constipation, depression, icterus, and weakness, and abortions often occur in advanced pregnancies. During the febrile period as many as 77 percent of the circulating erythrocytes contain bodies referred to as anaplasma bodies. Young calves develop a mild form of anaplasmosis; however, a splenectomized calf will develop acute or peracute anaplasmosis. Cattle which have recovered from anaplasmosis are immune to the disease but are permanent carriers. Minute amounts of blood from such cattle will reproduce anaplasmosis when injected into a susceptible bovine.

The etiology of the disease has not been established, but it is generally assumed that the anaplasma body is the etiologic agent of anaplasmosis. This body is generally considered to be a protozoan and is designated *Anaplasma marginale* (*2*). The anaplasma bodies are characteristically located at the periphery of the erythrocyte (Fig. 1 top left) and vary in size from 0.2 to 0.9 μ, the larger ones being composed of eight spherical "sporoid" bodies of equal size (*3*). They are said to be devoid of cytoplasm (*4*). The suggestion has been made that the anaplasma body is constituted of tightly packed submicroscopic elementary bodies and that the parasite undergoes multiple division instead of binary fission (*5*). A previous study by one of us (L.E.F.) demonstrated an ultrafiltrate of blood to be infective, thereby suggesting that the anaplasma body is a viral inclusion (*6*). The results of this investigation are presented to define better the nature of the anaplasma body.

Whole blood was drawn into a dry 10-ml syringe from the external jugular vein of a splenectomized calf affected with anaplasmosis. Seventy-seven percent of the erythrocytes contained anaplasma bodies. The blood was transferred immediately into a fixative composed of 1-percent osmic acid in a Veronal-acetate buffer, buffered to *p*H 7.4 (*7*) and maintained at 5°C. The fixation times used were 5, 10, 15, 20, and 30 minutes. Following fixation, the fixative was decanted from the cells and the cells were washed with distilled water and dehydrated with increasing concentrations of methyl alcohol. The cells were then embedded in a 1:3 mixture of methyl and butyl methacrylate. Sections of the embedded cells were cut on a Porter-Blum ultramicrotome at a thickness of 1/20 to 1/40 micron by means of a glass knife. The sections were mounted on copper grids with a Formvar film and viewed in an RCA EMU 3 electron microscope. Fixation for 20 minutes was found to be optimal.

With the electron microscope the anaplasma body is seen as a clear space at the margin of the erythrocyte, containing from one to seven masses of dense particulate matter (Fig. 1 top right and bottom left). The masses comprising the anaplasma bodies measure from 0.2 to 0.7 μ in diameter. The larger masses are seen in bodies containing single masses, while smaller ones are seen in bodies containing mutliple masses. The dense particulate matter typically consists of a central mass and a peripheral ring separated by a clear zone in which are seen a variable number of strands connecting the central mass and peripheral ring. Figure 1, bottom right, at greater magnification, demonstrates the particulate composition of the anaplasma body. The size of the particles is approximately 100 A. None of the organelles of a cell have been seen in the bodies—such as nucleus, mitochondria, and endoplasmic reticulum.

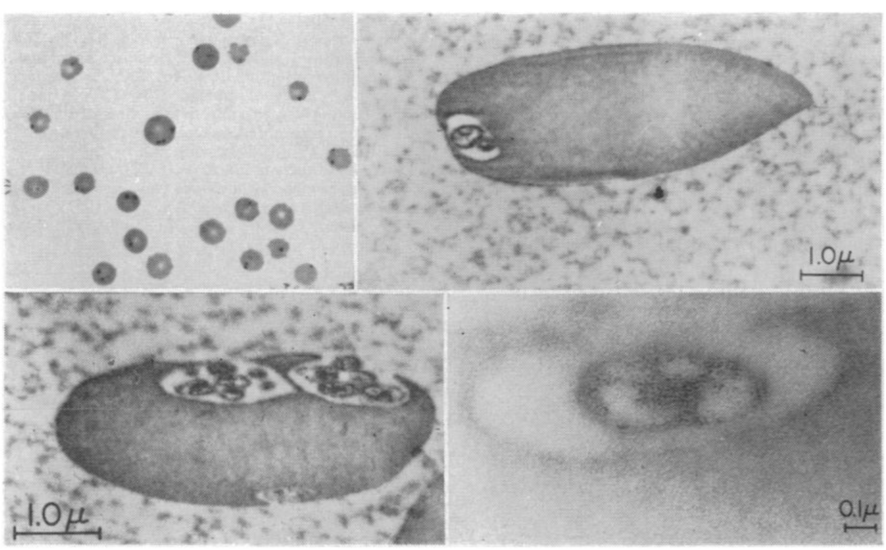

Fig. 1. (Top left) Peripheral blood smear with Giemsa stain (about × 730); (top right) anaplasma body in erythrocyte; (bottom left) anaplasma bodies in erythrocyte; (bottom right) anaplasma body in erythrocyte.

These observations (*8*) support the idea that the etiologic agent of anaplasmosis is a virus.

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3 February 1958

Spray Mechanism of the Cockroach Diploptera punctata

The cockroach *Diploptera punctata* (Eschscholtz) has a remarkable pair of glands, the secretion of which contains a mixture of *p*-benzoquinone and two of its derivatives (*1*). Each gland consists of a cluster of secretory cells surrounding a dilation of the trachea leading to the second abdominal spiracle. The secretion is stored within the tracheal dilations and is ejected through the second abdominal spiracles when the roaches are agitated, anesthetized, or otherwise disturbed (*1*).

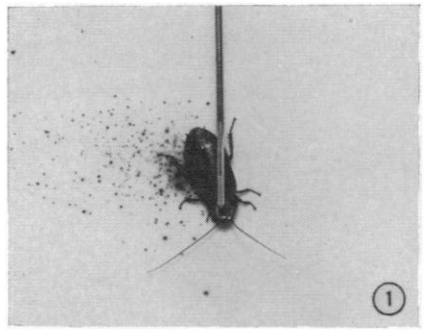
The very fact that disturbance elicits ejection suggests that this secretory apparatus is defensive in function. This contention was confirmed by a recent series of experiments (*2*), the main results of which are presented below.

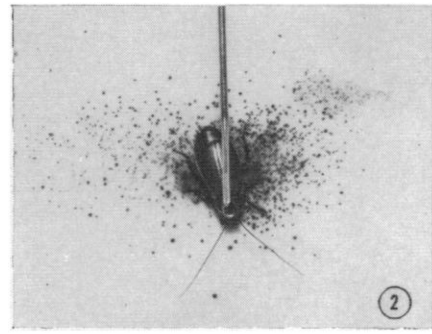
The secretion of *Diploptera,* like that of other arthropods that also secrete quinones, imparts an intense bluish-black coloration to acidulated KI-starch paper (*1, 3*). This indicator paper affords a convenient means of recording the direction, range, and degree of dispersion of the secretory discharge. Individual roaches, fastened to fixed rods and adjusted so that they assumed normal stances on sheets of indicator paper, were subjected to traumatic stimuli as follows: individual appendages were gently pinched with fine forceps or parts of the body were poked with a hot needle. Except for newly molted individuals, all adults and nymphs responded instantly to such stimuli by ejecting a discharge. The inability of newly molted individuals to discharge is undoubtedly due to the fact that at each molt the cuticular wall of the tracheal reservoirs and the quinones within are shed along with the other tracheal linings (*1*). The reservoirs were found to be replenished 8 to 24 hours after molting and the roaches were again able to discharge noticeably.

As evidenced by the pattern on the indicator paper (Figs. 1 and 2), the secretion is ejected in the form of a fine, broadly dispersed spray. Interestingly, both glands rarely discharged simultaneously. Usually, only the gland corresponding to the side of the body subjected to stimulation discharged at any one time (Figs. 1 and 2). A synchronous discharge of both glands occurred only when an appendage was persistently pulled, when appendages of opposite sides were pinched simultaneously, or when a persistent thermal stimulus was applied over a broad surface, as with a spatula. The number of consecutive discharges that could be produced by any one roach varied considerably. In general, up to four discharges could be elicited readily from each gland of large nymphs and adults, provided the roaches had not been disturbed for 2 or 3 days prior to stimulation.

In another series of experiments, aimed at evaluating the repugnatorial effectiveness of the spray, series of roaches were subjected to direct attacks of predators. The predators consisted of carabid beetles (*Galerita janus* Fabr.), praying mantids [*Hierodula patellifera* (Serville)], lycosid spiders (*Lycosa helluo* Walckenaer), and ants [*Pogonomyrmex badius* (Latr.)]. The mantids, spiders, and carabids were released individually into test arenas made of large finger bowls or beakers lined with indicator paper, in each of which they had access to a single *Diploptera*. The experiments with *Pogonomyrmex* were performed directly in front of the nest opening of a thriving laboratory colony of this ant, with the roaches affixed to rods and placed on sheets of indicator paper. All predators attacked readily, and in each case the roaches responded instantly by ejecting the spray. The discharge was usually unilateral, toward the particular appendage or quarter of the body seized by the predator.

The ants and carabids were in all cases repelled promptly by the secretion and retreated before they had inflicted noticeable injury. Conspicuous in the course of their escape was a series of abnormal seizures, during which leg movement became discoordinated and ineffectual, hampering and sometimes halting locomotion. These seizures, altogether, rarely lasted more than 2 minutes, and recovery was always complete. The spiders were also repelled, but only by the larger nymphs and adults; young nymphs were usually eaten promptly. Unlike the carabids and ants, the spiders never showed noticeable abnormalities following the impact of the spray. In a control study, all of the above-mentioned predators were offered freshly molted roaches and others from which the glands had been excised; such individuals proved vulnerable and were always eaten (Fig. 3).





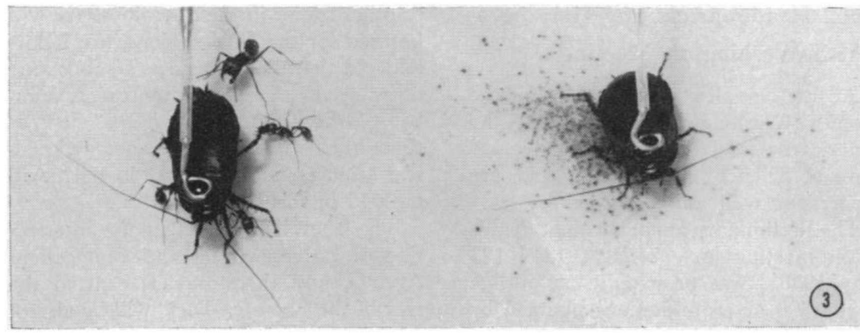


Fig. 1. Spray pattern (on KI-starch indicator paper) of discharge of the right gland of roach, following pinching of the right mesothoracic leg. Fig. 2. Same roach as shown in Fig. 1 after a second discharge but from the left gland, following pinching of the left mesothoracic leg. Fig. 3. Roach on left has had its glands excised and is under persistent attack by several ants (*Pogonomyrmex badius*). The intact roach on right was attacked, but it sprayed and repelled its assailants.

The praying mantids seemed to be completely unaffected by the spray, and every roach seized was invariably devoured. I am told by Susan Rilling, of Tufts University, that some of her mantids have been fed routinely with *Diploptera* for several days at a time, without apparent ill effects.

No extensive experiments with vertebrate predators have been made as yet. Some mice (*Mus musculus* L.), birds [*Cyanocitta cristata* (L.)], and lizards (*Anolis carolinensis* Voigt) were found to eat individuals of *Diploptera* when these were offered to them for the first time. However, before repugnatorial effectiveness of the secretion against vertebrates can be ruled out, it will be necessary to determine whether repeated encounters with this roach might not result in discrimination against it.

Quinone secretions are known to occur in a wide variety of other arthropods, including tenebrionid and carabid beetles, millipedes, and phalangids (*1*, *3*). Experimental and behavioral studies on many of these are currently being done at our laboratory, and some of this work has already been reported elsewhere (*4*).

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