

frequently—since they “feigned death” and tucked their legs under the body—they were grasped by the elytral margins. No sooner had an ant bitten than the beetle responded by bleeding from the nearest knee joint. The droplet of blood invariably spread onto the assailant, contaminating its antennae, and usually oozing between the gaping mouthparts. The ant immediately released its hold, backed away abruptly, and then began intensive cleansing activities, brushing its antennae with the forelegs, or walking along slowly, flailing its legs and dragging its blood-drenched mouth parts along the substrate (Fig. 12). [This peculiar dragging behavior has been noticed before in this ant in comparable encounters involving arthropods with defensive secretions (6)].

More pronounced signs of distress became apparent as the blood coagulated, becoming increasingly viscous and sticky. It was then not uncommon to see an ant with its antennae stuck together, with a leg stuck to an antenna, or with the mouth parts gummed up and virtually immobilized. Groups of ants would become stuck to one another (Fig. 13). Recovery is eventually always complete, but it may take an ant several minutes to over an hour to clear itself from the remaining flakes of clot. A few tests were also made by releasing ants and beetles onto the leaves of the beetle's food plant. The results differed only in that they pointed out clearly the protective value of “death-feigning”; when attacked, the beetles often simply let themselves drop from the leaves.

The experiments with larvae were all done on leaves. The ants attempted to bite, but would usually only succeed in breaking one or more of the larval spines. The droplets of blood released were instantly repellent to the ants, and affected them in the same way as did the blood of the adults. Unlike the adults, the larvae never dropped from leaves.

Evidently, the bleeding mechanism of *Epilachna* is a means of defense admirably suited for use against ants. It is certainly conceivable that the mechanism might be similarly effective against other arthropod enemies, but ants may well be one of the most important groups of predators—if not the most important group—responsible for the evolution of the mechanism. Most coccinellids are carnivorous, and the habit shared by so many of them of feeding

on aphids and coccids may well be primitive for the family (7). Though the tribe to which *Epilachna* belongs (Epilachnini) is predominantly herbivorous, it is “regarded as a relatively late and specialized offshoot of the Coccinellid stock . . .” (8). It seems that its hemorrhagic defense mechanism is an evolutionary legacy from its Homoptera-feeding forebears, whose exposure to the well-known aggressive tendencies of Homoptera-tending ants (9) provided the selective force that evoked the adaptation in the first place. It is interesting in this connection to note that the larvae of certain lycaenid butterflies that also feed on Homoptera tended by ants are protected from attack by secreting a substance that is not repellent but attractive to the ants (10).

In the past there has been some controversy as to whether the liquid exudate of adult coccinellids is indeed blood, rather than the product of special glands (11). Our own observations tend to confirm the prevalent view that it is blood. The liquid has all the diagnostic features of blood (Fig. 12): the same cells are present, and so are the tiny spherules known to be typical of coccinellid blood (12). One might add also that the size of the droplet released at the knee joint often distinctly exceeds the estimated volume of the leg that produces it. Histological studies (13) reveal no glandular reservoirs of appropriate capacity.

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1. This study was supported by grant No. E-2908 from the U.S. Public Health Service. It is the fifth paper of our series on defense mechanisms of arthropods.
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* Predoctoral fellow, National Science Foundation.

17 April 1961

Localization of Porphyrin Fluorescence in Planarians

Abstract. Two species of planarians were studied by fluorescence microscopy. Red fluorescence of uroporphyrin was observed localized in the epidermal rhabdites and subepidermal rhabdite-containing gland cells. Fluorescence was observed in isolated rhabdites of homogenates, but was not seen in rhabdites of the living animal. The identity of rhabdites was established by their location, shape, size, and acidophilic staining properties.

The occurrence of uroporphyrin in the planarian, *Dugesia dorotocephala*, has been described previously (1). This finding was based on analyses of acid extracts of the planarian. In that study, general porphyrin fluorescence of the acid-treated animal was observed; in the present study, however, higher intensity of ultraviolet light permitted the observation of fluorescence within definite morphological structures.

Detection of fluorescence was made microscopically; a Leitz HB 200 lamp was the source of ultraviolet light. A Leitz 2-mm BG-12 exciter filter was used, and a 2.5-mm OG-1 barrier filter was placed in the ocular. Observations and photographs were made with a dark-field condenser.

The two species of planarians studied were *Dugesia dorotocephala* and *D. tigrina*. Living animals were placed in water between a slide and cover slip, and exposed to ultraviolet light under the microscope. Red fluorescence observed in the living animal was restricted to a few irregular structures within cells lining the digestive tract, which probably were food particles or products of digestion. However, after treatment with a variety of chemical substances, a brilliant and extensive red fluorescence was observed in the epidermis and subepidermis of these animals.

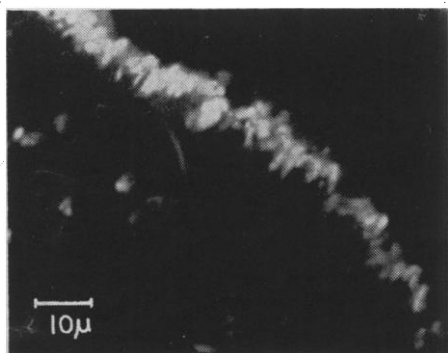


Fig. 1. Cross section of *Dugesia dorotocephala* showing fluorescence of epidermal rhabdites and subepidermal rhabdite clusters. The preparation was from an animal fixed in 100-percent ethyl alcohol; it was cleared and embedded in paraffin and sectioned at 5 μ . The section was then deparaffinized and mounted in nonfluorescing immersion oil for observation under the fluorescence microscope.

The substances used were 95-percent ethyl alcohol, 5-percent neutral formalin, 0.05N NaOH, 0.1N NH₄OH, and 50-percent H₂O₂. The appearance of fluorescence seemed to coincide with death of the animal. Observation was facilitated by applying pressure on the cover slip and flattening the animal. The red fluorescence was localized within rod- or crescent-shaped structures at the surface and just below the surface of the epidermis. The identification of these structures as rhabdites was based on their location, shape, and size (2). Their length varied from 2 to 8 μ , and their width from 1 to 3 μ . These structures could be seen as refractive bodies in the epidermis of the living animal under the phase-contrast microscope. The identity of the rhabdites was further checked by observing fluorescent rhab-

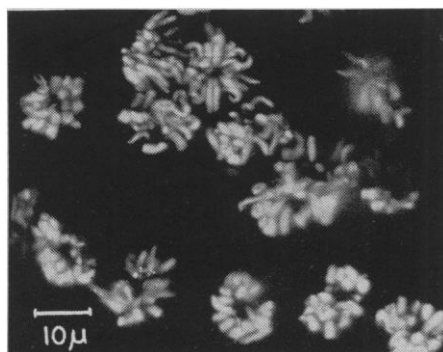


Fig. 2. Cluster of fluorescing rhabdites within gland cells of *Dugesia tigrina* from a squash of the whole animal treated with 0.05N NaOH, and observed under the fluorescence microscope.

dites in acetone-frozen and dried sections, and in sections of animals fixed in 100-percent ethyl alcohol, cleared in benzene, and embedded in paraffin. Although some porphyrin may have been extracted by the alcohol, deparaffinized sections under the microscope clearly revealed fluorescent rhabdites perpendicular to the surface of the animal (Fig. 1). In the subepidermis, rhabdites were noted in clusters, which were identified as rhabdite-forming gland cells. Fluorescent rhabdites were retained in a sacular gland cell when the whole animal was placed in 0.05N NaOH; the cells separated and flowed freely when slight pressure was placed upon the cover slip. This treatment allowed observation of the number and shape of rhabdites within the somewhat swollen gland cells (Fig. 2).

Sections were stained with acid dyes such as eosin, orange G, picric acid, and fast green. The rod-shaped structures which exhibited the red fluorescence also showed the marked eosinophilia and acidophilia described by other investigators as characteristic of rhabdites (3, 4). Rhabdites did not stain with basic dyes, such as methylene blue and toluidine blue.

Homogenates of planarians were made in a Potter homogenizer, and a drop of homogenate was examined under the fluorescence microscope. Red fluorescence was clearly visible in rhabdites isolated from other cellular materials. Fluorescence was also visible in clusters of rhabdites and in some smaller "subrhabdite" units, which may be structural precursors of rhabdites. These "subrhabdites" could also be observed within some gland cells after treatment with 0.05N NaOH. The fluorescence of the rhabdites in the homogenate appeared without any chemical pretreatment of the homogenate. When homogenates or whole planarians were subjected to HCl (1N or 2.5N), an extraction of red fluorescing material occurred. Examination under the microscope revealed an extraction of fluorescence from the rhabdites. The acid extract contained uroporphyrin as analyzed by the paper chromatography method of Nicholas and Rimington (5).

Rhabdites were observed to stain intensely with bromphenol blue, indicating protein in rhabdites; this confirmed observations of Prenant (3) and Pedersen (4) made on several other species of planarians. A linkage between protein and uroporphyrin or between a metal and uroporphyrin may account

for the lack of fluorescence in the rhabdites of living animals. Such a linkage would eliminate the alternating double-bond structure upon which the fluorescence of porphyrin depends. On the other hand, the uroporphyrin may be present as a nonfluorescing porphyrinogen (6).

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13 April 1961

Aging in Irradiated and Nonirradiated Hydras

Abstract. Evidence is presented that as hydras become older they bud more slowly and become more susceptible to adverse conditions. In these studies, a 2000-roentgen dose of x-radiation did not seem to affect the rate of these changes. The mean number of tentacles did not change with age.

It has been suggested by Brien (1) that individual hydras are theoretically immortal. He bases this suggestion on the fact that there seems to be no noticeable decrease in the budding rate of individuals maintained under observation for long periods (2). Also, in each individual hydra there is a continual replacement of the entire cell population (1, 3), and it may be that this produces a continual rejuvenescence. The facts that stimulation of the rate of cellular replacement by repeated injury seems to increase the

Table 1. Number of buds produced by offspring minus the number produced by parents ($\bar{x}$)

Group	Generation	$\bar{x}$	t	p
Control	4	-0.06	0.20	0.8
Control	8	.42	1.56	.07
Control	12	.63	3.07	<.005
Irradiated	4	.54	1.1	.15
Irradiated	8	.765	1.88	<.05
Irradiated	12	.53	1.59	.07