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# Defensive Secretions of Arthropods

## Insects, millipedes, and some of their relatives, discharge noxious secretions that repel predators.

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All organisms are chemosensitive, and all of them are also the source of substances to which others can potentially respond. In the course of evolution this potential for interaction has been thoroughly exploited, and organisms of the most diverse kinds have come to depend, in one adaptive context or another, on an exchange of chemical cues with other organisms in their environment. When the exchange is between members of the same species, the mediating substances are called pheromones (*1*). These interesting compounds, so important for the regulation of courtship and other social activities in animals, and including among others the sex attractants, alarm substances, aggregation-promoting substances, territorial markers, and trail substances, have recently been the subject of intensive research, and of excellent reviews (*2*).

Set apart from the pheromones, but certainly matching them in importance, is that vast array of substances—for which an adequate name has yet to be coined—that serve for the transmission of information between members of *different* species. Whether two species coexist in harmony (as in mutualistic associations) or in conflict (as in predator-prey or parasite-host relations), chances are that their interactions depend in some major way on chemical factors which they themselves produce. In fact, all living things—animals, plants, and microorganisms alike—are probably involved at one time or another in their existence with the emission or reception of extraspecific chemical messages. Surprisingly little is known about such cross-specific communication. Neither the chemistry of the "signals" themselves nor many of the factors relating to their reception and release are properly understood in most instances. This article deals with the chemical interactions that prevail between predators and certain of their prey. Defensive secretions, whether of arthropods or other organisms, are essentially chemical signals, since they represent the means by which predators and other potential enemies are "told" to desist or withdraw. Clearly, there is little room for ambiguity in the rather forceful message written in the stench of a skunk's spray. Among arthropods, chemical defenses comparable to that of the skunk are widespread. In fact, the vocabulary of protective chemical signals possessed by these animals may be one of the richest in the entire world of life.

### The Glands and Their Discharge Mechanisms

Unlike pheromones, which are often produced in vanishingly small quantities and may require the development of special bioassays to demonstrate their presence (*1–3*), defensive secretions may be detected with relative ease since they are often strongly odorous and discharged in substantial amounts. The literature abounds with references to insects, millipedes, phalangids, and other terrestrial arthropods that eject an obnoxious liquid when disturbed (*4*). The glands responsible for this output are integumental organs, consisting essentially of infoldings of the body wall. Their outer openings may be small and relatively inconspicuous, but the invaginated portion is usually large and readily seen in dissection (Fig. 1, A and B). Typically, a gland is made up of a sac-like reservoir in which the secretion is stored, and of a glandular tissue that may be a part of the wall of the reservoir itself (Fig. 1D) or may lie apart from the reservoir and be joined to it by one or more special ducts (*4*) (Fig. 1C). The secretory cells are often strikingly elaborate in structure, but in only a few instances have they been examined with the electron microscope (*5, 6*). The glands are so variable in number, distribution, and morphological detail that there can be no doubt that they have arisen many times independently in the course of evolution. They may occur singly, in pairs, or in multiple pairs, and they may be present on head, thorax, or abdomen.

As regards their mode of discharge, the glands fall into three main categories. In one type, present commonly in millipedes (*7, 8*), the secretion simply oozes from the glands onto the animal's own surface (Fig. 2, A and B). In another, found in some caterpillars (Fig. 2E) and beetles, the secretion is "aired" by actual evagination of the gland as a whole (*9, 10*). The third and perhaps most spectacular category includes those glands that dis-

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charge their contents as a spray (Fig. 2, C and D), sometimes to a distance of several feet. Among the many arthropods known to spray are certain cockroaches (11), earwigs (12), stick insects (13), "stink bugs" (Hemiptera) (14), notodontid caterpillars (15), grasshoppers (16), carabid (7, 17, 18) and tenebrionid beetles (19), whip scorpions (20), and millipedes (21).

Special behavioral and other adaptations enable arthropods to discharge their secretion with maximum effectiveness and minimum wastage. Species that eject a spray usually possess the ability to aim it precisely toward that portion of their bodies subjected to disturbance (Fig. 2, C and D), thus insuring that an attacking predator is met by the full impact of a discharge. There are different ways in which aiming may be accomplished. The caterpillar *Schizura concinna*, whose single gland opens ventrally just behind the head, directs the spray by pointing its front end in the appropriate direction (15). In the whip scorpion, *Mastigoproctus giganteus*, the two glands open at the tip of a short revolvable knob that acts in the manner of a gun turret (20). Aiming may also involve postural adjustments of the abdomen (12) or of the body as a whole (14).

Animals that possess more than one gland may call them into action singly or in groups. This is strikingly illustrated by millipedes, many of which have their glands arranged segmentally, one pair per most body segments. A sharply localized stimulus may cause the nearest glands to respond, but no others (Fig. 2A). Only after persistent or generalized disturbance do the glands discharge in large numbers (Fig. 2B) (7). Eversible glands may also be employed in a specialized fashion. In the caterpillar of the swallowtail butterfly *Papilio machaon*, the two-pronged gland (Fig. 2E) may be partially or completely extruded, depending on where and how intensely the stimulus is applied. Moreover, the larva arches its front end in such a way that the "horns" are wiped against the instrument used for stimulation (10).

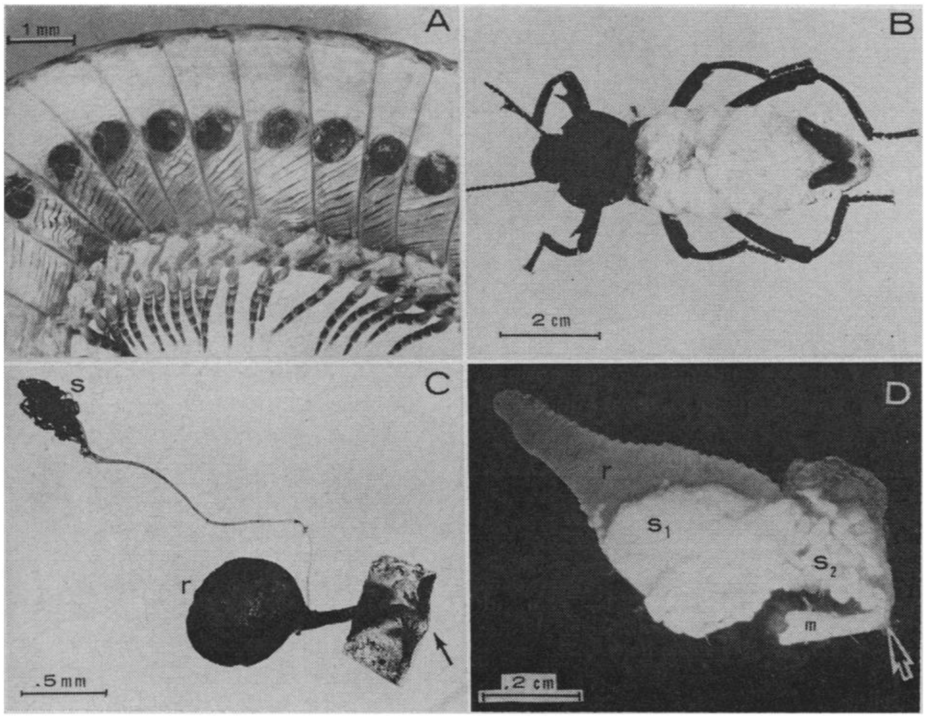


Fig. 1. (A) Midbody segments of a millipede (*Narceus gordanus*), sagittally dissected, showing spherical reservoirs of the defensive glands, amidst the intersegmental muscles of the body wall; the reservoirs are filled with dark-brown quinonoid secretion (see also Fig. 2, A and B). (B) A tenebrionid beetle (*Eleodes* sp.) with the dorsum of its abdomen dissected away to expose the two posterior defensive glands, turgid with quinonoid secretion (see also Fig. 2H). (C) Isolated gland of a carabid beetle (*Chlaenius cordicollis*); the secretory cells form a compact tissue (*s*), joined to the reservoir (*r*) of the gland by a narrow efferent tubule; a short duct (partly occluded by a rectangular piece of the body wall) leads from the reservoir to the outer orifice (arrow) of the gland. (D) Isolated defensive gland of the tenebrionid beetle *Eleodes longicollis*, showing two distinct secretory tissues ($s_1$, $s_2$) overlying the membranous reservoir (*r*) of the gland; the muscle (*m*) opens the valvular outer orifice (arrow) of the gland.

Although as a rule arthropods discharge their secretion only in response to direct contact-stimulation, exceptions are known. The walkingstick *Anisomorpha buprestoides* may eject its spray with accuracy toward a bird in its vicinity, even before any direct attack has taken place. Nothing is known about the particular sensory input that the insect relies upon for taking aim at the approaching predator (13).

The mechanisms whereby the discharge is effected vary. Sometimes muscular compression of the reservoir is involved (7, 13, 22). When intrinsic muscles are missing, the reservoir may be squeezed by blood pressure (7, 16). Blood pressure is also thought (9, 10) to force the extrusion of eversible glands, as in *Papilio* (Fig. 2E). Some glands are connected to respiratory tracheae and rely on air pressure for the discharge (Fig. 2G) (4, 23). In the remarkable bombardier beetles (*Brachinus* spp.), the secretion is expelled under pressure of gas generated in an explosive chemical reaction (24). Additional mechanisms have been postulated, some them perhaps unnecessarily elaborate (21, 25). An interesting discussion of the biophysics of the discharge mechanism of certain Hemiptera is provided by Remold (14).

## Chemistry of the Secretions

Although the earliest analytical work on arthropod secretions dates back to the 17th century (26), and occasional chemical studies have been made since then, the present widespread interest in this field was prompted largely by the recent studies of Pavan and his associates (27) on the toxins of ants and other insects. Several laboratories throughout the world are now actively concerned with elucidating the nature of the secretions, and dozens of active principles have been isolated and identified. Recent reviews have appeared in which these compounds are listed (4, 28), and in which some of the techniques that led to their isolation and identification are discussed (29). Most of the components identified to date are compounds of low molecular weight (about 30 to 200), belonging to a number of common chemical classes, including acids, aldehydes, ketones, esters, hydrocarbons, lactones, phenols, and *p*-benzoquinones. The group includes simple straight-chain compounds and both acyclic and cyclic monoterpenes (Fig. 3). With few exceptions they represent compounds previously known, including even common laboratory reagents. They are almost invariably strongly odorous, and they may occur within the glands at astoundingly high concentration [for example, acetic acid, 84 percent (*20*)]. Despite the simplicity of most of the compounds, their isolation and characterization have on occasion provided some challenge because of the small amounts of material available. In the few instances where new compounds have been discovered, and particularly in the case of the cyclopentanoid monoterpenes, their categorization and subsequent synthesis have been of considerable intrinsic chemical interest (*30*).

The secretions may be mixtures of some complexity. The odorous discharge of the pentatomid bug *Nezara viridula* contains 18 aliphatic components, including hydrocarbons, aldehydes, ketones, and esters (*31*). Quinonoid secretions usually contain two or three quinones, rather than a single one (*4, 28*), and the quinones may be accompanied by glucose, hydrocarbons, and traces of hydroquinones (*23, 32, 33*). The lesser components may offer clues to the biochemical mechanisms whereby the secretions are synthesized (*32*). In many mixtures only the dominant components have been identified, while minor ones remain unknown (*4, 28*). Secretions such as that of the cockroach *Eurycotis floridana* (*22*), which

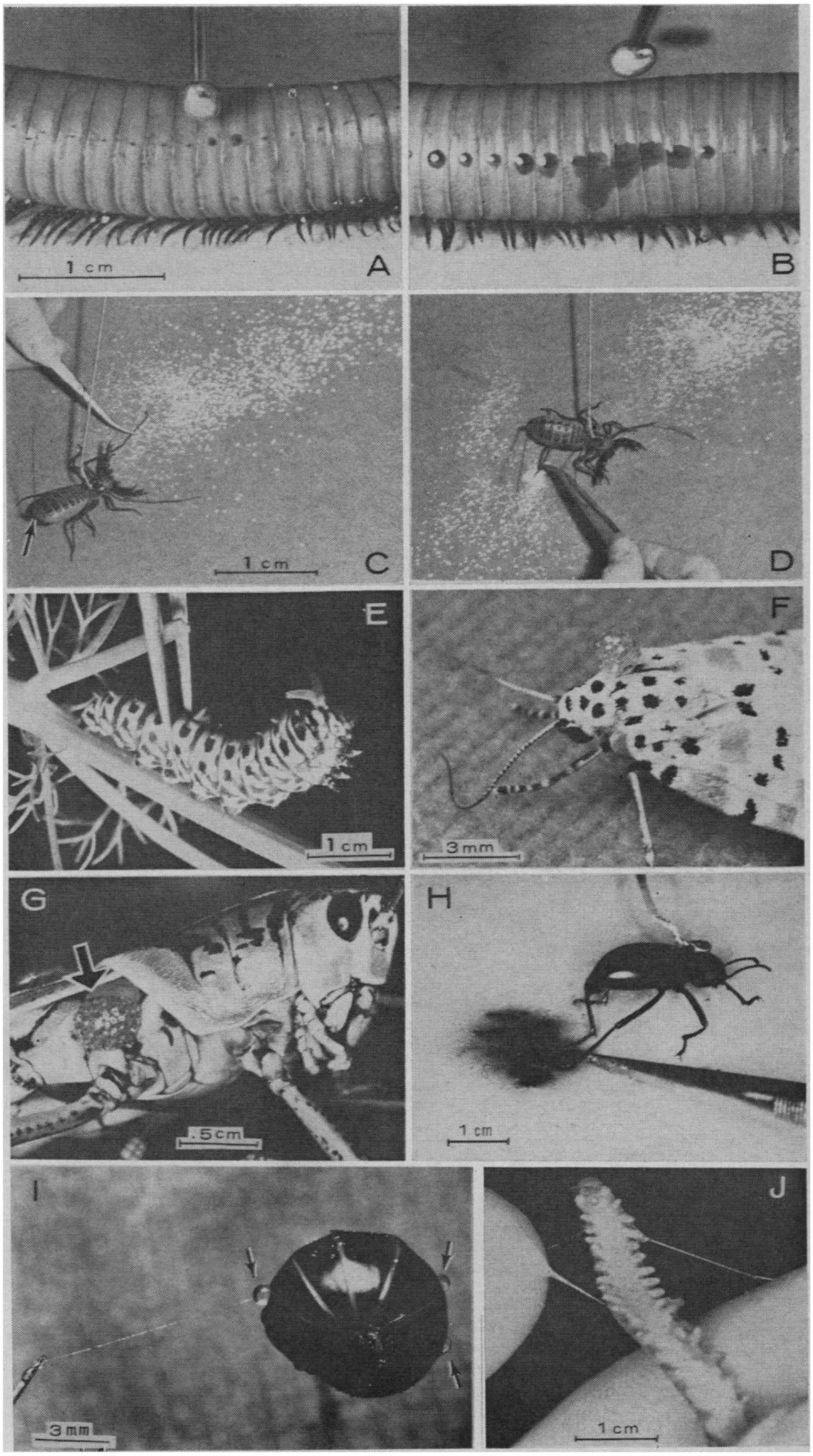


Fig. 2 (right). (A) A millipede, *Narceus gordanus*, being tapped with a metal mallet, has begun to discharge its quinonoid secretion from two of its segmentally arranged glands (see Fig. 1A). (B) The same millipede discharging profusely from several glands, after persistent tapping. (C) The whip scorpion, *Mastigoproctus giganteus*, discharging its aimed spray in response to the pinching of one of its appendages with forceps (the arrow points to gland openings); the animal has been affixed to a rod, and the acid spray is rendered visible on a background of filter paper impregnated with alkaline phenolphthalein. (D) The same scorpion discharging a second time, in response to pinching of the right rear leg. (E) Caterpillar of *Papilio machaon*, being pinched with forceps, everting its two-pronged postcephalic gland. (F) An arctiid moth, *Utetheisa bella*, emitting froth from its right cervical gland. (G) The grasshopper *Romalea microptera* discharging a froth (arrow) from its anterior thoracic spiracle; the respiratory tracheal tubes leading inward from the spiracle are beset with glandular tissue and filled with secretion; the froth is a mixture of secretion and respiratory air. (H) Tenebrionid beetle *Eleodes longicollis* ejecting quinonoid secretion in response to the pinching of one of its legs with forceps (see also Fig. 1B); the secretion is rendered visible on a substrate of filter paper impregnated with an acidulated solution of potassium iodide and starch. (I) An oniscomorph millipede (*Glomeris marginata*) coiled into a tight sphere in response to disturbance, has discharged three droplets of secretion (arrows) from glands opening on the dorsal midline; the sticky secretion can be drawn into fine threads, as is here being done with the tip of a needle (see also Fig. 6E). (J) Unidentified onychophoran from Panama, discharging its sticky secretion in response to handling.

consists of a single, virtually pure component (*trans*-2-hexenal), appear to be exceptional.

Arthropods of very diverse types may produce secretions containing the same or closely similar components. For example, *trans*-2-hexenal is known from Hemiptera (*31, 34–36*), cockroaches (*22, 37*), and a myrmicine ant (*38*). Similarly, formic acid is secreted by ants (*39*), carabid beetles (*29, 40*), and notodontid caterpillars (*15, 41*). Particularly widespread are the *p*-benzoquinones, which have been identified from beetles, earwigs, millipedes, a cockroach, and a phalangid (Fig. 5) (*4, 28*). The glands of arthropods producing similar secretions may be very different structurally and obviously not homologous.

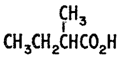
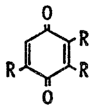
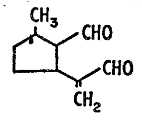
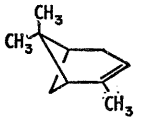
Instances of evolutionary diversification of the secretions are also known. In the beetles of the family Carabidae, the defensive (pygidial) glands (*42*), which are undoubtedly homologous throughout the group, have been shown in various species to produce compounds as different as formic acid (*29, 40*), *m*-cresol (*7*), *p*-benzoquinones (*24*), salicylaldehyde (*17*), and tiglic and methacrylic acid (*29, 43*) (Fig. 4). In millipedes, the orders Julida, Spirobolida, and Spirostreptida secrete *p*-benzoquinones (*29, 44, 45*), the Polydesmida produce cyanogenic secretions (*8, 46, 47*), and the single species of the Chordeumida that has been studied produces a phenol (*7*). Despite some differences in structure, the glands in these various orders are probably homologous.

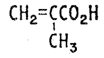
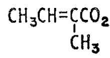
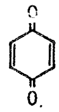
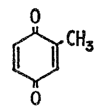
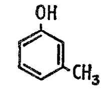
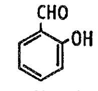
Future studies may yet uncover secretions containing entirely different compounds, perhaps even some of interesting pharmacological activity. Existing evidence is already suggestive in this respect. A pharmacologically active choline ester closely resembling $\beta,\beta$-dimethylacrylylcholine is present at high concentration in the cervical glands of an arctiid moth (*48*). The grasshopper *Poekilocerus bufonius* sprays a secretion containing a digitalis-like compound (*49*). The salivary secretion of the reduviid bug *Platymeris radamantus*, which is ordinarily used as a lethal venom for injection into prey but can also serve for defense by being sprayed against predators, contains at least six proteins, three of which show trypsin-like proteolytic activity; it also has strong hyaluronidase and weak phospholipase activity. The saliva resembles snake venom both in number of proteins and in enzyme activity (*50*).

Although as a rule the secretions retain their fluid consistency after discharge, there are some that undergo rapid transformation on exposure to air and become viscous and sticky. The spray of Onychophora (*Peripatus* and its relatives) (Fig. 2J), as well as the discharge of certain millipedes (for example, *Glomeris marginata*) (Fig. 2I) and isopod crustaceans, are of this type (*51, 52*). None of these secretions has been categorized chemically.

## Defensive Effectiveness of the Secretions

Although encounters between predator and prey have only rarely been witnessed in nature, it is evident from laboratory tests in which arthropods were pitted against caged predators that the glands are weapons of extraordinary effectiveness (Fig. 6, A–F). Among the numerous predators that were shown to be repelled by the discharge of their intended prey are ants, praying mantids, carabid beetles, solpugids, spiders, toads, lizards, jays, armadillos, and mice (*4, 7, 10–14, 17–20, 35–37*). As a rule, arthropods eject their secretion during the initial phases of an attack, thwarting their assailants before receiving disabling injury. The ability to eject an aimed discharge, or to discharge from only those glands

| COMPOUNDS | | SOURCE |
|---|---|---|
| hydrogen cyanide | HCN | polydesmoid millipedes |
| acetic acid | $CH_3CO_2H$ | whipscorpion, coreid hemipteran |
| caprylic acid | $CH_3(CH_2)_6CO_2H$ | whipscorpion, tenebrionid beetle |
| 2-methylbutyric acid |  $CH_3CH_2\overset{CH_3}{C}HCO_2H$ | papilionid caterpillar |
| trans-2-dodecenal | $CH_3(CH_2)_8CH{=}CHCHO$ | spiroboloid millipede |
| p-cresol |  | chordeumoid millipede |
| p-benzoquinones |  | see fig. 4 |
| dolichodial<br>(anisomorphal) |  | dolichoderine ants<br>walking stick |
| α-pinene |  | termite |

| COMPONENT(S) | | SOURCE |
|---|---|---|
| formic acid | $HCO_2H$ | Pseudophonus spp., unidentified species of Calathus, Acinopus, Carterus |
| methacrylic acid<br>(with or without)<br>tiglic acid |  $CH_2{=}\underset{CH_3}{C}CO_2H$<br> $CH_3CH{=}\underset{CH_3}{C}CO_2H$ | Carabus spp., Abax spp., Pterostichus spp., Apotomopterus spp.. Damaster oxuroides, Cychrus rostratus |
| p-benzoquinone<br>toluquinone | <br> | Brachinus spp. |
| m-cresol |  | Chlaenius cordicollis |
| salicylaldehyde |  | Calosoma prominens |

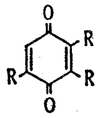
Figs. 3 and 4. Fig. 3 (left). Representative compounds from defensive secretions of arthropods. [Compiled from *4, 7, 10, 28, 69, 87, 88* and references therein] Fig. 4 (right). Components in defensive secretions of beetles of the family Carabidae. [Compiled from *4, 7, 28, 29*, and references therein] that are closest to the site of attack, adds considerably to the efficiency of the weapon (Fig. 6D) (*11–13, 18, 20*).

The secretions act as topical irritants, causing the predators to desist from the attack and to perform more or less vigorous cleansing activities. Among vertebrates, the sensitive areas of the face—mouth, nose, and especially the eyes—appear to be the most vulnerable. Mice respond to the discharge by wiping their eyes and snout with their front feet, or by scurrying away, plowing their muzzle in the earth (*7, 17, 20*). Birds ruffle their plumage and rub their head against the feathers of the body; their eyes are cleansed by the wiping action of the nictitating membranes, which, in the manner of "windshield wipers," are drawn quickly back and forth over the eyeballs (*13, 17, 20*). Frogs and toads are sensitive over their entire body surface, and scratch affected regions with their feet (*12, 19*). Carabid beetles, ants, and other predaceous insects brush their sensitive antennae with their feet, or flee, dragging their mouthparts (*18, 52*). Small predators such as ants, whose entire bodies may become contaminated, literally attempt to "bathe" in the soil, rubbing their bodies against it while flailing their outstretched legs (*12, 20, 52*). Although prolonged confinement with the vapors of a secretion may eventually prove fatal to a predator (*53*), under conditions of adequate ventilation such as are likely to prevail in nature the secretions usually cause only transient effects. Predators may recover in a matter of seconds or minutes, depending on the species, the nature of the secretion, and other factors (*7, 11–13, 18, 20*). Future studies should attempt to establish quantitative criteria by which the repellent effectiveness of the secretions may be more precisely evaluated. Such studies should take into account the intriguing possibility that predators are more or less sensitive to the chemical defenses of their prey depending on the time of day. Rhythmic daily fluctuations in the susceptibility of animals to certain drugs is an established pharmacological fact (*54*).

No experimental studies have been made to determine how—that is, through which particular sensory receptors—the secretions exert their irritant effect on predators. In the case of vertebrates, it seems likely that the principal receptors are the peripheral "free" nerve endings which, acting in the capacity of relatively insensitive and nonspecific chemoreceptors, mediate the so-called "common chemical" or "general chemical" sense (*55*). Highly vulnerable regions, such as the cornea, are indeed densely innervated by free nerve endings (*56*). Arthropods also possess a common chemical sense (*57*), but it is not clear whether free nerve endings—which they are known to possess (*58*)—are the receptors involved. As regards the precise mode of action of the secretions, it remains to be seen whether they act directly on the receptors or indirectly through induction of biochemical changes in nonnervous cells and in tissue fluids around the nerve endings. The growing literature on this general subject has recently been summarized (*59*). To some extent, the secretions may also act by way of true olfactory receptors. This is suggested by the fact that even the dilute vapors that linger after a discharge may be highly repellent. Such vapors are obnoxiously odorous, certainly to man, and have been shown to prevent some predators from closing in on their prey (*7, 18, 20, 35*). In the case of the odorless sticky secretions discharged by Onychophora and some millipedes (Fig. 2, I and J), the primary (but not necessarily exclusive) mode of action may be mechanical rather than toxicological (Fig. 6E). Small predators such as ants become trapped in the gummy discharge and may struggle for minutes or even hours before freeing themselves (*52, 60*). The secretion of certain termites has a similar immobilizing effect (*61*).

p-BENZOQUINONES

R = H, $CH_3$, $C_2H_5$ or $OCH_3$



| CLASS | ORDER | FAMILY |
|---|---|---|
| Arachnida | Phalangida (harvest spiders) | Gonyleptidae 1/1 |
| Diplopoda (millipedes) | Spirobolida | Spirobolidae 2/3 |
| | | Rhinocricidae 1/1 |
| | | Trigoniulidae 2/2 |
| | | Floridobolidae 1/1 |
| | Spirostreptida | Spirostreptidae 3/6 |
| | | Harpagophoridae 1/1 |
| | | Cambalidae 1/1 |
| | Julida | Julidae 4/4 |
| Insecta | Coleoptera (beetles) | Tenebrionidae 12/21 |
| | | Alleculidae 1/1 |
| | | Carabidae 2/4 |
| | Dermaptera (earwigs) | Forficulidae 1/1 |
| | Blattaria (cockroaches) | Blaberidae 1/1 |

Fig. 5. List of arthropods producing secretions containing *p*-benzoquinone or derivatives thereof. In the fraction given after each family, the numerator indicates the number of genera examined; the denominator, the number of species examined. [Compiled from *29, 44*]

Although as a rule the defensive glands possess both the capability and sufficient secretion to discharge several times in succession (*7, 11, 12, 14, 18, 20*), they may ordinarily be called upon to discharge only once during a given attack. This is because residual secretion, remaining around the gland openings or elsewhere on the animal's body after the first discharge, acts to deter the predator from resuming its assault (*7, 18, 20, 35*). The beetle *Chlaenius* remains invulnerable to ants 8 to 13 minutes after a discharge. The estimated 100 meters it can cover during this period at its normal ambulatory pace must suffice for outdistancing even a dense swarm of ants (*7*). Since depleted glands may require several days to be completely replenished (*13, 18, 20*), it is obviously of adaptive value that the glands should ordinarily be required to expend no more than a minimum amount of secretion.

Vertebrates may quickly learn to discriminate against obnoxious prey. After having been sprayed by the walkingstick *Anisomorpha*, blue jays remained aloof from them even when the insects were presented to the birds no more often than every 2 to 3 weeks (*13*). Discrimination by learning on the part of predators has led to the evolution of mimicry, and some arthropods with defensive glands are known to have mimics. The tenebrionid beetle *Eleodes longicollis*, which when disturbed does a headstand and sprays a quinonoid secretion from its abdominal tip, is mimicked by another tenebrionid beetle (*Megasida obliterata*) that lives in the Arizona desert together with *Eleodes*. *Megasida* also lifts its rear when disturbed, but it lacks defensive glands (Fig. 6I) (*19*).

Some predators, although obviously sensitive to the secretions, overcome their prey through sheer persistence. In laboratory tests, grasshopper mice (*Onychomys torridus*) relentlessly pursued whip scorpions and, although temporarily deterred by each discharge, eventually subdued their quarry (*20*). Comparable results were obtained with

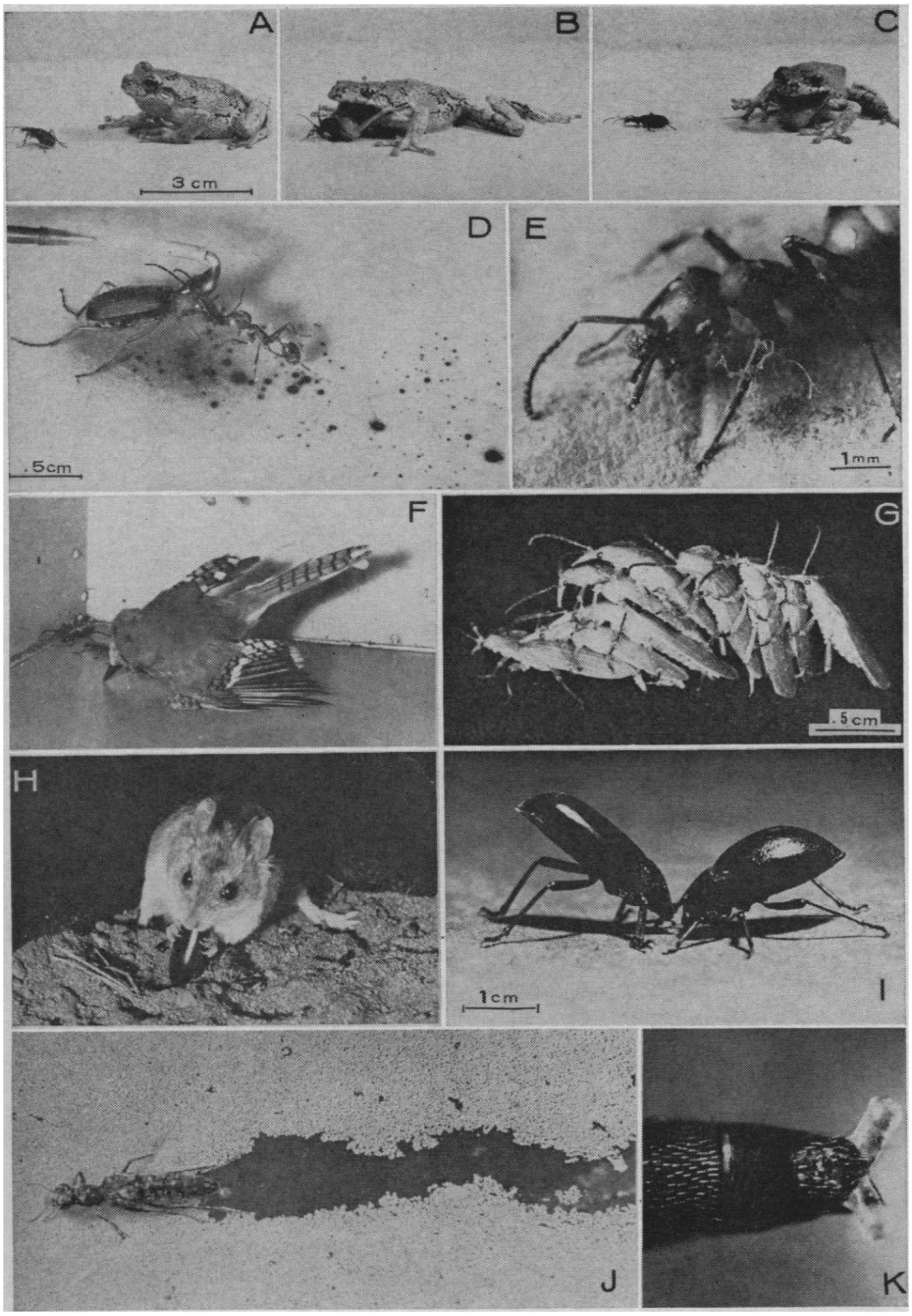


Fig. 6. (A–C) Toad (*Hyla versicolor*) being repelled by a bombardier beetle (*Brachinus* sp.). The toad (A) eyes the beetle, (B) strikes at it with its sticky tongue, and (C) rejects it. (D) Bombardier beetle (*B. ballistarius*), affixed to a rod, spraying in the direction of an attacking ant (*Pogonomyrmex badius*); the pattern of secretion is visible on a background of filter paper similar to that of Fig. 2H. (E) An ant (*Formica polyctena*), after having been contaminated with the sticky secretion of the millipede *Glomeris marginata* (see Fig. 2I); the tip of the left antenna is glued to the cranium; threads of dried secretion bind the left front leg to the head. (F) Blue jay (*Cyanocitta cristata*) at the moment of being sprayed by the stick insect *Anisomorpha buprestoides* (the prey is partly visible in the corner of the cage). (G) Group of caged male lycid beetles (*Lycus loripes*) drawn into a cluster by their own attractant pheromone. (H) Grasshopper mouse (*Onychomys torridus*) feeding on the beetle *Eleodes longicollis* (see also Figs. 1B, 2H); the beetle is held in such a way that its secretion, ejected from the tip of the abdomen, is discharged into the soil. (I) *Eleodes longicollis* (left) has defensive glands in the abdomen (see Figs. 1B, 2H) and performs a headstand when disturbed; its mimic, *Megasida obliterata* (right), also lifts its rear when annoyed, but lacks glands. (J) Staphylinid beetle (*Stenus* sp., about 5 millimeters long) propelling itself forward over water through touching the water surface with glands at the tip of its abdomen; the glands produce a secretion that depresses the surface tension of water; for experimental purposes the surface had been covered with *Lycopodium* powder; the clear trail behind the beetle marks the region of depressed tension where the powder has withdrawn. (K) Abdominal tip of *Stenus*, showing everted glands. [Photographs J and K, courtesy of K. E. Linsenmair (*66*)]

a rat opossum (*Marmosa demararae*) feeding on *Anisomorpha* (*13*). Other predators may attack their prey in such a way as to avoid exposure to the secretions. Grasshopper mice feed on certain beetles (*Eleodes, Chlaenius*) by holding them upright in their front paws, while jamming them butt-end down into the earth; the secretion, which is ejected from glands that open at the rear of the beetles' abdomen, is harmlessly expended in the soil (Fig. 6H) (*7, 19*). Toads, which characteristically catch their prey by a quick flip of their sticky tongue, may swallow millipedes (*Narceus*) or beetles (*Eleodes*) before the quinone-producing glands of the prey are discharged. The toads betray no immediate or long-range ill effects from secretion that might subsequently be ejected inside their stomach (*19, 52*). Bombardier beetles (*Brachinus*), which also produce quinones (*24*), avoid capture. They discharge the moment they are struck by the sensitive tongue of the toad, and are rejected instantly (Fig. 6, A–C).

The overall defensive effectiveness of a secretion may not be attributable solely to its chief constituents. Lesser components, even at concentrations of a few percent, may have important ancillary functions. The defensive spray of the whip scorpion, *Mastigoproctus giganteus*, consists of acetic acid (84 percent), water (11 percent), and caprylic acid (5 percent). Although the primary irritant in the mixture is acetic acid, caprylic acid adds to the effectiveness of the secretion as it is used against arthropod predators. The cuticular exoskeleton of terrestrial arthropods has a thin outermost layer, the epicuticle, which has the important function of waterproofing the cuticle, and which, by virtue of its high wax content, can act as a limiting barrier to the penetration of wax-insoluble compounds such as acetic acid. Caprylic acid is a wax solvent, and it apparently acts by disrupting the epicuticle, thereby facilitating the penetration of acetic acid. Caprylic acid also serves as a wetting agent that promotes the spread of the individual spray droplets over the cuticle of the enemy, thus increasing the area of contact of the poison. Actually, caprylic acid at a concentration of 5 percent can fulfill neither of these functions. However, the ejected droplets, during their aerial trajectory, undergo a differential evaporative loss of acetic acid, which insures that they arrive on target with an effective caprylic acid concentration of 10 to 20 percent (*20*). "Additives" other than caprylic acid are also known to occur. The aliphatic hydrocarbons in the secretion of Hemiptera promote not only the spreading and penetration of the mixture but also its seepage into the respiratory tracheae of insects (*14*).

The secretions may or may not have a noticeable effect on the arthropods that produce them (*22, 62*). In some cases there may be a high level of tolerance. The millipede *Apheloria corrugata*, which secretes a cyanogenic secretion (*47*), usually outlives other arthropods confined with it in a cyanide killing jar (*52*). Similarly, whip scorpions are apparently unaffected by the incidental dousing of acid spray that they inevitably receive when they eject their secretion (*20*). Whether insensitivity is attributable to an impervious integument or to the existence of special systemic detoxification mechanisms has not been determined. Some species can tolerate only limited contact with their own secretion, and they react, in much the same way that predators do, by cleansing sensitive regions (*17*). In certain Hemiptera the exoskeleton around the gland openings is elaborately sculptured to prevent the discharged secretion from spreading over the body (*14*).

## Other Functions of Defensive Secretions

Some secretions, although clearly defensive in function, may in addition serve as pheromones. The soldiers of the so-called nasute termites possess a pointed cephalic "nozzle" from which they eject a defensive spray. The secretion is used against insects or other small animals that venture into the termites' nest or foraging territory, and it has a rapid incapacitating effect. It also acts as an "alarm" substance, alerting additional soldiers to the state of emergency and inducing them to discharge spray toward the intruder (*61*). The ant *Acanthomyops claviger* produces a secretion in its mandibular glands that also serves both for defense and as an "alarm" pheromone (*63*).

Adults of the beetle *Lycus loripes*, an inedible, aposematically colored species, form dense and conspicuous aggregations on the plants upon which they mate and feed. The clustering behavior (Fig. 6G) is mediated by a volatile pheromone, produced by the males alone, capable of attracting both males and females. Aggregation in *Lycus* is more than just a means for bringing the sexes together. It is an important defensive behavior that enables the beetles to "pool" their aposematic resources and advertise themselves *en masse*, thereby more effectively warning visually oriented predators of their presence. The pheromone thus fulfills both a reproductive and a protective role (*64*).

Defensive secretions may also possess entirely different, nonpheromonal functions. Mention has already been made of the salivary secretion of *Platymeris*, which is sprayed in defense against predators, while also serving as a lethal offensive venom when injected into prey (*50*). Some defensive secretions have antimicrobial activity and may conceivably provide protection against both predators and microorganisms (*4, 36*). A mutant of the flour beetle *Tribolium confusum* has recently been discovered whose defensive glands contain far less quinone than those of the wild type. The nutrient medium in crowded populations of the mutant tends to become moldy, suggesting that the glands ordinarily serve to inhibit fungal and bacterial growth in the beetle's environment (*65*). A truly anomalous function is possessed by the eversible defensive glands in the abdominal tip of certain staphylinid beetles (*Stenus* spp.) (Fig. 6K). These small insects, which are normally terrestrial but may on occasion forage on water or be blown by wind onto water, rely on the surface-tension-depressant properties of their secretion to propel themselves across the surface of the liquid. By touching their everted glands to the water they weaken the surface tension behind them, and thus are carried forward by the "contracting" surface as it withdraws before them (Fig. 6J). The beetles employ this type of locomotion, appropriately called *Entspannungsschwimmen*, only when they are threatened by an emergency. Ordinarily they simply paddle along by walking (*66*).

## A Remarkable Parallel

Many plants are characteristically odorous, and their fragrance does not always originate from the flowers alone. Leaves, stems, and roots may also be scented, as is often especially apparent after they have been crushed. Although the attractant properties of floral perfumes have long been recognized, the adaptive significance of the odorous compounds of vegetative structures has remained a matter of some controversy. Organic chemists have long been interested in these natural products, many of which have been isolated and identified. Since the odorous principles appear not to play a major role in the fundamental biochemical processes of the living plant, they are usually classed—together with such other "irrelevant" compounds as saponins, alkaloids, and tannins—among the so-called "secondary" plant substances (*67*). An excellent catalog has recently appeared, in which these substances (except for alkaloids) are listed by chemical structure and source (*68*).

It is a striking fact that the active components in the defensive secretions of arthropods also occur, almost without exception, as secondary substances of plants. Some of the compounds, such as 2-hexenal, produced in the defensive spray of several insects (*4, 28*), and $\alpha$-pinene, discharged by certain termites (*69*), are extraordinarily widespread in plants (*68*). Others, such as isobutyric acid and 2-dodecenal, found in the secretion of a caterpillar (*10*) and a millepede (*45*), are more restricted in distribution (*68*). Sometimes the parallel even extends to the mechanism whereby the odorous principles are released. In cyanogenic plants, hydrogen cyanide is generated by hydrolysis of cyanohydrin glycosides (*70*). In the polydesmoid millipede *Apheloria*, cyanogenesis is effected by dissociation of mandelonitrile, the cyanohydrin of benzaldehyde (*47*).

The fact that the active principles in the defensive secretions of arthropods are effectively repellent to predators raises the possibility that these same principles, as secondary substances of plants, serve to protect plants against herbivores. This possibility has been advanced before, notably by Fraenkel (*67*). Irrespective of how one may feel about the hypothesis, it can lead to useful predictions. Among the more interesting compounds recently isolated from defensive glands of insects are certain cyclopentanoid monoterpenes, including iridodial and iridomyrmecin from ants, and dolichodial (= anisomorphal) from ants and a walkingstick (*30, 71*). Similar terpenes have been found in plants (*72*). One of these, nepetalactone or catnip, had long been of interest because of its peculiar excitatory effect on cats, a property that is surely irrelevant to the actual adaptive function of the terpene in the mint plant that produces it. Since the cyclopentanoid terpenes in the secretions of insects were known to be repellent to other insects, it seemed likely that nepetalactone might be similarly repugnatorial and that it might protect the mint plant against herbivorous insects. Actual tests showed nepetalactone to be an insect repellent of considerable effectiveness (*73*). Clearly, new insect repellents of potential practical significance may yet be discovered among the secondary substances of plants. Some of the oldest repellents known to man are, in fact, derived from plants. One of the most familiar, citronellal, is now known to be produced also in the glands of an ant (*63*).

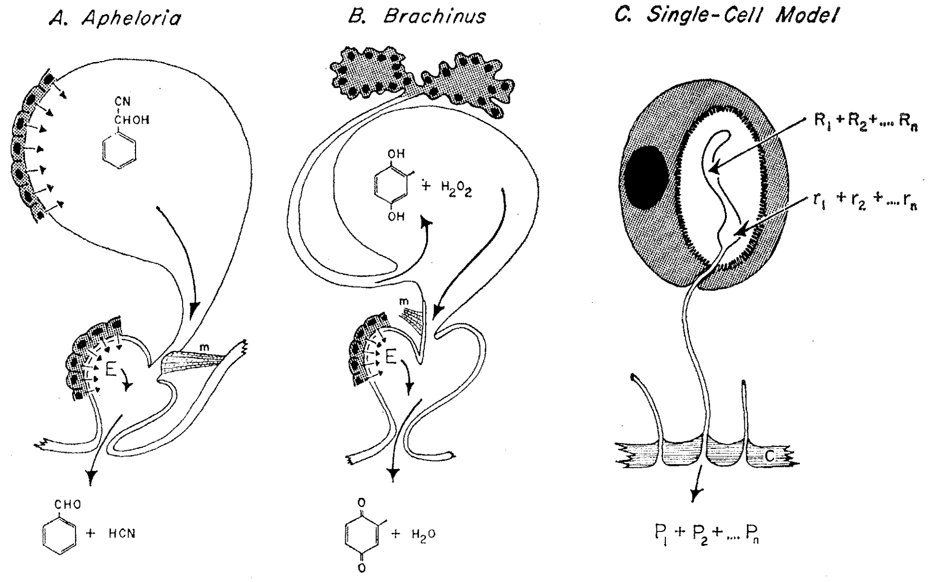


Fig. 7. (A–B) Diagrammatic representation of the known mechanism of operation of the defensive gland of a millipede, *Apheloria* [after Eisner *et al.* (*47*)], and a bombardier beetle, *Brachinus* [after Schildknecht and Holoubek (*24*)]. (C) Postulated mechanism of toxicant synthesis, such as may prevail in a single secretory cell; within the vesicle of the cell lies the hollow cuticular "reaction chamber," into which the cell passes sets of reactants (*R, r*) that interact to form the toxic products (*P*); a slender cuticular duct conveys the products from the reaction chamber to the wall (*C*) of the gland (see text).

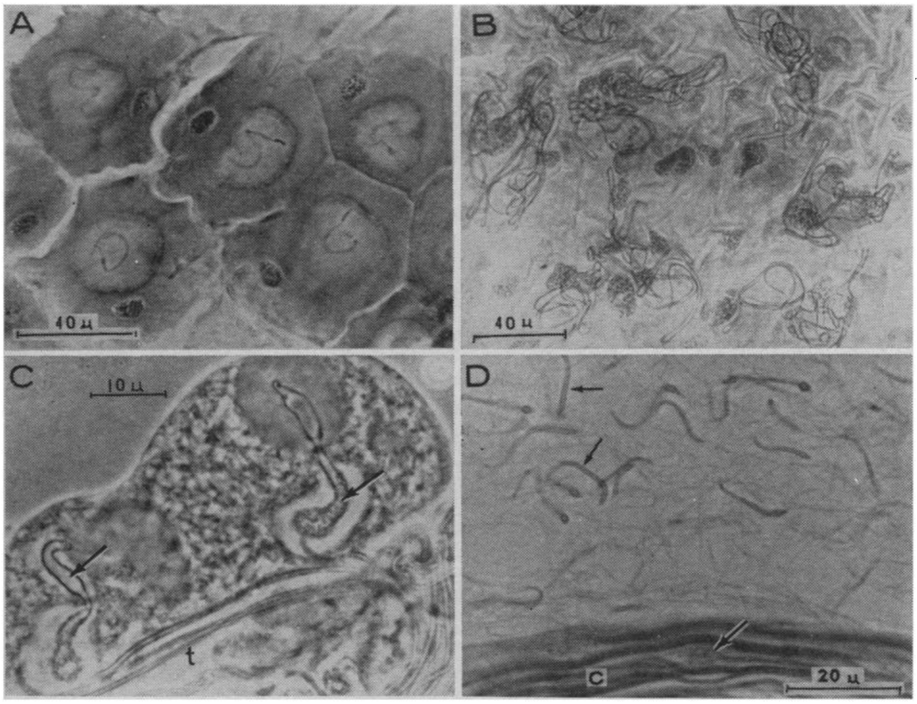


Fig. 8. Tubular cuticular organelles associated with secretory cells of defensive glands. (A) Secretory cells (from tissue $s_1$, Fig. 1D) of glands of *Eleodes* sp.; the cells have an eccentric nucleus, a large central vesicle, and within the vesicle a twisted cuticular organelle that, presumably, functions as a "reaction chamber" for synthesis of the poisons. (B) Surface view of a portion of the secretory epithelium of the quinone-producing gland of the cockroach *Deropeltis erythrocephala*; the long cuticular tubules lead from the cells to the lumen of the gland. (C) Cuticular "reaction chambers" (arrows) in secretory cells of the abdominal quinone-producing glands of the flour beetle *Tribolium castaneum;* the chambers are drained by cuticular tubules (*t*) that lead to the reservoir of the glands. (D) Isolated cuticular organelles from secretory cells of the quinone-producing tracheal gland of the cockroach *Diploptera punctata*; the organelles were isolated by dissolving all soft cellular components in hot aqueous potassium hydroxide; the "reaction chambers" (small arrows) are drained by long thread-like tubules that open (large arrow) on the cuticular wall (*c*) of the gland. [Photograph D, courtesy of D. W. Alsop]

## Biosynthesis of the Secretions

Most of the relatively meagre evidence available to date suggests that the active components in the defensive secretions of arthropods are synthesized by the animals themselves from readily available metabolic precursors. Thus, the ant *Acanthomyops*, which produces citral and citronellal, synthesizes these acyclic monoterpenes from acetate units, by way of mevalonate, according to the usual terpenoid biosynthetic pathway (*74*). The cyclopentanoid monoterpene anisomorphal, secreted by a stick insect (*71*), is also synthesized from acetate and mevalonate (*75*). The results of such biosynthetic studies are sometimes unexpected and of intrinsic biochemical interest. In the beetle *Eleodes longicollis*, which secrets three closely similar quinones—*p*-benzoquinone, toluquinone, and ethylquinone (*76*)—the three compounds are apparently produced by two different routes. Whereas *p*-benzoquinone is synthesized from preformed aromatic precursors (phenylalanine, tyrosine), the two alkylated quinones are synthesized from acetate (*77*).

Herbivorous insects might conceivably be expected to make use of secondary plant substances obtained in their diet by incorporating such substances unchanged into their secretion. This does not appear to occur in pentatomid Hemiptera, one of which (*Nezara viridula*) was shown to be capable of synthesizing virtually all its many secretory products from acetate (*78*). And it evidently does not take place in the caterpillar of *Papilio machaon*, whose secretion had been thought to contain only the essential oils of the caterpillar's food plant but in fact contains two acids (2-methylbutyric and isobutyric acid) that are apparently absent from the umbelliferous species on which the caterpillar feeds (*10*). However, in the grasshopper *Poekilocerus bufonius*, the digitalis-like material isolated from its secretion (*49*) may stem from the diet. The insect feeds on Asclepiadaceae (milkweeds), which are known to be rich in cardiac glycosides (*79*). In view of the limited ability of insects to synthesize steroids from nonsteroidal precursors (*80*), this particular instance of a possible utilization by one of them of a preformed stereoidal compound of plant origin takes on special significance.

The active principles of defensive secretions are, for the most part, relatively small, highly reactive molecules potentially capable of interfering with any number of vital chemical processes. It is therefore a matter of some interest that there should exist animals capable of producing such general toxicants, let alone capable of storing them at high concentrations. How do living cells make such poisons without poisoning themselves? There is as yet no final answer to this question, although suggestive clues have emerged from work done recently on millipedes and bombardier beetles. Paradoxical as it may seem, the gland cells responsible for the production of the poisons may themselves never really be exposed to them.

In the millipede *Apheloria*, which produces a cyanogenic secretion (*8, 47*), each gland is a two-compartmented organ (Fig. 7A). In the larger compartment is stored a compound, mandelonitrile, that consists of a stable combination of benzaldehyde and hydrogen cyanide; the smaller compartment contains a factor E (presumably an enzyme), capable of catalyzing the dissociation of mandelonitrile. A tight valve ordinarily blocks the tubular connection between the compartments, but at the moment of discharge—which occurs by compression of the larger compartment—the valve is opened by a special muscle, *m*, and the contents of the two compartments are mixed and ejected through the single outer orifice of the gland. Hydrogen cyanide emanates as a gas from the discharged mixture (*8, 47*). Aside from the intrinsic elegance of the mechanism, the point worth noting is that the final step in the production of the poison occurs, not inside the living gland cells, but outside them.

A remarkably similar mechanism occurs in bombardier beetles (*Brachinus* spp.), which eject a secretion containing quinones. The discharge is accompanied by an audible detonation, and the spray is hot to the touch. In *Brachinus*, each gland is also two-chambered (Fig. 7B). The large inner chamber is the source of phenolic precursors of the quinones, and of hydrogen peroxide, while the smaller, outer chamber produces the enzyme catalase (*E*). When the contents of the two chambers are mixed, catalase promotes the liberation of oxygen from hydrogen peroxide and the phenols are oxidized to quinones in an exergonic reaction. Under pressure of the liberated oxygen, the hot mixture suddenly "pops out" (*24*). To all intents and purposes this is an explosive mechanism. Unique in its own way, it resembles the mechanism in *Apheloria* in that the toxic end products are synthesized outside rather than inside the cells.

The majority of defensive glands differ from those of bombardier beetles and cyanogenic millipedes in that they consist of single instead of double compartments and the poisons are stored within them as finished products rather than as precursors. It is surprising in itself that the animals should be able to store the concentrated poisons without detriment to themselves, but the lumen of the glands is always lined with a cuticular membrane (*4*), which apparently acts as an effective insulating barrier (*14*). However, this does not explain how the individual secretory cells—which evidently must manufacture the finished toxins before pouring them into the lumen of the gland—withstand exposure to the poisons. The answer may eventually be found in the function of certain peculiar tubular organelles with which these cells are characteristically endowed (Fig. 8) (*22, 23, 81*). Cuticular in nature, the tubules are essentially links between the cells and the wall of the gland and, offhand, appear to be more than efferent ducts that drain the cells of their secretion. However, it is conceivable that the tubules fulfill a more specialized role, and that their lumen is actually the site where the final synthesis of the poisons takes place. At the free end of the tubule there is frequently to be found an expanded (Fig. 8D), sometimes elaborately specialized (Fig. 8C), bulbar portion, and this might possibly function as a "reaction chamber." One might imagine a mechanism whereby the cell manufactures a series of harmless precursors, which are prevented from interacting to form the final poisons until they are passed into the lumen of the organelle. The cuticular wall of the organelle could be of such nature as to permit the inward passage of the precursors, but not the outward diffusion of the poisons. Trapped within the organelle, the poisons would then be channeled along the tubule to the lumen of the gland (Fig. 7C). If this mechanism is correct —and the evidence for it is only circumstantial (*5*)—then it would be essentially similar to that occurring in bombardier beetles and cyanogenic millipedes. The difference is that, whereas in the latter animals the "reaction chamber" is the impervious two-compartmented lumen of the gland itself, in the other forms the reaction chamber consists of hundreds of tiny individual units, distributed singly among the secretory cells. It may be possible, by histochemical techniques, to reconstruct the sequence of biosynthetic events taking place in the cells and their cuticular organelles, and some efforts in this direction have already been made (*22, 23*).

## Nonexocrine Chemical Defenses

Most of what we know about the chemical defenses of arthropods stems from studies of such integumental glands as are discussed here. But arthropods also possess repellent substances in the blood (*82*), and possibly in other parts of their bodies, and only a very few of these systemic factors have been investigated to date. Although such factors are likely to be more difficult to extract and isolate than exocrine products, the efforts should be well worth while. Substances such as cantharidin and pederin, present in the body fluids of meloid and some staphylinid beetles, are not only unusual chemically but also of some medical and pharmacological significance (*83*). The compounds responsible for distastefulness in the many insects that figure as "Müllerian" elements of mimetic complexes would appear to be of particular interest. The pharmacological properties of a genin-like substance in the Monarch butterfly has recently been investigated (*84*), as well as cyanogenesis in the tissues of a moth (*85*), but literally hundreds of other species remain to be studied, including many butterflies, moths, and beetles. Interesting ecological and evolutionary publications have appeared recently that are likely to provide fruitful leads for experimental work in this general area (*86*).

There is also a good deal yet to be learned about the use that insects may make of secondary plant substances in their diet. Although, as pointed out above, such substances may not necessarily be appropriated intact for incorporation into defensive secretions, they may exert their protective action in other ways. When an insect feeds on a cyanogenic plant, the very act of chewing is likely to trigger cyanide emission at the sites of injury. Might the insect derive some protection from the emanating vapors, perhaps against small predators such as ants? The digestive tract of herbivorous insects often bulges with contained plant matter. Is it possible that intact secondary substances remaining in the gut act to deter appropriately sensitive predators? Many insects respond to disturbance by regurgitating some of their fluid intestinal contents. Is the regurgitate repellent to enemies? Is repellency attributable to factors in the diet or to endogenous substances supplied by the insect itself?

Evidently, the subject of chemical defenses, even as it pertains to the arthropods alone, is still far from exhausted. Like so many other areas of research concerned with the chemical interactions of organisms, it is likely to blossom for some years to come, if for no other reason than that chemists and biologists are now jointly involved in its exploration.

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