samples were protected from change in composition by sealing in airtight containers and by freezing between the times of collection and analysis.

The samples, subjected to appropriate cleaning, were analyzed for endrin by gas-liquid chromatography, with an electron-capture detector (Table 1). Homogenates of fish were subjected to hydrolysis by alcoholic potassium hydroxide and were extracted several times with normal hexane. Samples of shrimp and oyster were homogenized, extracted with a mixture of hexane and isopropanol (3 : 1), and filtered through glass wool. Samples of mud and water were extracted with a mixture of ether and isopropanol 2 : 1.

The following procedure was used with all samples. Extraction solvents were freed of alcohol (and of alkali, when present) by repeated washings with water. Drying with sodium sulfate, concentration, and liquid-phase chromatographic cleanup through a magnesium oxide–Celite No. 545 column preceded quantification. The appropriate portion of the eluate was analyzed by gas-liquid partition chromatography, with an electron-capture detector. Quantification was determined from calibration curves prepared by analysis of fortified endrin solutions in hexane. Analyses were repeatedly verified by recovery experiments; recovery averaged 85 percent.

The lower limit of confident analysis is estimated to be 0.005 parts per million; this is the lowest analytical value that is considered discernible from the analytical response of interfering substances found occurring naturally in the samples analyzed. Analyses yielding less than 0.005 ppm are reported as negative. Three readings per sample per month were usually taken. Oysters and shrimp were negative throughout. Catfish yielded 0.01, 0.02, and 0.01 ppm of endrin in July 1964; and one reading of 0.01 ppm in each of August and October 1964 and June 1965. Bream yielded one reading of 0.01 ppm in each of July and October 1964 and February 1965. Mud and water were negative throughout apart from two readings of 0.01 ppm in July 1964 and one of 0.01 ppm in each of February and June 1965.

The data show neither a high level of endrin nor a time-ordered change in the endrin level. The general absence of endrin from the samples indicates that there is no significant contamination of the environment by the pesticide, but the possibility of localized and sporadic occurrence of residues is not ruled out. We also conclude that the portion of human food supply represented by the organisms studied is not contaminated by endrin.

Table 1. Conditions for gas-chromatographic analysis for endrin with a Micro-Tek model 2000 R.

Item	Condition
Column	
Substrate	5 per cent silicone D-11
Support	Chromosorb W, 60–80 mesh
Length, shape	91 cm, coiled helix
Bore	6 mm, Pyrex
Carrier gas	Methane-argon, 5:95
Pressure	2.7 atm (g)
Flow rate	142 cm^3/min
Operating temp.	200°C, column; 210°C, flash heater; 205°C, detector
Output polarity	Negative
Isothermal	Negative
Output attenuator	8–16
Input attenuator	1
Chart speed	1.2 cm/min
Detector	Tritium Electron affinity

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Defensive Secretion of a Caterpillar (Papilio)

Abstract. *The eversible cervical gland (osmeterium) of the caterpillar of the swallowtail butterfly,* Papilio machaon, *produces a secretion containing isobutyric and 2-methylbutyric acid. The gland is effective in defense against ants. Even when caterpillars were fed on one of three unbelliferous plants (fennel, carrot, parsnip) the secretion contained the same two acids.*

The caterpillars of butterflies of the family Papilionidae (swallowtails and parnassians) possess a remarkable defensive gland, the osmeterium, situated middorsally just behind the head. Consisting essentially of a two-pronged invagination of the neck membrane, the gland is ordinarily tucked away invisibly beneath the integument, but it is forcibly everted when the animal is disturbed. A characteristic intense odor invariably emanates from the two extruded "horns," which glisten with a coating of secretion. When the disturbance subsides, the horns are retracted. The gross morphology of the osmeterium and the cytology of the secretory cells associated with it have been described (*1, 2*). The mechanism of operation of the gland can be inferred from its structure—the horns are evaginated by blood pressure and withdrawn by special retractor muscles. We undertook this study, on the larva of the European swallowtail, *Papilio machaon,* because very little was known about the protective effect of the osmeterium, and the active principles of the secretion had never been identified.

To observe in detail the behavior of the larva when it brings the osmeterium into action, we subjected individual caterpillars to simulated attack by prodding, tapping, or pinching them. As a first response to such disturbance, a larva usually assumes a characteristic "threat posture," in which it suddenly thrusts its front end into an uplifted position. Poised in this fashion, it is prone to extrude the osmeterium (Fig. 1*B*), and it does so inevitably, if not at the very onset of the "attack," then certainly after sustained or repeated stimulation. Whether the horns are partially or completely everted depends on the intensity of the stimulus. Mild disturbance, such as poking the animal gently with a blunt probe, may elicit no more than incipient evagination. More complete (Fig. 1*B*) or even total eversion occurs only when considerable trauma is induced, as it is when the body is pinched with forceps. While everting the gland, the larva arches its front end toward the region traumatized, and may then wipe its horns against the instrument used for stimulation (Fig. 1*C*). The two horns are not necessarily equally protruded.

If a stimulus is applied to only one side of the body, then the particular horn of that side invariably everts further than its partner (Fig. 1*C*). As a rule, matched eversion occurs only in response to generalized trauma (as, for example, when the animal is roughly handled, which causes total eversion of both horns) or when a more restricted but balanced bilateral stimulus is applied (Fig. 1*B*). These findings apply to larvae of all instars.

The protective effect of the osmeterium became apparent when individual caterpillars were placed near the entrance to the nest of a laboratory colony of the ant *Formica polyctena*. As long as the ants attacked singly or in small numbers, the larvae had no difficulty in repelling them. In response to an ant's bite, a larva would instantly revolve its front end so as to wipe the extruded osmeterium against the assailant (Fig. 1*D*), and the ant, visibly contaminated with secretion, would promptly flee, pausing occasionally along the way to cleanse itself vigorously (*3*). Eventually, after the larva had apparently exhausted its supply of secretion, the ants converged upon it in swarms, doused it with their own spray which contains formic acid, killed it, and dragged it into the nest. Although scarcity of larvae precluded experimentation with other predators, it seems unlikely that the secretion is repellent to ants alone. The suggestion (*1*, *2*) that the gland is not primarily a defensive device is unwarranted. Whereas it is true that certain lizards and rats have been seen to take papilionid larvae into captivity (*1*, *4*), no critical experimentation with vertebrates has been done to determine whether long-term experience with the larvae would eventually lead predators to discriminate against them. Experiments with birds should be fruitful. It is also conceivable that the osmeterium offers protection against some entomophagous parasites, although it certainly does not do so against all of them (*1*). Statements to the effect that the osmeterium serves for thermoregulation (*2*) or excretion (*1*, *4*) are purely speculative.

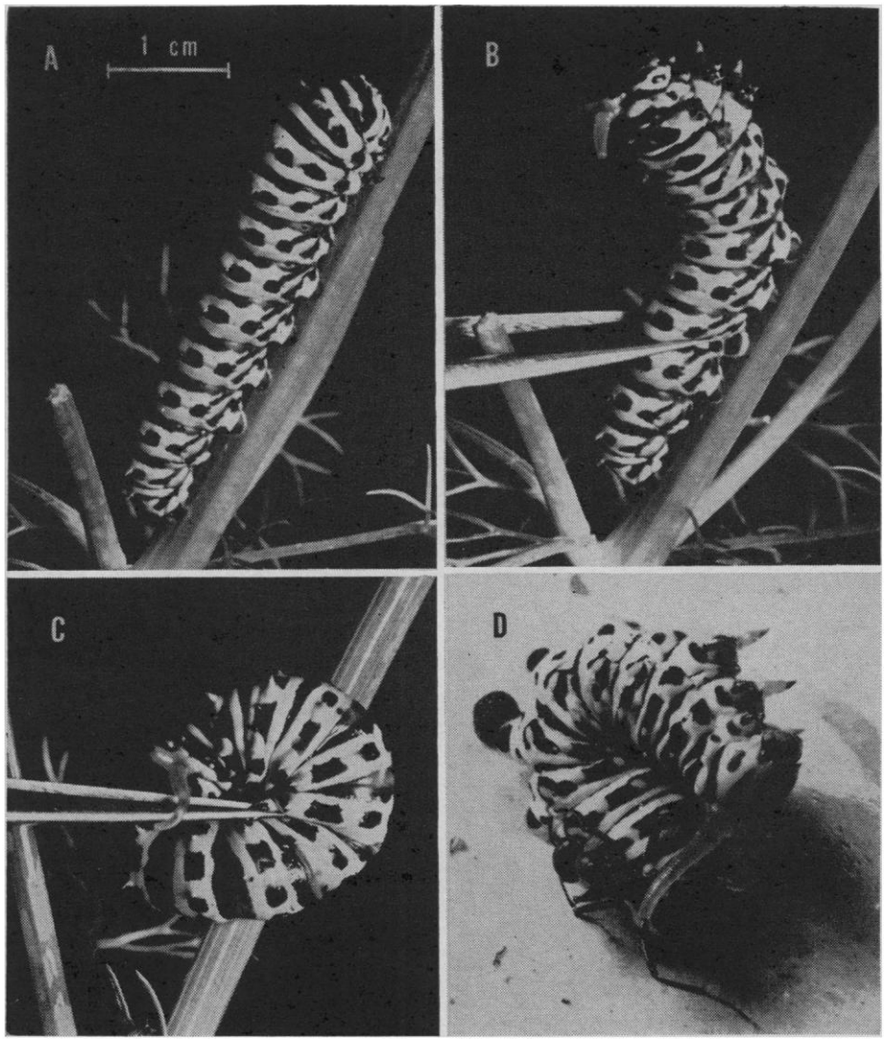
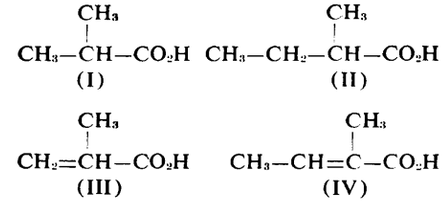


Fig. 1. (*A*) Larva of *Papilio machaon* at rest on food plant. (*B*) Same, "rearing up" and partially everting its osmeterium in response to mild pinching with forceps; note that the stimulus is bilaterally applied and that the two "horns" are about equally extruded. (*C*) Larva, being pinched with forceps, wiping its osmeterium against the instrument; note that the "horn" of the side stimulated is more fully extruded. (*D*) An ant (*Formica polyctena*), having just bitten the larva's rear, flees as the osmeterium is being brushed against it.

A supply of the secretion was obtained for chemical analyses from a group of 30 larvae reared on fennel plants (fam. Umbelliferae; *Foeniculum vulgare*) and "milked" by swabbing their extruded horns with small discs of filter paper. The discs were extracted with methylene chloride, and the solution was dried by filtration through anhydrous magnesium sulfate. An infrared spectrum of the filtrate was indicative of a carboxylic acid group (broad hydroxylic absorption at 3 to 4 μ and carbonyl absorption at 5.88 and 5.75 μ). Extraction with 10 percent aqueous sodium bicarbonate, followed by acidification and reextraction of the basic solution, gave an acid fraction whose infrared spectrum was identical with that of the original secretion. No neutral product was obtained.



Since the acidic fraction was not amenable to thin-layer chromatography or gas-liquid chromatography, it was treated directly with ethereal diazomethane to form the corresponding methyl esters. The ethereal solution of methyl esters was subjected to gas-liquid chromatography (5 percent SE30 column, 50°C). Two major peaks were apparent, with retention times of 2.0 and 3.5 minutes, respectively, indicative of esters of low molecular weight.

Infrared spectra of a large number

Table 1. Chromatographic retention times and boiling points of various methyl esters of C_3 to C_5 carboxylic acids. A 5-percent SE-30 column at 50°C was used. All boiling points are at atmospheric pressure except that marked with an asterisk (13 mm-Hg).

Methyl ester	Boiling point (°C)	Retention time (min)
Propionic	79.7	1.4
Butyric	102	2.6
Isobutyric	93	2.0
Cyclopropane carboxylic	119	3.5
n-Valeric	127.3	5.0
Isovaleric	116	3.5
2-Methylbutyric	113*	3.5
Pivalic	100–102	2.4
Cyclobutane carboxylic	136	7.1

of authentic C_3 to C_6 fatty acids were examined. The spectrum of the secretion was closely similar to the spectra of the simple saturated acids, but differed distinctly from those of the unsaturated or cyclic acids. Authentic samples of methyl esters of every possible C_3 to C_5 saturated acid were prepared and subjected to direct comparison by gas-liquid chromatography with the esters from the caterpillar secretion (Table 1). From these comparisons it is clear that one of the acids in the secretion (retention time, $t = 2.0$ min) is isobutyric acid (I). The other acid ($t = 3.5$ min) could be cyclopropane carboxylic acid, isovaleric acid, or 2-methylbutyric acid. The cyclopropane structure was excluded by direct infrared comparison. The distinction between the remaining two possibilities was made by gas-liquid chromatography on a column of 15-percent Apiezon grease-L at 100°C, which separates methyl 2-methylbutyrate ($t = 7.5$ min) from methyl isovalerate ($t = 8.0$ min). On this column, the esterified secretion showed two peaks at 4.0 minutes (methyl-isobutyrate) and 7.5 minutes (methyl 2-methylbutyrate). The second acid in the secretion is evidently 2-methylbutyric acid (II).

Additional milkings were made from larvae that had been reared on two other umbelliferous plants, carrot (*Daucus carota*) and parsnip (*Pastinaca sativa*). In both cases the principal components of the secretion were again isobutyric acid and 2-methylbutyric acid, although the same chromatographic method showed the presence of additional minor components that were not pursued further. Evidently, the nature of the food plant does not grossly affect the constitution of the mixture. It might be added that neither of the two acids figures among the many compounds that have been isolated from fennel, carrot, or parsnip (*5*). There is certainly no reason to presume that the larvae need rely on any but readily available metabolic precursors for the synthesis of these simple aliphatic acids. The suggestion (*4*) that the gland provides a vehicle for the elimination of essential oils obtained with the diet is evidently contraindicated, at least for *Papilio machaon*.

The relative proportion of the acids in the secretion was estimated. This was done by comparison with gas-liquid chromatograms of known mixtures of the authentic methyl esters. Caterpillars fed on carrot and fennel produced isobutyric and 2-methylbutyric acid in the ratio of 70:30. For those fed on parsnip the ratio was 52:48.

Although neither of the acids produced by *Papilio* is known to occur in the secretions of other arthropods, it is interesting that certain beetles (Carabidae) (*6*) should produce a defensive spray containing methacrylic (III) and tiglic acid (IV), the unsaturated analogues of isobutyric and 2-methylbutyric acid. Additional acids secreted by arthropods include formic, acetic, and caprylic (*7*). Certain caterpillars of the moth family Notodontidae discharge a spray containing formic acide (*7, 8*). Their gland is entirely different from the osmeterium and is clearly no homologous to it.

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Lactate Dehydrogenase Isozyme Patterns of Human Platelets and Bovine Lens Fibers

Abstract. *Human platelets and bovine lens fibers contain lactate dehydrogenase-3 (LDH-3) as the predominant isozyme but only very faint traces of LDH-5. Since the platelets and lens fibers, like mature human erythrocytes, lack a nucleus, the results strengthen the case for a previously developed association between LDH-5 and the cell nucleus. These three cell types are mainly anaerobic, and therefore their isozyme patterns are incompatible with the theory that anaerobic tissues exhibit predominantly LDH-5 and aerobic tissues mainly LDH-1.*

The lactate dehydrogenase (LDH) isozyme pattern of platelets and bovine lens fibers is of interest in light of a previously developed association between LDH-5 and the cell nucleus or direct nuclear products such as RNA (*1*). Most tissues exhibit varying proportions of at least five LDH isozymes (*2*). The mature human erythrocyte, devoid of a nucleus, displays mainly LDH-1 and -2 with small amounts of LDH-3 and -4 (*3*). By use of a sucrose-saline lysing solution traces of LDH-5 have been identified on starch-gel electrophoresis (*4*). The association between LDH-5 and the nucleus was also suggested by a comparative study of LDH isozymes in erythrocytes from various classes of vertebrates in which only species with circulating nucleated erythrocytes revealed cathodal isozymes (*3*). Furthermore, LDH-5 appeared in hemolyzates from anemic patients with reticulocytosis and from guinea pigs developing reticulocytosis after administration of phenylhydrazine (*1*). Finally, the isozyme pattern of nuclei purified from duck erythrocytes showed predominantly LDH-5 whereas the cytoplasmic pattern exhibited mainly LDH-1, -2, and -3 (*1*). Since the human platelet and the bovine lens fiber also lack a nucleus, they provide a unique opportunity to investigate further this association between LDH-5 and the cell nucleus or direct nuclear products.

Platelets were prepared from each of six normal individuals. Citrated blood (20 ml) was centrifuged for 5 minutes at 600*g*, and the supernatant of platelet-rich plasma was centrifuged at 1200*g* for 20 minutes. The sedi-