Table 1. Interconversion of *cis-trans* isomers of vitamin K_1.

Quinone	Amount of quinone added (μmole)		Added quinone recovered (μmole)		Newly formed quinone recovered* (μmole)	
	Cis	*Trans*	*Cis*	*Trans*	*Cis*	*Trans*
Synthetic† K_1	4.0‡	36.7‡	4.8	22.6		
Trans K_1	0	11.0		8.7	0.31	
Cis K_1	11.0	0	5.8			0.3

* These values represent the quantity of the purified isomer former from either *cis* or *trans* K_1 which was not present in the initial starting material. † Four identical flasks, each containing 5 mg of synthetic K_1, were incubated separately, and the acetone extracts were combined prior to purification. ‡ The amount of each of the geometric isomers present in the synthetic naphthoquinone was obtained spectrophotometrically after chromatographic resolution. The synthetic vitamin K_1 was incubated without prior separation into isomers.

tween the two isomers is not completely understood in terms of their possible participation in oxidative phosphorylation, the fact that incubation of the pure *trans* isomer resulted in the production of the *cis* isomer provided a clue (Table 1). The system consisted of 0.5 ml of washed irradiated particles (30 to 40 mg of protein per milliliter), 5 mg of *cis*, *trans*, or synthetic vitamin K_1 (10 ml/ml suspended by sonic oscillation in the presence of dialyzed irradiated supernatant), 0.2 ml of untreated supernatant (15 to 10 mg of protein per milliliter), 1.5 μmole of nicotinamide-adenine dinucleotide, 40 μmole of β-hydroxybutyrate, 20 μmole of inorganic phosphate, 10 μmole of adenosine diphosphate, and 30 μmole of $MgCl_2$. The reaction was terminated by the addition of 10 ml of cold acetone. The precipitated protein was separated from the organic phase and reextracted with a further 10 ml of acetone. The acetone extracts were combined and mixed with 20 ml of diethyl ether and then with 30 ml of water. The ether layer containing the quinone was washed three times with water, dried over sodium sulfate, and evaporated to dryness under nitrogen. The lipid extract was then fractionated by thin-layer chromatography as previously described and eluted from the silica gel. The amount of quinone was measured spectrophotometrically. In addition, it was observed that the ratio of *trans* to *cis* decreased after incubation of the unresolved synthetic mixture and that the amount of *cis* K_1 recovered in this experiment was actually higher than in the starting material, an indication of the possible formation of the *cis* isomer from the *trans* quinone. Nevertheless, a precise quantitative estimation of this conversion could not be made due to the variation in the recovery of quinone which was observed with different preparations. The enzymatic interconversion of the *cis* and *trans* isomers of vitamin K_1 was also observed with the resolved isomers as starting material. Even though the *cis* isomer was relatively inactive in terms of its ability to restore oxidative phosphorylation (Fig. 3), *trans* vitamin K_1 was produced from the *cis* isomer when the latter was added to an irradiated particulate preparation. The interconversion of the isomers was not observed with zero time controls.

Although a structural and stereospecific requirement for restoration of oxidative phosphorylation in quinone-depleted *M. phlei* preparations was demonstrated with vitamin K_1, this synthetic quinone differs from the natural quinone in this microorganism. Nevertheless, the ability of vitamin K_1 to restore oxidative phosphorylation and the interconversion of the isomers suggest that the natural quinone in *M. phlei* may also be present as *cis* and *trans* isomers. Dunphy *et al.* (*13*) demonstrated the existence of two isomers of the natural naphthoquinone vitamin K_9 (II-H) in *M. phlei* (Fig. 1). The two isomers were shown to be of identical molecular weight by mass spectroscopy, and the identification of the two isomers as *cis* and *trans* was confirmed by NMR analysis. The requirements for restoration of oxidative phosphorylation have been shown to be stereospecific and dependent on *trans* vitamin K_1. Nevertheless, *cis* vitamin K_1 is enzymatically formed from the *trans* isomer. Preliminary studies have shown that the conversion of *trans* vitamin K_1 to the *cis* isomer requires conditions of oxidative phosphorylation, whereas the conversion of the *cis* isomer to *trans* may be independent of this process.

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22 June 1967; revised 30 October 1967

Defensive Use of a "Fecal Shield" by a Beetle Larva

Abstract. *The larva of* Cassida rubiginosa *carries a tight packet of cast skins and feces on a fork held over its back. The packet is a maneuverable shield used by the larva to protect itself against attack. It is highly effective in blocking the bite of ants.*

The larva of *Cassida rubiginosa*, a beetle of the family Chrysomelidae widely distributed through the Northern Hemisphere, has long been known to naturalists for its odd habit—shared with other members of the genus—of carrying a trash packet above its back (*1*) (Fig. 1A).

The packet, which is held by a two-pronged fork that projects forward from the tip of the abdomen (see Fig. 1C), consists of the molted skins previously shed by the larva, together with the dried remains of its accumulated fecal wastes. The mechanism whereby the cast skins are retained on the fork, and the procedure by which the fecal droplets are discharged onto the packet by the protrusible anal turret (Fig. 1B), have been described (*2*). The function of this "stercoraceous parasol" (*3*)—or "fecal shield" as we shall call it—has remained a matter of dispute. It has been said to serve for camou-

flage, for protection against desiccation or rain, and for defense (*1*, references in *2*). We here present evidence in support of its defensive role.

The first clue to the significance of the shield stemmed from the observation that it is a highly maneuverable device, capable of being oriented by the larva in virtually any direction through flexion and rotation of the abdomen. That this maneuverability is specifically adapted for defense became apparent when the body of the larva was subjected to localized stimulation by prodding with a small blunt probe or by pinching with fine forceps. No matter where the stimulus was applied, the larva responded by pressing the shield against the offending instrument. Stimulation at or near the head caused the larva to uplift its rear and to bring the shield forward and downward over the cephalic region (Fig. 1H). Stimuli applied to the flanks brought about a sharp tilting of the shield toward the affected side (Fig. 1G). Disturbance at the posterior end caused erection (Fig. 1F) and partial backward deflection of the shield. The responses were immediate. Stimuli that were applied consecutively at different sites caused the shield to be shifted quickly from one appropriate defensive posture to the next. After cessation of stimulation, the shield was always returned promptly to its resting position above the body.

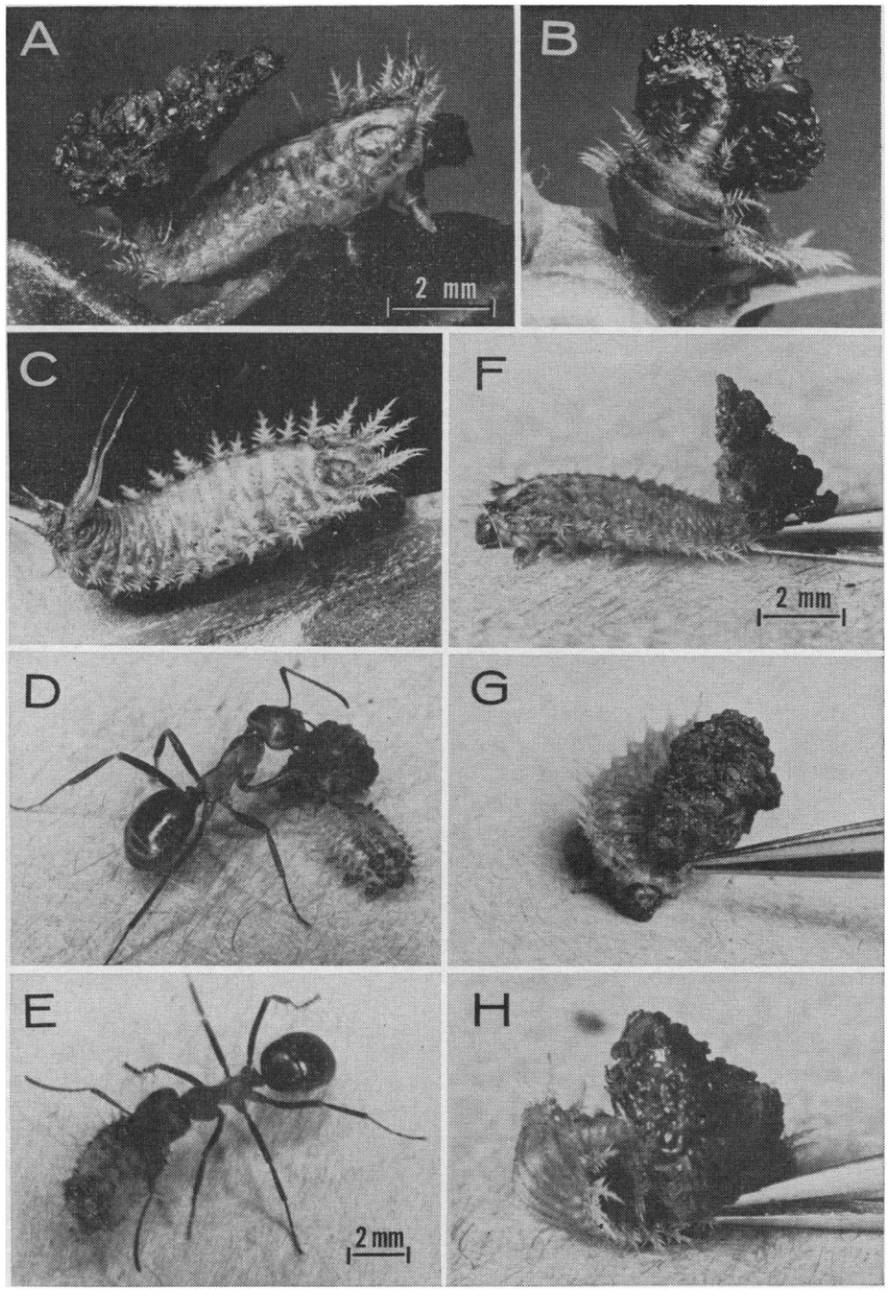

Fig. 1. (A) Larva of *Cassida rubiginosa* on its food plant (*Cirsium arvense*). (B) Rear view of larva; note the glistening fecal droplet freshly deposited on the shield by the extended anal turret. (C) Shieldless larva, showing fork that ordinarily carries the shield. (D) An attacking ant biting into the shield of a young larva. (E) An ant carrying in its mandibles a larva that has been rendered defenseless by deprivation of its shield. (F–H) Larvae responding to localized pinching with forceps by tilting their shield toward the site stimulated.

Projecting outward from the flattened body of the larva is a marginal fringe of long branched spines (Fig. 1C). These are particularly sensitive to contact stimulation and are evidently adapted to alert the larva to the initial probings of a predator. Stimulation of a single spine, and sometimes even the mere prodding of one of its branches, elicited an immediate defensive positioning of the fecal shield. The larva also responds, although much less readily, to stimulation of the shield itself. Mere contact with the shield had no effect, but persistent tapping or prodding eventually did result in its being deflected toward the stimulus.

To determine how effectively the shield is employed in defense against actual predators, we exposed individual larvae to attack by ants. These insects are undoubtedly among the chief natural enemies of *Cassida* and were always seen foraging in large numbers in the dense herbage that included the beetle's host plant (*Cirsium arvense*). For convenience, the attacks were staged in the laboratory, on a platform that served as a foraging arena for a thriving colony of the ant *Formica exsectoides*. The results were clear-cut. The moment an ant encountered a larva, it paused and began to "inspect" its find. The larva showed no initial responses and simply remained motionless while the ant stroked it with its forelegs and antennae. But then, as the ant became more persistent, and as it bore down with gaping jaws upon some of the sensitive spines of the larva, the latter abruptly mobilized its shield. Tilting it over the accosted region when the attack had taken place from in front or from the sides, or flipping it backward against the ant when the attack occurred from the rear, the larva usually took its action before the ant had secured a hold with its mandibles. Confronted with the inanimate shield rather than with the body of the larva,

the ant was quick to lose interest in its quarry. It paused momentarily to palpate the shield and sometimes even to bite into it (Fig. 1D), but eventually the ant always abandoned the attack and walked away.

In only two of the several dozen encounters witnessed did an ant succeed in biting a larva before the shield was mobilized against it. In one of these cases the larva was fatally injured, but in the other the ant held on only momentarily. Prodded by the shield, it relinquished its hold, briefly shifted its attention to the shield itself, and departed.

The presence of fresh wet feces near their site of deposition at the base of the shield (Fig. 1B) adds to the defense potential of the weapon. Ants that made casual contact with this pasty material when they first encountered the larva, or those that had it smeared upon them by the activated shield, usually fled promptly. During their escape they often paused to clean appendages or to drag their contaminated mouthparts against the substrate. Ants are known to react in this fashion to chemical repellents (*4*).

Larvae that had been artificially deprived of their shield were highly vulnerable. They attempted to defend themselves by maneuvering the naked fork, but they were nevertheless bitten and killed, or carried away live into the ant's nest (Fig. 1E).

Judging from its effect on ants, one might expect the shield to be deterrent also to other small predators. Yet it does not protect against all of them. Tests with an unidentified lycosid spider invariably resulted in the larva's being crushed by the chelicerae and sucked dry. Vulnerability to spiders had already been reported for the larva of another species of *Cassida* (*2*). It has been suggested, with some evidence (*5*), that the shield also protects against entomophagous parasites. But effectiveness is here again not absolute. Our own populations of *Cassida* were heavily parasitized by a chalcidoid wasp (*Tetrastichus* sp.).

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1 September 1967

Fast Potential Spike of Frog Skin Generated at the Outer Surface of the Epithelium

Abstract. *Experiments were performed to test which structure of frog skin epithelium is responsible for the electrical excitation (fast, all-or-none potential spike) displayed by this tissue during stimulation with a current pulse. Preselected cells of the outer epithelial surface were punctured by microelectrodes under microscopical observation. The major part of the transepithelia resting resistance and the major part of the spike were recorded between microelectrode tip and outside bathing solution. A leak between microelectrode and punctured membrane is made responsible for the attenuation of spike amplitude observed under these recording conditions. It is shown that if the spike is generated at but one of the series membranes of the epithelium, this membrane must be at the outermost border of the tissue.*

During the passage of constant inward current the transepithelial electrical potential of frog skin develops a spike-shaped all-or-none transient with a duration of about 10 msec and an amplitude of 200 mv. Its rising phase is characterized by an impedance increase, while its falling phase shows an impedance drop followed by a refractory state of depressed d-c resistance lasting up to 1 minute (*1*). Presently, we prefer to use the merely descriptive term "potential spike" for this phenomenon (*2*). Requisites for the spike are: (i) the presence of Li^+ or Na^+ in the solution bathing the outside of the skin, and (ii) an inward current density sufficiently high to bring the potential to a threshold value (*1*). The spike is of interest because of its similarity to the hyperpolarizing response of nerve and muscle plasma membranes. Also, its study may further illuminate the permeability properties of frog skin epithelium. In order to determine where in the epithelium (*3*) the transient impedance changes take place we used microelectrodes, the tips of which were inserted into individual, preselected surface cells under microscopical observation. The experimental arrangement is shown in Fig. 1. With transillumination and a magnification of 400, nuclei and cellular borders of the outermost cell layer were clearly discernible. When necessary, the contrast of these structures was improved by phase contrast or by vital staining with basic dyes. For staining, a few crystals of methylene blue or toluidine blue were added to the outside solution and washed away after a few minutes. Dye accumulated at the "chicken fence," in cellular nuclei, mainly in the second cellular layer below the outer surface, and in the lumen and cellular nuclei of the ducts of subepithelial glands. The degree of staining varied among skins, possibly depending on the regenerative phase of each epithelium. Acid dyes were ineffective, except fluorescein, which would sometimes stain the chicken fence and nuclei of the surface cellular layer when added to the outside solution. No harmful effects were noted when concentration of dye and time of exposure were kept low (*4*).

The surface cells had diameters of 20 to 40 μ when viewed from above (Fig. 1) and were 5 to 10 μ deep. Their depth was measured in unfixed 10-μ sections of rapidly frozen epithelia after reimmersion in Ringer fluid, by use of phase-contrast or dark-field microscopy. The total thickness of the epithelia was 40 to 60 μ in these sections or when estimated by means of the microscope focus (*5*) in the living preparation.

The solution facing the corium (inside solution) was isotonic chloride-Ringer, while that facing the outer epithelial surface was either isotonic chloride-Ringer or this solution diluted tenfold with distilled water. The probing electrode tip resistance of 5 to 50 megohms was monitored with a repetitive calibration pulse (pulse *A* of Figs. 2 and 3) of 1 msec duration, applied to the input of the microelectrode follower stage (*6*). This pulse was followed in time by a 25-msec rectangular current pulse of subrheobase strength (pulse *B*) passed inward through the epithelium between Ag-AgCl electrodes on either side of the skin. The overall skin potential (*V*)