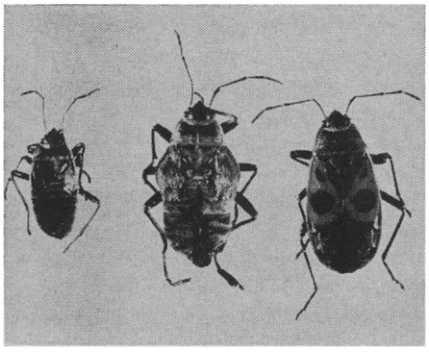

Fig. 1. The difference between the giant sixth-instar supernumerary nymph (center), normal fifth-instar nymph (left), and normal adult (right) of *P. apterus*.

are similar to those reported for todomatuic acid (*6*, *7*), m-p. 58° to 58.5°C, $[\alpha]_D^{13} = +85°$ (ethanol). Ultraviolet analysis in ethanol showed a band at 222 mμ (extinction, $\epsilon = 13{,}600$), indicative of the expected α,β-unsaturated acid. It is apparent that juvabione is the methyl ester of todomatuic acid.

Throughout purification, activity was followed by applying the material topically in 0.5 μl of acetone to newly molted last instar *P. apterus* nymphs. Supernumerary molting to giant nymphs signaled high activity (Fig. 1), while nymphal-adult intermediates were obtained with less-active fractions.

The conclusions of Carlisle *et al.* (*2*) that the balsam material produced a "pathological growth pattern" in *P. apterus* rather than juvenile hormone activity led us to compare the effects of juvabione and *trans-trans*-10,11-epoxyfarnesenic acid methyl ester on several species of Hemiptera. The latter compound is the most active synthetic juvenile and gonadotropic hormone of known structure reported to date (*8*). Table 1 shows that *trans*-10,11-epoxyfarnesenic acid methyl ester is active on all of the Hemiptera investigated; juvabione is active only on *P. apterus*, which tends to support the previously reported specificity of the balsam extract for this species. However, at higher levels (100 μg), the box elder bug [*Leptocoris trivittatus* (Say)] did molt to nymphal-adult intermediates but had such difficulty during the molt that the dorsal thoracic suture of the new cuticle ruptured and the intestines were everted through the body wall. In addition, we found that juvabione was as active as farnesol in the *Tenebrio* genitalia juvenile hormone test (*9*). In all insects responding to juvabione, the immature characters retained were like those obtained by treatment with 10,11-epoxyfarnesenic acid methyl ester. In *Tenebrio*, the juvenilizing effects of juvabione were identical with those produced by the cecropia hormone. Thus juvabione does not enjoy even ordinal specificity, despite the greater sensitivity of *P. apterus* to it. These data also indicate that the activity of the pure methyl ester of todomatuic acid is somewhat less on *P. apterus* than the activity recorded for the semipurified extracts of others (*10*); however, we were unable to detect significant activity in any other fraction from the balsam extractives. Perhaps other substances in the extract, not in themselves active, may augment the effect of juvabione, or a strain difference in test organisms may exist.

Table 1. The effect of juvabione and 10,11-epoxyfarnesenic acid methyl ester on several species of Hemiptera. The degrees of juvenilization are: O, none; N-A, nymphal-adult intermediate; and S, supernumerary sixth instar nymph. Each value represents the average modification of ten insects.

Species	Juvenilization			
	Juvabione		Epoxide	
	1 μg	5 μg	1 μg	5 μg
P. apterus	N-A	S	N-A	S
L. trivittatus	O	O	N-A	S
O. fasciatus	O	O	N-A	S
Lygaeus kalmii Stål	O	O	N-A	S

One of the most interesting aspects of our study was the discovery of a monocyclic sesquiterpene with juvenile hormone activity. Although derivatives of certain straight-chain alcohols have very high juvenile hormone activity, most mimicking compounds of known structure are derivatives of the acyclic sesquiterpenoid alcohol farnesol, which is the often proposed parent of such monocyclic sesquiterpenes as bisabolene, zingiberene, perezone, atlantone, and others, and possibly, of juvabione. Possibly the acyclic derivatives are active by virtue of their ability to cyclize within the insect, and if this is true, it follows that the natural insect juvenile hormone or hormones may also be a monocyclic sesquiterpene.

Note added in proof: We have now obtained an authentic sample of todomatuic acid through the courtesy of Dr. S. Isoe (NIH, Bethesda, Maryland). The methyl ester of this compound and juvabione are biologically equivalent, and identical by infrared spectra and gas-liquid chromatography.

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3. Gas-liquid chromatography was performed on a Barber Colman Model 500 instrument equipped with an all glass column 1.8 m by 6 mm (inside diameter) containing 0.75 percent SE-30 on Gas Chrom P (100 to 140 mesh), at 150°C, and with the gas flow (argon) being 15 lb/in² (1 atm). The solvent system used with both preparative and analytical thin-layer chromatography was a mixture of chloroform and methanol (97 : 3).
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Proliferation of Cells in the Central Cylinder of the Reduced Mutant in Lanceolate Tomato

Abstract. *When the* reduced *phenotype in homozygous* lanceolate *tomato is cultured on a sterile nutrient medium, there is a considerable amount of cell division within the central cylinder. Such proliferation may occur in response to a stimulus furnished to the shoot by the root.*

Lanceolate tomato, a leaf-shape mutant determined by a single gene, has been described by Mathan and Jenkins (*1*) and Stettler (*2*). If the mutant allele is present in double dosage, different phenotypes are produced, one of which has a hypocotyl that lacks coty-

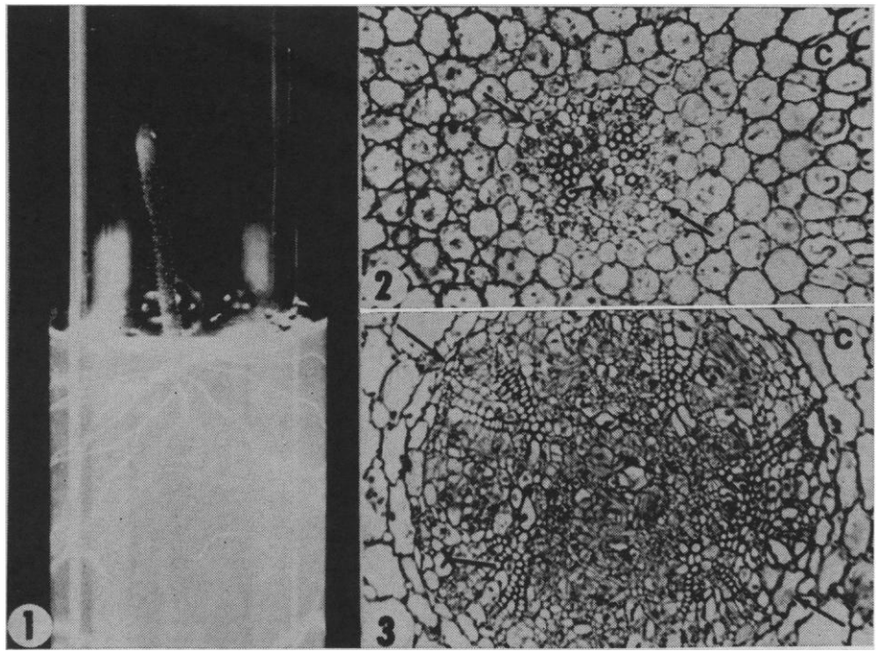

Fig. 1. Hypocotyl of the *reduced* tomato mutant cut basally, growing on a modified Murashige-Skoog basal medium supplemented with 2 percent sucrose. Adventitious roots are evident as white lines in the medium (× 1.6). Fig. 2. Transverse section of 1-week-old *reduced* hypocotyl, grown in a greenhouse, showing cortical cells (*C*) and xylem elements (*X*). Arrows delimit central cylinder (× 130). Fig. 3. Transverse section of 6-week-old *reduced* hypocotyl grown on culture medium (as cited above) and having enlarged cortical cells and proliferated cells in central cylinder (× 130).

ledons, foliage leaves, and an organized shoot apex. This plant is short-lived when grown in soil and is appropriately referred to as *reduced*.

Although Mathan and Jenkins grew the *reduced* mutant in sterile culture and described gross morphogenetic effects resulting from the addition of certain growth factors, the anatomy of this unusual plant and its internal structural changes under experimental conditions have not been described in detail. For our study, surfaces of seeds from heterozygous *lanceolate* and homozygous *normal* parents were sterilized with 5 percent Clorox, and the seeds were sown in autoclaved petri plates on moistened, sterile blotter paper. *Reduced* and *normal* seedlings (6 to 7 days old) were transferred to test tubes (25 by 150 mm), each tube containing one plant on 10 ml of a modified Murashige-Skoog medium solidified with 0.8 percent agar (*3*). Either basal medium (*3*) alone or that supplemented with 2 percent sucrose was used. The plants were grown under an illumination of 5500 lumens/m^2 in an 18-hour-day photoperiod with temperatures averaging between 23° and 25°C. Duration of the experiments varied from 3 to 6 weeks. Hypocotyls obtained from these experimental seedlings and also from 1- to 2-week old seedlings grown in soil in the greenhouse were fixed in FAA (formalin, ethyl alcohol, and acetic acid) or Craf III (chromic acid, acetic acid, and formalin), stained in safranin-fast green, and examined microscopically.

If the primary root in the *reduced* seedling is left intact upon its transfer to nutrient medium, the root appears to grow normally. If the hypocotyl is cut horizontally at the base, and if the primary root is removed during transfer, many adventitious roots are formed at the cut surface (Fig. 1). The *reduced* hypocotyl in sterile culture is similar in shape to that of the greenhouse-grown plant, but is slightly taller and several times larger in diameter. The *normal* plant in culture, although typical in its morphology, is much smaller in all its parts than a *normal* tomato plant grown in the greenhouse.

For a plant that lacks leaves, the *reduced* mutant has a very generous amount of xylem. However, the central cylinder is relatively small in diameter in those specimens grown in soil (Fig. 2). The great increase in diameter in the hypocotyl of *reduced* plants maintained in sterile culture is due to a proliferation of cells within the central cylinder and a concomitant cell enlargement in the cortical tissue (Fig. 3). The increase in cell size in the cortical tissue enables the cortex to keep pace in an orderly manner with the unorganized, callus-like growth in the central cylinder. Some cells within the resulting massive central cylinder differentiate as xylem, but these are not arranged as the xylem elements in the hypocotyl of *normal* tomato. The behavior of the mutant on medium with and without sucrose is the same whether the plant is intact or cut at the base. Added sucrose apparently permits, in some instances, greater cellular proliferation and production of a larger plant. Cells in the central cylinder of the *normal* hypocotyl appear in sterile culture as do their counterparts in those plants grown in the greenhouse; no proliferation is observed.

In our experiments there is a correlation between this phenomenon of cell proliferation in the *reduced* mutant and the presence of roots, whether these are primary roots of the seedlings or adventitious roots formed after the base of the hypocotyl is cut. In other species, phytokinin-like compounds are transported upward in the stem via the tracheary elements (*4*). It thus seems reasonable to postulate that a stimulus for cell division moves acropetally from the root to the shoot tip in these tomato seedlings. In a plant having a shoot apex and leaves, as do the *normal* seedlings, the stimulus would be used in the customary processes of leaf formation and cell division within the apex. The absence of an unusual amount of cell division within the central cylinder of *normal* hypocotyls grown in sterile culture is compatible with this view, in terms of which the shoot apex is essentially acting as a "physiological sink" (*5*). In a plant lacking leaves and shoot apex, such as the *reduced* mutant we studied, such a stimulus might well be held within the central procambial cylinder, virtually the only meristematic tissue present in the stem, and there exert its effects. This would result in the proliferation that is found in the *reduced* hypocotyl. Although the *reduced* plants growing in soil have primary roots, the shorter life span of these plants appears to preclude the behavior of the cells in the central cylinder which we see when the *reduced* mutant is grown under sterile culture conditions.

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Curare as a Neuromuscular Blocking Agent in Insects

Abstract. *Topical application of curare produced no effects on electrical activity in single flight-muscle fibers of the fly* Sarcophaga. *However, the intra-abdominal injection of curare induced a neuromuscular block similar to that described for vertebrates. The general refractoriness of some insects to chemical agents may well be due to the method of application.*

The question of the effectiveness of curare as a neuromuscular blocking agent in insects has recently been reopened by the demonstration that solutions containing large doses of curare, when injected into the body of an insect, resulted in a "complete flaccid paralysis" (*1*). Although the conclusion was drawn that this represents a vertebrate-type response to curare—that is, a neuromuscular block—no direct evidence was presented. Since it has long been held that curare is without effect on neuromuscular transmission in insects, this paper reports the results of experiments designed to test the electrical response of a single muscle cell to curare.

The fly *Sarcophaga bullata* Parker was mounted ventral side down on a mound of Tackiwax and fastened to a lucite peg. The peg fitted into the bottom of a lucite chamber filled with a physiological salt solution (*2*). Two fine Ag–AgCl wires were inserted into the thorax and directed downward toward the central thoracic ganglion. Stimuli delivered to this area elicited action potentials in the dorsal longitudinal flight-muscles. The chitin covering the scutellum was excised, thereby exposing the flight muscles. Conventional glass-capillary microelectrodes were used to impale individual fibers for measurement of transmembrane electrical responses.

Curare was administered in two ways: first, by topical application to the surface of the exposed muscle fibers, and second, by intra-abdominal injection. The topical application of curare solution produced no observable effects over a period of 2 hours. Curare as the pure crystalline powder was sprinkled directly onto the surface of

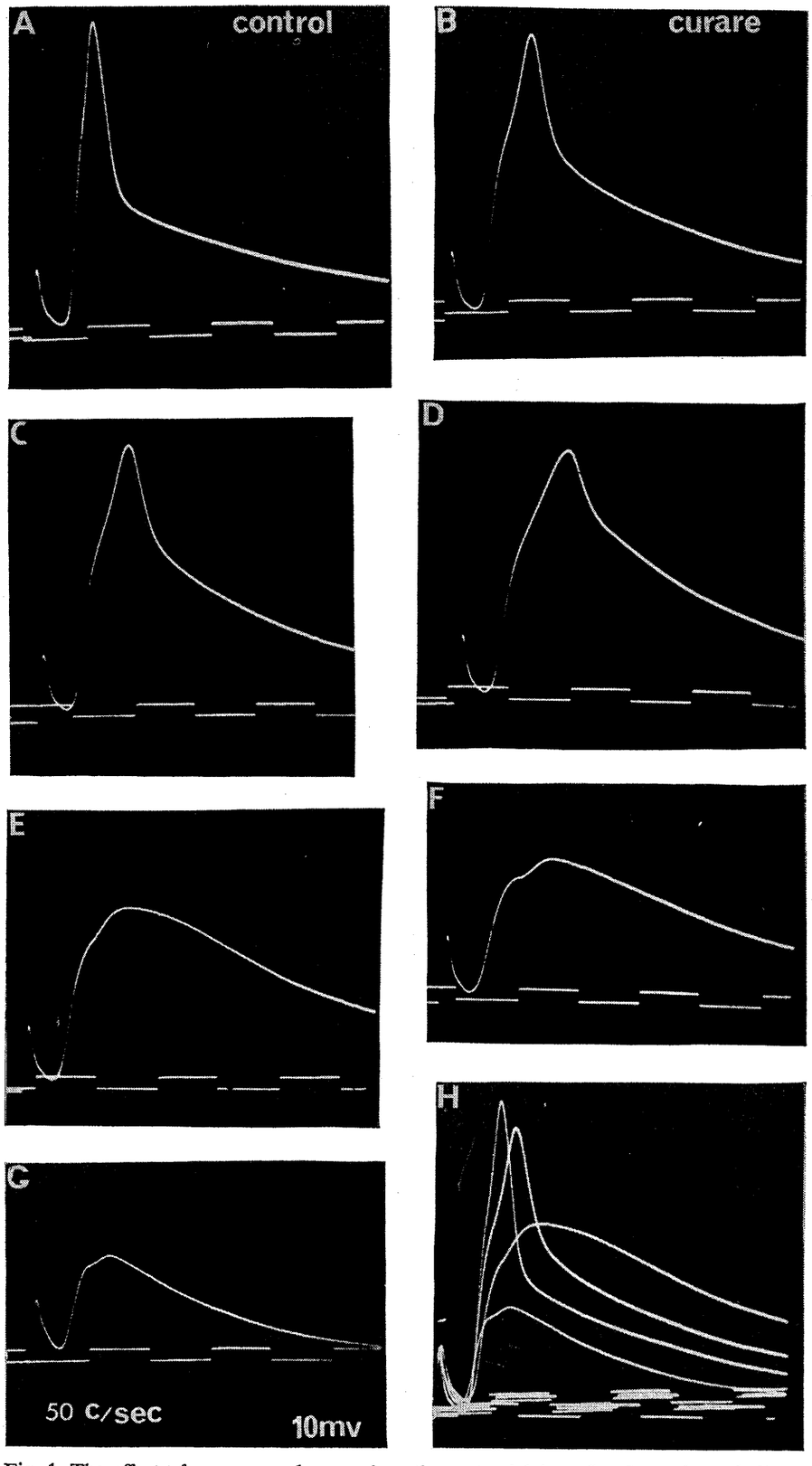

Fig. 1. The effect of curare on the muscle action-potential in a fly, *Sarcophaga bullata* Parker. (A) Control; (B–G) progressive decrement of intracellular response after intra-abdominal injection of curare; (H) composite of A, B, E, and G.