Conversion of Beta Sitosterol to Cholesterol Blocked in an Insect by Hypocholesterolemic Agents

Abstract. *Two vertebrate hypocholesterolemic agents (triparanol and 22,25-diazacholesterol) block the conversion of β-sitosterol to cholesterol in the larva of the tobacco hornworm,* Manduca sexta *(Johannson). A primary site of inhibitory action is the terminal step in this conversion—the reduction of desmosterol (24-dehydrocholesterol) to cholesterol. This is also the site at which these compounds inhibit* de novo *cholesterol biosynthesis in higher animals. Both agents severely inhibit growth and maturation of the tobacco hornworm.*

Phytophagous and certain omnivorous insects obtain their essential cholesterol through the dealkylation of dietary C_{28} and C_{29} phytosterols. One such conversion is the dealkylation of β-sitosterol to cholesterol (*1*), and we have shown that in the tobacco hornworm, *Manduca sexta* (Johannson), this transformation proceeds through the intermediate desmosterol (24-dehydrocholesterol) (Fig. 1) (*2*).

We are currently studying an unidentified compound (or compounds) that blocks the conversion of β-sitosterol to cholestrol in the tobacco hornworm. This substance, found in a commercial preparation of plant origin, inhibits the terminal step of this conversion, that is, the reduction of desmosterol to cholesterol (*2*). Certain hypocholesterolemic agents, including triparanol (*3*) and diazasterols (*4*), have previously been found to block the conversion of desmosterol to cholesterol in vertebrates—in which this reduction is the terminal step in the *de novo* biosynthesis of cholesterol. The similarity in the sites of action of these inhibitors prompted us to examine the effect of two vertebrate hypocholesterolemic agents on the metabolism of β-sitosterol in the tobacco hornworm.

The hornworms were reared as reported previously (*2*), and either triparanol (MER-29) or 22,25-diazacholesterol dihydrochloride was added to the artificial diet. Each compound was tested at concentrations of 0.013 and 0.026 percent (wet weight); β-sitosterol (99+ percent pure), at a concentration of 0.026 percent (wet weight), was used as the sole added sterol in both control and test diets. The insects were weighed and frozen when they reached the prepupal stage (normally at about 15 days) or at 20 days. The total sterols were subsequently isolated and analyzed by column, thin-layer, and gas-liquid chromatography (GLC) (*2*).

Both diazacholesterol and triparanol severely retarded larval growth and development (Table 1), and fewer than 3 percent of the larvae on these diets reached the prepupal stage. Analysis of sterols of the tobacco hornworms fed these diets revealed that both diazacholesterol and triparanol significantly blocked the conversion of β-sitosterol to cholesterol and brought about an accumulation of both desmosterol and unchanged β-sitosterol (Table 1). Diazacholesterol was more effective than triparanol in blocking cholesterol formation, and at the higher concentration it also caused the most severe retardation of growth. Data in Table 1 are average values obtained by weighing and analyzing larvae of varied sizes. Analyses of some of the smaller and more severely inhibited larvae indicated a more complete interference with the dealkylation mechanism; in these insects β-sitosterol accounted for more than 80 percent of the sterol present, which suggests that there is a direct relation between inhibition of the metabolism of β-sitosterol and inhibition of growth.

Earle *et al.* (*5*) recently reported partial inhibition of growth in the boll weevil, *Anthonomus grandis* Boheman, when two different azasterols (20,25-diazacholesterol dihydrochloride and 25-azacholesterol hydrochloride) were fed in combination with β-sitosterol or cholesterol. They concluded that inhibition was not due to prevention of the conversion of β-sitosterol to cholesterol, but no analyses of the sterol content of insects that had been fed diets containing the azasterols were reported.

The site of in vivo inhibition by these compounds in the tobacco hornworm has been further substantiated by in vitro studies. In the hornworm, both triparanol and diazacholesterol inhibit the Δ^{24}-sterol reductase activity in gut tissue that converts C^{14}-desmosterol to C^{14}-cholesterol. Triparanol and a diazasterol have previously been reported to inhibit this conversion in an in vitro system from rat liver homogenates (*6*). The Δ^{24}-sterol reductase enzymes of a vertebrate and this insect are thus so similar that they are both inhibited by two structurally dissimilar hypocholesterolemic agents, in spite of the difference in the physiological roles of these enzymes in the two organisms. In one, the enzyme functions in the *de novo* biosynthesis of cholesterol; in the other, an organism lacking the mechanism for endogenous biosynthesis of sterols, it functions in the formation of cholesterol through the degradation of a preformed sterol molecule from an exogenous source. Speculation as to the phylogenetic relations between these enzyme systems, however, must wait for a more complete knowledge of the presence

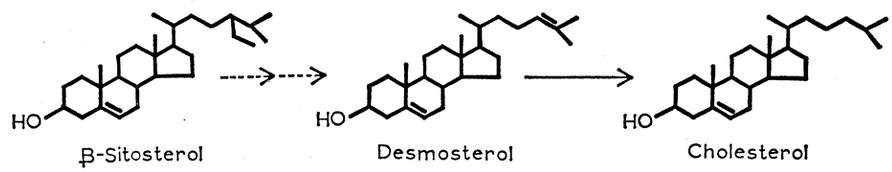

Fig 1. Partial scheme of the conversion of β-sitosterol to cholesterol.

Table 1. Average weight and the sterol composition of tobacco hornworms reared on an artificial larval diet containing 0.026 percent β-sitosterol (wet weight) alone (control) and in combination with triparanol and 22,25-diazacholesterol. Percentages of relative sterol composition are based on GLC quantitation of cholesterol, desmosterol, and β-sitosterol. Certain other minor sterol components were also detected. Average weights are for six to eight insects per test. Control insects were weighed at the prepupal stage (approximately 15 days) and test insects at 20 days.

Hypocholesterolemic agents (% wet weight)	Average weight (g)	Relative sterol composition of hornworms (%)		
		β-Sitosterol	Dealkylated sterols	
			Cholesterol	Desmosterol
Control	8.6	15.5	83.0	1.5
Triparanol				
0.013	2.1	40.2	39.5	20.3
.026	3.5	41.3	15.5	43.2
22,25-Diazacholesterol				
0.013	3.5	63.5	4.9	31.6
.026	1.2	69.0	6.2	24.8

or absence of sterol biosynthesis throughout the animal phyla.

The effects of the two compounds on larval growth and metamorphosis of the hornworm may be explained in a number of ways. They could be caused by an accumulation of large quantities of desmosterol in the tissues, in spite of our previous finding that the hornworm grows and develops normally on an artificial diet in which desmosterol is the sole added dietary sterol (*2*). Dietary desmosterol is nearly quantitatively converted to cholesterol by the hornworm (*2*), and in vitro studies indicate that the hornworm intestinal tract is a good source of Δ^{24}-sterol reductase activity. Thus, desmosterol may not gain access to the internal tissues or organs when it is obtained from the diet. Apparently certain sterols (for example, β-sitosterol and desmosterol) are not normally transported across the tissues of the intestinal tract in any quantity, since normally there is little accumulation of β-sitosterol or desmosterol in the tissues. Perhaps the transport of sterols might be altered to permit passage of these two compounds when certain steps in the dealkylation mechanism are blocked.

In addition to an accumulation of desmosterol, three to four times more unchanged β-sitosterol is present in hornworms that have been fed the inhibitors than in the controls, possibly because of the rate-limiting effect of the accumulated desmosterol on dealkylation of β-sitosterol. This increase in β-sitosterol is accompanied by a decrease in the relative content of cholesterol, which may be reduced to less than 10 percent of the content in normal insects. The low titer of cholesterol in these insects could limit the availability of this precursor for the molting hormones (ecdysones) and thus potentially be another factor in growth inhibition. The house fly, *Musca domestica* L., does not dealkylate but can use either β-sitosterol or desmosterol as a sparing sterol to replace more than 99 percent of its total dietary cholesterol requirement (*2, 7, 8*). In the latter insect, a ratio of β-sitosterol or desmosterol to cholesterol as great as 100 to 1 in the tissues still allows normal growth and metamorphosis. However, since the hornworm efficiently dealkylates plant sterols, perhaps it has not evolved a mechanism whereby it may spare its cholesterol requirement by using structurally related sterols as tissue components. Indeed, the plant sterol β-sitosterol or the intermediate desmosterol may actually inhibit growth and metamorphosis of the hornworm when they are incorporated in appreciable quantities into tissues of this phytophagous insect.

Other possible explanations of the inhibitory action of the hypocholesterolemic agents are (i) they may bring about formation and accumulation of minor steroid metabolites (other than desmosterol) which may act as growth inhibitors or (ii) they may affect physiological systems other than dealkylation. Minor steroid metabolites have been detected (*2*); these are currently under study, and the second possibility is being tested by feeding the hypocholesterolemic compounds in diets containing cholesterol.

Insects require a dietary source of sterol for normal growth, metamorphosis, and reproduction, and this essential sterol serves both as a structural component of the tissues and as a precursor of the steroid molting hormones, the ecdysones. Insects that feed on plants must derive most, if not all, of their essential cholesterol through the dealkylation of phytosterols such as β-sitosterol. This study demonstrates that in the tobacco hornworm the conversion of β-sitosterol to cholesterol can readily be blocked and that this interference severely inhibits larval growth and metamorphosis. These results suggest this to be an area worthy of intensive research both for its comparative biochemical and biomedical importance and for its potential in the development of chemicals that might be used to disrupt the development of plant-feeding insects.

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Synaptic Connections between a Transplanted Insect Ganglion and Muscles of the Host

Abstract. *When a metathoracic ganglion from one cockroach* (Periplaneta americana) *is transplanted into the coxa of another cockroach, it innervates only those leg muscles that have been previously denervated. The transplanted ganglion evokes hyperpolarizing synaptic potentials in the host muscles that it innervates. These potentials are correlated with twitching of the host limb.*

The pattern of connections among members of a population of neurons may in large part determine the form of the behavior evoked by that group of cells. To examine the factors controlling the formation of connections between one unit and another, it is desirable to have a small population of cells in which each member can be identified and in which the connections between units can be experimentally manipulated. If the system also yields defined bits of behavior that can be related to the development of specific connections, then one might have a model for examining cellular events responsible for the behavioral capacity of a nervous system. Our study indicates that transplanted central ganglia of insects, and the connections they form with the host cells, have many of the required elements for such a model system.

Bodenstein (*1*) demonstrated that the thoracic ganglion of the cockroach *Periplaneta americana* can be transplanted into the coxa of a host cockroach and that it survives. He reported that the transplant causes fibrillation of the host coxal muscles, presumably by