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Hormonal Termination of Larval Diapause in Dermacentor albipictus

Abstract. *The molting hormone, α-ecdysone, and an analog Δ⁷-5β-cholestene-2β,3β,14α-triol-6-one when applied externally, terminates diapause in larvae of the winter tick,* Dermacentor albpictus. *This is the first reported hormonal termination of diapause in an arthropod other than an insect.*

Under outdoor conditions in the United States, the eggs of the winter tick *Dermacentor albipictus* (Packard) hatch during the winter or early spring months; but the larvae diapause and do not seek hosts or attach if they are placed on hosts during the subsequent spring or summer. In the laboratory, larvae exposed to a cycle of 16 hours of light, 8 hours of dark (LD 16:8) at a temperature of 27°C and 80 percent relative humidity diapause in much the same way, but comparable larvae kept in LD 8:16 for 4 weeks will attach and feed on a guinea pig or a cow (*1*).

Juvenile hormone or juvenile hormone analogs will initiate feeding activity (*2*), yolk deposition (*3*), oviposition (*4*), and morphogenetic effects (*5*) in diapausing insects. Also, molting hormones (10 μg or less per insect) will terminate diapause in several species of insects when injected (*6*). I have investigated the influence of the two major molting hormones (α-ecdysone and 20-hydroxyecdysone), an analog of molting hormone (Δ⁷-5β-cholestene-2β,3β,14α-triol-6-one), and an analog of juvenile hormone (*trans,trans*-10,11-epoxyfarnesenic acid methyl ester), on the termination of larval diapause in *D. albipictus.* The insect molting hormones were dissolved in methanol (*7*). The analogs of molting hormone and of juvenile hormone were dissolved in acetone.

Groups of 25 diapausing larvae exposed to LD 16:8 from the time the female parent detached from the host were treated with either 0.1 or 1 percent solutions (1 μl) of one of the four compounds applied topically to each larva with a microapplicator. Then the larvae, placed on filter paper in a Büchner funnel, were dried by air gently pulled through the funnel. One day later the larvae were placed inside small plastic containers (*8*) attached to closely clipped guinea pigs; the percentage of attached (and feeding) larvae was determined at the end of 48 hours. Other samples of 100 diapausing larvae were each immersed in one of the same solutions for less than 15 seconds and handled in the same way as those with topically applied solution. Untreated controls from both short- and long-day photoperiods were placed on guinea pigs at the same time as the treated larvae. In other tests, the effects of the solvents on mortality and attachment were shown to be insignificant.

Treatment with either concentration of the triol compound terminated larval diapause in this tick (Table 1). Treatment with the α-ecdysone produced positive effects. The triol compound was the most effective of the four compounds tested by topical application. Immersion in a 1 percent solution of 20-hydroxyecdysone seemed to be more effective than the topical application of the same material (Table 2). The analog of juvenile hormone, *trans,trans*-10,11-epoxyfarnesenic acid methyl ester, was ineffective when applied topically, but a variable response was observed when the larvae had been immersed in it. During immersion, the compound might have entered by ingestion as well

Table 1. Comparison of the effect of hormones on termination of diapause in *Dermacentor albipictus* larvae as determined by the percentage of attachment to guinea pigs when treated topically with 1 μl per larva to 6 replicates of 25 diapausing larvae 28 days old. Some larvae died or escaped, so the percentage of attachment represents the ratio of the number of larvae that attached to the total number present and alive at the end of 48 hours.

Concentration (%)	Larval attachment (%)						
	1	2	3	4	5	6	Average
			20-Hydroxyecdysone				
0.1	8	12	4	4			7
1.0	12	8	4	32	10	44	18
			α-Ecdysone				
0.1	28	48	25				34
1.0	32	24					28
			trans,trans-*10,11-Epoxyfarnesenic acid methyl ester**				
0.1	0						
1.0	0						
			Δ⁷-5β-Cholestene-2β,3β,14α-triol-6-one				
0.1	56	30	25	32	24	40	35
1.0	68	21	32	64	60	36	47
			Long-day control				
	8	4	12	4	6	4	6
			Short-day control				
	43	56	56	52	32	32	45

* Methyl-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoic acid.

Table 2. Percentage of larval attachment to guinea pigs of 100 diapausing larvae immersed at indicated ages (days) in solutions. The percentage of attachment represents the ratio of the number of larvae that attached to the total number present and alive at the end of 48 hours.

Concentration (%)	Larval attachment (%) at day:				
	18	28	28	28	42
			20-Hydroxyecdysone		
0.1	4				
1.0		36	29	21	40
			trans,trans-*10,11-Epoxyfarnesenic acid methyl ester**		
0.1	8				
1.0		8	37	5	21
			Δ⁷-5β-Cholestene-2β,3β,14α-triol-6-one		
0.1	18				
1.0		20	18		44

* Methyl-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoic acid.

as by penetration of the integument and may account for the increased activity of the 20-hydroxyecdysone and the variable response to the *trans,trans*-10,11-epoxyfarnesenic acid methyl ester.

Endocrine activity regulates growth and metamorphosis in insects, and the hormones involved, such as 20-hydroxyecdysone, have been isolated from insects (*9*) and from crustacea (*10*). In addition, the juvenile hormone or its analogs terminate photoperiodically induced diapause in insects (*2–4*). My results demonstrate that molting hormones can be used to terminate larval diapause in a tick and are the first to demonstrate this phenomenon in an arthropod other than an insect. These results, added to evidence cited above, strongly suggest that there are similar hormonal systems among the Acarina, Crustacea, and Insecta.

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Nicotine Hydrogen Tartrate: Effect on Essential Fatty Acid Deficiency in Mature Pigs

Abstract. *Nicotine (as the acid tartrate) prevented the development of essential fatty acid deficiency symptoms in animals receiving a linoleate-deficient diet.*

Uncastrated male pigs, 6 months old (grown on a normal commercial pig diet) and weighing about 100 kg, were used to study the effects of nicotine on tissue and serum lipid concentration and composition (*1*).

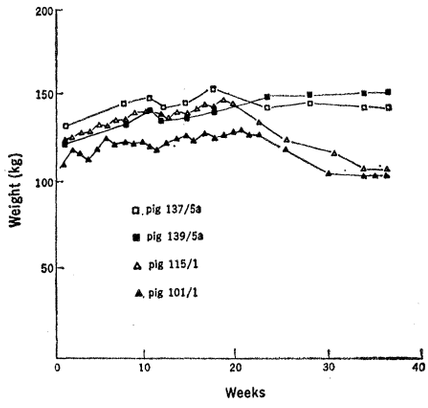

Fig. 1 (left). Weight of four pigs. Numbers 137/5a and 139/5a were fed a low fat diet with nicotine supplement; 115/1 and 101/1 had no supplement. The other animals are omitted for reasons of clarity.

Table 1. Composition of isocaloric diet.

Substance	Composition (%)
Barley meal	70.0
Fine Millars Offal	20.0
Extracted soya bean meal	7.5
Salt	0.5
Ground limestone	0.5
Sterilized bone meal	1.0
"Eves" No. 32 (totally digestible)	0.25

Animals were kept in individual pens with concrete floors; all received an isocaloric diet containing 0.3 percent linoleate calories and yielding 4500 calories per day (Table 1). Twenty-two animals received this diet alone. Fifteen other animals received a daily injection (before feeding) of an aqueous solution of nicotine hydrogen tartrate equivalent to 1 milligram per kilogram of body weight, administered subcutaneously at the top of the forehock, in addition to the control diet. All animals were weighed weekly. Animals were killed at intervals for morphological study; thus only a relatively small number of animals remained for long-term observation.

All animals thrived for the first 6 months of the study, after which eight pigs on the low fat diet (without nicotine) remained and these lost weight steadily although they continued to consume their entire ration (Fig. 1). These pigs suffered from severe skin irritation with scaling, and seven of them died. Autopsy revealed no recognizable organic disease. The eighth pig was saved by isocaloric substitution of maize oil (28 g/day), from the age of 20 months, and gained 14 kg in the 21st month.

The changes observed are those produced by essential fatty acid deficiency (*2*). This is supported by the reversal of the condition by increasing linoleate calories to 2 percent with the maize oil supplement. In contrast, none of the 15 pigs receiving nicotine hydrogen tartrate showed either loss of weight or skin irritation, and all of them continued to thrive. By 35 weeks there were marked differences in the weight of the eight pigs receiving a low fat diet alone and the five receiving a low fat diet with injections of nicotine hydrogen tartrate (Fig. 1), and marked physical differences were observed between animals of the two groups.

The manner in which the nicotine salt produces this striking effect is not clear, but it may act by sparing the polyunsaturated fatty acid stores of the body.

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Histone Structure: Asymmetric Distribution of Lysine Residues in Lysine-rich Histone

Abstract. *Structural studies on a very lysine-rich histone show that the carboxyl-terminal half of the molecule is enriched in lysine (and proline), which suggests that it is a site for binding to DNA. The amino-terminal half, containing most of the acidic residues, resembles small, nonhistone proteins and so might have specificity for factors other than DNA.*

The lysine residues in lysine-rich histones are not uniformly spaced (*1*). We now present evidence that most of the lysine residues are packed within the carboxyl-terminal half of the polypeptide chain and that other amino acids are clustered within certain regions of the histone molecule (*2*).