

In the present situation, at $L=2$ mm, $V_L/V_0 = 0.70$; therefore, $\lambda \approx 2.2$ mm.

10. The characteristic length, λ , is a function of the internal and external resistances per unit length of axon, r_i and r_o , respectively, and of the membrane resistance of a unit length of axon, r_m . It is given by the following expression:

$$\lambda = [r_m/(r_i + r_o)]^{1/2}$$

[W. A. H. Rushton, *J. Physiol.* **82**, 332 (1934)]. If the axon is bathed in a large volume of conducting medium then r_o is essentially zero; and if we consider the axon to be a cylinder of radius a , then $r_i = \rho_i/\pi a^2$, where ρ_i is the resistivity of the axoplasm, and $r_m = R_m/2\pi a$, where R_m is the resistance of a unit area of axon surface. In the present study $\lambda = 0.22$ cm (6), $a = 0.75 \times 10^{-3}$ cm (2), and $\rho_i = 60$ ohm-cm² [A. L. Hodgkin and W. A. H. Rushton, *Proc. Roy. Soc. London Ser. B* **133**, 444 (1946)]; therefore, $R_m = 7800$ ohm-cm².

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12. The action of picrotoxin is to compete with GABA for sites on the postsynaptic membrane. In the presence of picrotoxin a reduction in the amount of GABA released by the presynaptic fiber due to shadowing the median ocellus would have little or no effect on the membrane potential of the postsynaptic cells; therefore, the shadow-evoked activity would be blocked.
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analyzed reagent containing 99.9 percent dimethyl sulfoxide), a method successfully employed in the investigation of *p,p'*-DDT effects on cultured tissues (10). A portion of the juvenile hormone-dimethyl sulfoxide solution (50 $\mu\text{g}/\mu\text{l}$) was mixed with the culture medium before addition of the disks. An equivalent amount of dimethyl sulfoxide was added to the control dishes. Thus, the disks were exposed to juvenile hormone or dimethyl sulfoxide alone for 24 hours before the addition of 20-hydroxyecdysone. The cultured disks were observed regularly over a period of 3 to 4 weeks. Our first experiments confirmed that 20-hydroxyecdysone without fat body stimulated tracheal migration and elongation of the disks as reported previously for cultured *Galleria* disks (7). A concentration of 0.05 μg of 20-hydroxyecdysone per milliliter of medium stimulated tracheal migration alone, and higher concentrations caused hypertrophy of the peripodal sac, elongation, and, in some cases, tracheal migration.

We first examined the effects of 20-hydroxyecdysone and fat body on wing disks taken from mature, feeding larvae (18 to 21 mg). None of the 70 disks cultured without hormone produced cuticle. Treatment with 0.5 μg of 20-hydroxyecdysone induced cuticle deposition in 13.3 percent of the disks. By contrast, 63.3 percent of the disks cultured with 0.5- μg hormone and fat body made cuticle. Similarly, at higher concentrations of hormone (2 $\mu\text{g}/\text{ml}$, 5 $\mu\text{g}/\text{ml}$, and 50 $\mu\text{g}/\text{ml}$), fat body increased the percentage of disks depositing cuticle (Table 1). The cuticle produced by the cultured disks was tanned, lacked scales or hairs, and looked like pupal cuticle. Typically, this cuticle was seen beneath the peripodal sac covering one side of the disk. This is similar to

Cuticle Deposition in Imaginal Disks:

Effects of Juvenile Hormone and Fat Body in vitro

Abstract. *Wing disks from the last larval instar of the Indian meal moth, Plodia interpunctella* (Hübner), were successfully cultured in modified Grace's medium. 20-Hydroxyecdysone induced cuticle deposition in these disks in vitro. This response was enhanced by treating the medium with larval fat body and was inhibited by application of juvenile hormone.

Increasingly, attention has been focused on the action of insect hormones on target tissues which grow and metamorphose in vitro (1). After the first report of hormone-induced metamorphosis in insect tissues cultured in vitro (2), the action of the ecdysones, but not of other hormones or tissues, on in vitro cuticle deposition has been studied (3). Some effects of juvenile hormone in vitro have been reported, but none of these involved cuticle deposition (4). We report that 20-hydroxyecdysone (also known as β -ecdysone, crustecdysone, or ecdysterone) induced cuticle deposition in cultured wing disks of the Indian meal moth, *Plodia interpunctella* (Hübner). The deposition of cuticle in vitro was enhanced by fat body and inhibited by *Cecropia* juvenile hormone (5)—a mixture of isomers of methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate. This is the first example of an effect of juvenile hormone in vitro on cuticle deposition in imaginal disks.

The insects were reared according to the methods of Silhacek and Miller (6). The culture and dissection procedures were the same as those described for *Galleria mellonella* (Linnaeus) disks (7, 8). Mesothoracic wing disks were removed from final instar larvae and cultured in modified Grace's medium (Grand Island Biological) (9), which contains 10 percent whole egg ultrafiltrate, 7 percent fetal calf serum, and 1

percent albumin fraction 5. Unlike *Galleria* wing disks, *Plodia* disks did not survive in chemically defined Grace's medium, but remained healthy for about 1 month in the modified medium. Fat body was taken from the mesothoracic region of the same larvae as were the disks, except that fat body from 18- to 21-mg larvae was used with disks from the 8- to 11-mg larvae. Groups of ten disks were cultured with or without fat body for 24 hours before 20-hydroxyecdysone, in 10 percent ethanol, was added. Some of the control cultures received an equivalent amount of 10 percent ethanol. Because of the difficulty of solubilizing juvenile hormone in the culture medium, this hormone was first dissolved in dimethyl sulfoxide (Baker

Table 1. Effects of fat body on cuticle deposition in cultured imaginal disks of *Plodia interpunctella*. The cultures contained 10 percent ethanol.

Donor larvae (mg)	Disks examined (No.)	Fat body	20-Hydroxyecdysone ($\mu\text{g}/\text{ml}$)	Disks with tanned cuticle (%)
18-21	20	—	0 (no ethanol)	0
18-21	10	+	0 (no ethanol)	0
18-21	50	—	0	0
18-21	20	+	0	0
18-21	20	—	0.05	0
18-21	20	+	0.05	0
18-21	30	—	0.5	13.3
18-21	30	+	0.5	63.3
18-21	20	—	2.0	10.0
18-21	80	+	2.0	95.0
18-21	40	—	5.0	2.5
18-21	30	+	5.0	46.7
18-21	20	—	50.0	0
18-21	20	+	50.0	70.0

Table 2. Effects of juvenile hormone on cuticle deposition in cultured imaginal disks of *Plodia interpunctella*. The fat body, 10 percent ethanol, and dimethyl sulfoxide were present in each of these cultures.

Donor larvae (mg)	Disks examined (No.)	20-Hydroxyecdysone ($\mu\text{g/ml}$)	Juvenile hormone ($\mu\text{g/ml}$)	Disks with tanned cuticle (%)
18-21	10	0	0	0
18-21	50	2.0	0	54.0
18-21	50	2.0	100.0	38.0
12-15	90	2.0	0	72.2
12-15	80	2.0	100.0	2.5
12-15	20	2.0	50.0	45.0
12-15	20	2.0	25.0	70.0
8-11	40	2.0	0	25.0
8-11	20	2.0	100.0	5.0

the development in vivo in which only the external surface of the pupal wing has tanned cuticle. Further comparisons with in vivo cuticle await a detailed histological study. However, as we know larval wing disks lack cuticle, the deposition of cuticle in vitro is by itself a suitable criterion of metamorphosis for this tissue.

We next determined whether or not juvenile hormone would prevent deposition of cuticle induced by 20-hydroxyecdysone in vitro. Juvenile hormone (100 $\mu\text{g/ml}$) caused a moderate inhibition of cuticle formation in disks from 18- to 21-mg larvae, and a pronounced inhibition in disks from 8- to 11-mg larvae. The most striking inhibition was observed with disks from 12- to 15-mg larvae. Only 2.5 percent of these disks made cuticle in response to 20-hydroxyecdysone at 2 $\mu\text{g/ml}$, fat body, and juvenile hormone at 100 $\mu\text{g/ml}$, compared to 72.2 percent with 20-hydroxyecdysone, dimethyl sulfoxide, and fat body. Dimethyl sulfoxide alone had no effect on disks cultured with fat body (Table 2). Disks cultured with juvenile hormone appeared healthy. Tracheal migration and elongation occurred even in disks in which cuticle deposition was prevented by the juvenile hormone.

Our observation that fat body potentiated the stimulation of cuticle deposition in vitro by 20-hydroxyecdysone in *Plodia* imaginal disks is of interest in view of the hypothesis that an interaction between fat body and α -ecdysone may be important in the development of the wing disks of *Galleria* (8). Observations by Kambyssellis and Williams (11) show that fat body may contain a macromolecular factor (MF) which promotes spermatogenesis in cultured *Cynthia* testes. As they also report that fetal calf serum, present in modified Grace's medium used in our cultures, contains MF, we believe that the

fat body in our experiments supplied an additional factor or that it may have modified the 20-hydroxyecdysone molecule to a more active form.

Our results show that *Plodia* disks produced cuticle in response to 20-hydroxyecdysone in modified Grace's medium, although in an earlier experiment *Galleria* disks did not do so when cultured in chemically defined Grace's medium, which does not contain hemolymph or other serum (7). To test whether our new results were achieved by changing the medium or the species, we cultured disks from mature *Galleria* larvae in modified medium with fat body and 20-hydroxyecdysone. In these experiments, small patches of cuticle were observed in some disks, but there was not the extensive cuticle deposition seen in *Plodia* disks. The improved in vitro responses in cultured *Plodia* disks compared with the responses in *Galleria* disks were due to a change in species and perhaps, also, to a change in culture medium.

Our experiments in vitro suggest that,

as the wing disks of the last larval instar of *Plodia* mature, they become less responsive to juvenile hormone but more responsive to 20-hydroxyecdysone. We believe that the inhibition in vitro of ecdysone-induced cuticle deposition may be an appropriate system for investigating juvenile hormone action.

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12. We thank Hoffman-La Roche for providing the *Cecropia* juvenile hormone which they synthesized, Dr. D. L. Silhacek for supervising the rearing of the *Plodia*, and Professors Howard A. Schneiderman and Carroll M. Williams for comments on a preliminary draft of this manuscript. We also thank Professor Williams for making the report by Kambyssellis and Williams (11) available to us prior to publication.

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Water-Soluble Derivatives of Δ^1 -Tetrahydrocannabinol

Abstract. Δ^1 -Tetrahydrocannabinol, which is resinous and insoluble in water and therefore difficult to study pharmacologically, can be converted to a water-soluble derivative without loss of its biological activity. This has been achieved by preparing esters bearing a nitrogen moiety with the use of carbodiimide as the condensing agent. The availability of such water-soluble derivatives will allow the evaluation of Δ^1 -tetrahydrocannabinol in self-administration studies in monkeys for its addiction liability potential in man. This technique of water solubilization is also applicable to other compounds of chemical and biological significance.

Δ^1 -Tetrahydrocannabinol (Δ^1 THC) is a resinous material which is insoluble in water and is administered in various solvents, such as polyethylene glycol, Tween, triton, and alcohol, which are not without pharmacological activity (1). Hence, the need for a water-soluble derivative of Δ^1 THC is apparent

(2). The availability of such a derivative should facilitate pharmacological studies and allow evaluation in self-administration monkey studies (3). An ester derivative of Δ^1 THC is an obvious choice, but so far the conventional methods of esterification have not been successful.