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Some effects of the anti-juvenile hormone fluoromevalonate in three lepidopterous species[1]

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Fluoromevalonate (FMev) has been tested for its anti-juvenile hormone activity in pupae and adults of *Pieris brassicae* (Pieridae), larvae, pupae, and adults of *Cydia pomonella* (Tortricidae), and larvae, pupae, and adults of *Ephestia kuehniella* (Pyralidae). Anti-juvenile hormone activity expressed as anti-gonadotropic effect, i.e. reduced egg production, was found in all three species, if they were treated in the sensitive period where the juvenile hormone dependent vitellogenesis takes place: the freshly eclosed imagos of *P. brassicae* and *C. pomonella* and the pharate adult of *E. kuehniella*. No precocious metamorphosis or prepupal behaviour could be induced in third and fourth instar larvae of *C. pomonella*, though stationary moulting occurred. This unexpected effect of FMev does not speak in favour of its anti-juvenile hormone activity in *C. pomonella* larvae but may result from an unspecific effect on the larval brain.

Fluoromevalonate (tetrahydro-4-fluoromethyl-4-hydroxy-2H-pyran-2-one = FMev) is a compound that inhibits steroid synthesis and is known as a hypocholesterolaemic substance in vertebrates (SINGER *et al.* 1959). More recently QUISTAD *et al.* (1981) and FARAG & VARJAS (1983) reported anti-juvenile hormone activity (AJHA) of FMev in seven lepidopterous species: *Maduca sexta* (L.) (Sphingidae), *Samia cynthia* (DRURY) (Saturniidae), *Phryganidia californica* PACKARD (Dioptidae), *Galleria mellonella* (L.) (Pyralidae), *Spodoptera exigua* HÜBNER), *Heliothis virescens* (FABR.) (Noctuidae), and *Hyphantria cunea* (DRURY) (Arctiidae). The AJHA of FMev was expressed by precocious metamorphosis or prepupal behavior. QUISTAD *et al*. (1981) also showed that the AJHA of FMev could be averted by concurrent application of the juvenoid hydroprene. A high dose (2.5 mg) injected into adult *M. sexta* 1–2 h after eclosion also prevented the maturation of eggs. All these effects are consistent with AJHA of the compound, which was further proved by EDWARDS *et al.* (1983) who demonstrated the inhibition of juvenile hormone biosynthesis by FMev in larvae of *M. sexta*. The AJHA of FMev seems to be specific for Lepidoptera, since QUISTAD *et al.* (1981) could not find AJHA in insects of other orders (Diptera, Coleoptera, Heteroptera, and Orthoptera). A review on FMev and other anti-juvenile hormones (AJHs) was presented by STAAL (1986).

BOWERS (1976) had found two compounds (precocenes 1 and 2) with good AJHA in Heteroptera and Orthoptera, but no strong AJHA of these compounds could be demonstrated in holometabolous insects. Thus FMev with its specific AJHA in Lepidoptera closes a gap and might be a useful tool for «chemical

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allatectomy» in Lepidoptera.

In order to prove the anti-gonadotropic activity of FMev we tested the substance in the three species *Pieris brassicae* L. (Pieridae), *Cydia pomonella* (L.) (Tortricidae), and *Ephestia kuehniella* ZELLER (Pyralidae). In the first species egg maturation is strictly imaginal and starts only a few days after eclosion from the pupa (KAISER, 1949; KARLINSKY, 1963; BENZ, 1970a,b) and egg maturation depends on the presence of JH (KARLINSKY, 1963; BENZ, 1970a). *C. pomonella*, too, is a species with egg maturation in the imaginal stage, though the ovaries of some freshly eclosed females may contain up to 20 mature eggs (WIESMANN, 1935).

E. kuehniella, on the other hand, hatches from the pupa with a large number of fully mature eggs (NORRIS, 1933), egg maturation starting in the pharate adult at the stage when the compound eyes are fully pigmented and pigmentation of the legs begins (BENZ, unpubl.).

In order to find out whether or not FMev can be used for «chemical allatectomy», we tested the substance in third and fourth (penultimate) instar larvae of *C. pomonella*, a species on which we had much information concerning the effects of real allatectomy (JANS, 1982).

MATERIAL AND METHODES

Fluoromevalonate and its application

The fluoromevalonate was kindly supplied by Dr. D. BASSAND of Sandoz Ltd., Basle. It was diluted with acetone to the concentration of 10% or 1% (v/v) FMev. These dilutions were topically applied to the insects with either a 1 µl or a 0.5 µl micro-pipette. The doses are indicated a µg FMev/insect, 1 µl of a 10% dilution containing 100 µg of FMev. Controls were treated with pure acetone.

The insects

Pieris brassicae ist reared in our Department on a natural cabbage diet under long day photoperiod (16 L : 8 D) at 25° C during the light and 22° C during the dark period. It is a laboratory line inbred for more than 100 generations.

Two-day-old pupae and freshly eclosed male and female adults were treated with FMev and then mated. The mated females were placed in 1 l plastic boxes containing a fresh leaf of cabbage for oviposition and a source of sugar water for nutrition. The leaf was replaced every 5 days and the eggs laid were counted. The dead females were dissected and the mature eggs in the ovaries were counted.

Cydia pomonella is reared on a semisynthetic diet after the method of HUBER *et al.* (1972). It is also a laboratory line inbred for more than 100 generations.

Third and fourth (penultimate) instar larvae as well as pupae and adults were treated with FMev. Mortality of the treated larvae and other abnormities were recorded. Eclosed adults from treated larvae and pupae were mated and the eggs laid were counted (method of HUBER *et al.*, 1972).

Ephestia kuehniella was kindly supplied by Dr. F. BIGLER of the Federal Agricultural Research Station Reckenholz, Zürich, and was reared in our Department in 500 ml glass jars containing 200 ml of a mixture of one half of corn semolina and one half of whole wheat flour supplemented with 1% of dried brewer's yeast.

Last instar larvae, pupae, and adults were treated with FMev. Mortality of treated larvae and pupae was recorded. Surviving adults were mated and the eggs laid were counted.

RESULTS

Pieris brassicae

Since preliminary experiments with pupae had shown that treatment with 100 µg FMev resulted in high mortality, the main experiments were made with adults. The results are summarized in Tab. 1.

Tab. 1: Effect of FMev on adults of *Pieris brassicae*

Dose of FMev µg	Pairs copulated	Nr. of eggs per female laid	in ovar	produced
control	24	269.2	28.2	297.4
100	39	83.8	23.8	107.6[a]
200	13	64.6	26.5	91.1[a]
300	14	20.5	0	20.5[a]

[a] All values significantly different from control at 0.1% level ($p < 0.001$).

Cydia pomonella

Third and fourth instar larvae

The results are summarized in Tab. 2. Larval mortality after treatment at the third instar resulted from difficulty of ecdysis; especially the head capsules could not be shed. Sometimes up to 3 capsules could be found on the head of one larva. The body cuticle of these larvae often looked crumpled. On closer examination it proved to be the ecdysed old cuticle that had not been shed. If it was removed, smooth skin was found below. At the highest concentration the skin of the larval body always looked white, as if freshly moulted. There was no real growth of these insects, although they moulted several times before dying. One of these dauerlarvae stayed alive for more than two months without ever reaching another stage than the fourth instar.

Treatment of the fourth instar with the two lower doses resulted in no significant larval mortality, and the surviving larvae formed normal pupae that gave rise to adults which laid a normal number of eggs (Tab. 2). However, the highest dose produced significant larval and larval/pupal mortality. The occurrence of larval-pupal intermediates was quite remarkable. Only a comparatively small proportion of the treated individuals reached the pupal and adult stage. The number of eggs laid by the surviving females was not significantly reduced.

Independent of the stage of treatment, all treated larvae that pupated did so at the same time as the controls, i.e. 14 to 15 days after hatching from the eggs. Precocious pupation was not observed.

Tab. 2: Effects of FMev in third and fourth instar larvae of *C. pomonella*

Instar	dose of FMev µg	larvae treated	larvae dead	larvae %	pupae N	pupae %	adults N	adults %	pairs N	eggs per ♀
L_3	control	38	3	*7.9*	35	*92.1*	35	*92.1*	15	184.9
	10	45	7	*15.6*	38	*84.4*	36	*80.0*	15	177.5
	50	35	19[a]	*54.3*	9[b]	*25.7*	8	*22.9*	-	-
	100	40	27[a]	*67.5*	3[b]	*7.5*	2	*5.0*	-	-
L_4	control	30	4	*13.3*	26	*86.7*	26	*86.7*	8	165[c]
	10	40	3	*7.5*	37	*92.5*	35	*87.5*	14	145[c]
	50	40	3	*7.5*	37	*92.5*	29	*72.5*	12	147
	100	52	14[d]	*26.9*	19[e]	*36.5*	15	*28.8*	5	123.4[f]

[a] Differences to control significant at 0.1% level.
[b] Plus respectively 8 and 10 larval-pupal intermediates. Differences to control significant at 5% level.
[c] In supplementary experiments 15 control pairs laid an average of 184.8 eggs and 20 pairs treated with 10 µg FMev laid an average of 187.3 eggs.
[d] Difference to control not significant at 5% level.
[e] Plus same number of larval-pupal intermediates. Difference to control significant at 0.1% level.
[f] Difference to control not significant at 5% level.

Pupae and adults

The results are summarized in Tab. 3. The treatment of pupae had some lethal effects but did not significantly reduce the oviposition rate of the surviving adults. Treatment of the pharate adult within the pupal cuticle had no effect at the dose 50 µg/♀. However, even 10 µg per adult female reduced the oviposition rate significantly.

Tab. 3: Effects of FMev (dose in µg) on female pupae, pharate adults in pupae (phad.) and adults of *C. pomonella*

Stage	dose(µg)	treated		adults	eggs/♀
pupa	control	20		19	184.2
	10	24		16	144.7
	20	26		12	146.5
phad.	control	20		20	195.1
	50	20		20	194.1
adult	control	15	=	15	182.7
	10	20	=	20	57.2[a]
	50	20	=	20	69.3[a]

[a] Differences to control significant at 0.1% level.

Last instar larvae

The low dose of 10 µgFMev only has been tested with last instar larvae. No larval mortaility could be registered, but pupal mortality was significant (Tab.4). The number of eggs per female laid by the treated group was not significantliy different from that of the control group.

Pupae and adults

The results of these experiments are summarized in Tab. 5. Mortality of treated pupae was high with the lowest dose and 100% with all higher doses. The surviving adults tended to lay less eggs but the difference to the control was not fully significant at the 5% level.

Tab 4: Effect of FMev on last instar larvae of *Ephestia kuehniella*

Dose in µg	Nr. treated	pupae N	pupae %	adults N	adults %	eggs per ♀
contr.	70 ♂	56	80.0	55	78.6	
	70 ♀	66	94.3	61	87.1	258
10	118 ♂	102	86.0	62	52.5	
	100 ♀	89	89.0	60	60.0[a]	236

[a] Differences to control significant at 1% level.

Tab. 5: Effects of FMev on pupae, pharate adults, and adults of *E. kuehniella*

Stage	dose(µg)	Nr.treated	adults		eggs/♀
pupa	control	89♂	77	86.5%	
	"	67♀	59	88.1%	324
	10	197♂	21	10.7%	
	"	218♀	28	17.4%	261
	20	45♀	0	-	-
	40	45♀	0	-	-
	100	40♂	0	-	-
		41♂	0	-	-
phad.	control	41♀	39	95.1%	324
	10	77♀	62	80.5%	237[a]
	control	20♀	18	90.0%	305
	40	20♀	14	70.0%	103[b]
adult	control	20♀			292
	10	20♀			263
	20	20♀			239

[a, b] Differences to controls significant at respectively 1% and 0.1% level.

Treatment of the pharate adults with 10 and 40 µg FMev had far less toxic effect than the treatment of the pupae. The number of eggs produced by the 70–80% surviving adults was significantly reduced (Tab. 5: phad.). Neither 10 nor 20 µg FMev had a measurable effect in adult *E. kuehniella* (Tab. 5).

DISCUSSION

Our experiments, particularly the results regarding the pupae of *E. kuehniella* and third instar larvae of *C. pomonella*, are biased by the nonspecific toxicity of FMev already mentioned by QUISTAD *et al.* (1981). These authors considered the metabolic conversion of FMev to the potent biocide fluoroacetate as a possible explanation for the nonspecific toxic effects.

Of the specific anti-JH effects of FMev reported by QUISTAD *et al.* (1981) only the anti-genadotropic effect was found in our experiments. Treating adult *P. brassicae* (Tab. 1) and penultimate instar larvae of *C. pomonella* (Tab. 2) with high doses of FMev, and adult *C. pomonella* Tab. 3) and pharate adult *E. kuehniella* (Tab. 5) with low doses of FMev significantly reduced egg maturation. EIDMANN (1929, 1931) and MELL (1940) called attention to the fact that Lepidoptera may be divided into three groups: (i) those in which all or nearly all eggs mature in the pupal stage (the pharate adult stage was meant, as we know today), (ii) those in which part of the eggs mature in the «pupal» stage, and (iii) species with strictly imaginal (postmetabolic) oogenesis. Since *P. brassicae* and *C. pomonella* belong to the third type and *E. kuehniella* to the second, it is not astonishing that in the first two species FMev reduced oogenesis only when applied in the imaginal stage and in *E. kuehniella* in the pharate adult stage, i.e. in the JH dependent stage of vitellogenesis. An apparent exception found in the reduced egg production of *C. pomonella* treated in the penultimate larval stage with high doses of FMev does not necessarily contradict this finding. It is possible that the reduced egg production resulted either from the nonspecific toxic effects mentioned above, or from a fraction of the applied dose having remained unmetabolized up to the sensitive imaginal stage.

It is remarkable that there was no precocious metamorphosis in the treated *C. pomonella* larvae. Except for the highest dose the fourth instar larvae produced fifth instar larvae and then pupae, whereas a large number of the treated third instar larvae moulted to the fourth instar and then either stopped moulting or moulted without changing the instar. Such dauerlarvae may be the result of a raised JH titer early in the last instar larvae (BENZ, 1973) and correspond with the diapausing stages of certain Lepidoptera. In fact the diapause of last instar larvae is caused by a raised JH titer in the Pyralidae *Diatraea grandiosella* (YIN & CHIPPENDALE, 1973), *Chilo suppressalis* (YAGI & FUKAYA, 1974), and *Ostrinia nubilalis* (YAGI & AKAIKE, 1976) as well as in the tortricide *C. pomonella* (SIEBER & BENZ, 1977, 1980a). These authors also succeeded in initiating larval diapause by the topical application of JH to fresh last instar larvae. Injecting the diapausing larvae with ecdysone resulted in additional stationary moults as long as the JH titre was high (YIN & CHIPPENDALE, 1973; YAGI & FUKAYA, 1974), but caused pupal moults in late diapausing larvae, i.e. after the JH titre had declined to a threshold permitting metamorphosis (YIN & CHIPPENDALE, 1973, 1979; SIEBER & BENZ, 1980a). Moreover, SIEBER & BENZ (1980a) were able to prevent the manifestation of diapause in diapause induced *C. pomonella* by treating the freshly moulted last instar larvae with precocene 2, thus demonstrating AJHA of this substance in *C. pomonella.*

The phenomena in the fourth instar larvae of *C. pomonella* treated with FMev in the third instar resemble the diapause phenomena mentioned above and may be interpreted as an indication for the presence of JH in the treated insects. This would imply that FMev has no AJHA at all in the larvae of *C. pomonella*. The hypothesis seems to be strengthened by the finding that all larvae moulting several times moulted larval, not pupal, as would be expected in the absence of JH (JANS, 1982). However, SIEBER & BENZ (1980b), when treating larvae of *C. pomonella* with a juvenoid, induced either diapause (i.e. suppression of moulting) or moulting to an additional larval instar (L_6) or larval-pupal intermediates. Moultings therefore, if they occur in the presence of a high dose of JH, seem to be progressive rather than stationary in *C. pomonella*. On the other hand, JANS (1982) was able to induce stationary larval moults in the absence of JH by injecting allatectomized debrained 4-day-old last instar larvae of *C. pomonella* with ecdysterone. In that case the stationary moults depended on debraining the larvae on the first day after the last moult, because pupal moulting occurred, whenever the larvae were debrained later on, i.e. after the cells had already changed from larval to pupal commitment.

In the light of these observations it is not easy to come to a conclusive interpretation of the action of FMev in *C. pomonella* larvae. FMev seems to stimulate the biosynthesis of moulting hormone, as indicated by the repeated stationary moultings. However, it cannot be decided yet, whether or not the nonprogressive moulting indicates the presence of JH or a nonspecific effect of FMev inhibiting the larval brain to initiate the change from larval to pupal commitment.

ZUSAMMENFASSUNG

Fluoromevalonat (FMev) wurde auf Antijuvenilhormonaktivität (AJHA) in drei Lepidopterenarten geprüft. Puppen und Imagines des Kohlweisslings *Pieris brassicae* (Pieridae), des Apfelwicklers *Cydia pomonella* (Tortricidae) und der Mehlmotte *Ephestia kuehniella* (Pyralidae) sowie – bei den zwei letztgenannten Arten – auch bei Larven. Die Substanz wurde mit Mikropipetten topical appliziert. AJHA in der Form reduzierter Eireifung wurde in allen drei Arten gefunden, wenn das sensitive Stadium behandelt wurde, in dem die JH-abhängige Dotterbildung und die Einlagerung des Dotters in die Eier erfolgt, nämlich: frisch geschlüpfte Imagines von *P. brassicae* und *C. pomonella* und pharate (d.h. noch in der Puppe eingeschlossene) Adulte von *E. kuehniella*. Hingegen konnte keine verfrühte Metamorphose von behandelten Dritt- und Viertstadium-Larven von *C. pomonella* beobachtet werden, obwohl dies erwartet werden mußte, wenn FMev wirklich die JH-Biosynthese blockieren sollte. Statt dessen traten wiederholte stationäre Häutungen auf, was eher für einen erhöhten als einen reduzierten JH-Spiegel spricht. Es wäre allerdings auch möglich, daß JH zwar die JH-Synthese unterdrückt, gleichzeitig aber das Gehirn daran hindert, die Umstellung von der larvalen zur pupalen Programmierung vorzunehmen. Bei gleichzeitiger Aktivität der Häutungsdrüsen müßte es dann auch ohne JH zu larvalen Häutungen kommen.

REFERENCES

BENZ, G. 1970a. *The influence of the presence of individuals of the opposite sex, and some other stimuli on sexual activity, oogenesis and oviposition in five lepidopterous species.* In: L'influence des stimuli externes sur la gamétogenèse des insectes. Coll. Internat. C.N.R.S. *189*: 175–206.

BENZ, G. 1970b. *The stimulation of oogenesis in Pieris brassicae by the juvenile hormone derivate farnesenic acid ethyl ester.* Experientia *26*: 1012.

BENZ, G. 1973. *Reversal of spinning behaviour in last instar larvae of Pieris brassicae treated with juvenile hormone derivates.* Experientia *29*: 1437–1438.

BOWERS, W.S. 1976. *Discovery of insects antiallatotropins.* In The Juvenile Hormones, ed. L.I. GILBERT, pp. 394–408. Plenum,New York.

EDWARDS, J.P., BERGOT, B.J. & STAAL, G.B. 1983. *Effects of three compounds with antijuvenile hormone activity and a juvenile hormone analogue on endogenous juvenile hormone levels in the tobacco hornworm, Manduca sexta.* J. Insect Physiol. *29*: 83–89.

EIDMANN, H. 1929. *Morphologische und physiologische Untersuchungen am weiblichen Genitalapparat der Lepidopteren. – I. Morphologischer Teil.* Z. ang. Ent. *15*: 1–66.

EIDMANN, H. 1931. *Morphologische und physiologische Untersuchungen am weiblichen Genitalapparat der Lepidopteren.* – II. Physiologischer Teil. Z. ang. Ent. *18*: 57–112.

FARAG, A.I. & VARJAS, L. 1983. *Precocious metamorphosis and moulting deficiencies induced by an anti juvenile hormone compound FMev in the fall webworm Hyphantria cunea.* Entomol. Exp. Appl. *34*: 65–70.

HUBER, J., BENZ, G. & SCHMID, K. 1972. *Zuchtmethode und semisynthetische Nährmedien für Apfelwickler.* Experientia *28*: 1260–1261.

JANS, P. 1982. *Untersuchungen zur Funktion des Juvenilhormones bei der fakultativen Diapause des Apfelwicklers Cydia pomonella L.* Ph.D. Thesis Nr. *7186*, ETH Zürich.

KAISER, R. 1949. *Histologische Untersuchungen über die Corpora allata und Prothoraxdrüsen der Lepidopteren im Bezug auf ihre Funktion.* Arch. Entwicklungsmech. Organ. *144*: 99–131.

KARLINSKY, A. 1963. *Effets de l'ablation des corpora allata imaginaux sur le développement ovarien de Pieris brassicae L. (Lépidoptère).* Compt. Rend. Acad. Sci. Paris, *256*: 4101–4103.

MELL, R. 1940. *Eiproduktion bei Lepidopteren in Tropenrandgebieten.* Z. ang. Ent. *27*: 503–539.

NORRIS, M.J. 1933. *Contribution towards the study of insect fertility. – II. Experiments on the factors influencing fertility in Ephestia kuehniella Z. (Lepidoptera, Phycitidae).* Proc. Zool. Soc. Lond. *4*: 903–934.

QUISTAD, G.B., CERF, D.C., SCHOOLEY, D.A. & STAAL, G.B. 1981. *Fluoromevalonate acts as an inhibitor of insect juvenile hormone biosynthesis.* Nature *289*: 176–177.

SIEBER, R. & BENZ, G. 1977. *Juvenile hormone in larval diapause of the codling moth, Laspeyresia pomonella L. (Lepidoptera, Tortricidae).* Experientia *33*: 1598–1599.

SIEBER, R. & BENZ, G. 1980a. *The hormonal regulation of the larval diapause in the codling moth, Laspeyresia pomonella (Lep., Tortricidae).* J. Insect Physiol. *26*: 213–218.

SIEBER, R. & BENZ, G. 1980b. *Hormonal regulation of pupation in the codling moth, Laspeyresia pomonella.* Physiol. Entomol. *5*: 283–290.

SINGER, F.M., JANOSZKA, J.P. & BORMAN, A. 1959. *New inhibitors of in vitro conversion of acetate and mevalonate of cholesterol.* Proc. Soc. exp. Biol. Med. *102*: 370–373.

STAAL, G.B. 1986. *Anti juvenile hormone agents.* Ann. Rev. Entomol. *31*: 391–429.

WIESMANN, R. 1935. *Untersuchungen über den weiblichen Genitalapparat, das Ei und die Embryonalentwicklung des Apfelwicklers Carpocapsa (Cydia) pomonella.* Mitt. Schweiz. Ent. Ges. *16*: 370–377.

YAGI, S. & AKAIKE, N. 1976. *Regulation of larval diapause by juvenile hormone in the European corn borer, Ostrinia nubilalis.* J. Insect Physiol. *22*: 389–392.

YAGI, S. & FUKAYA, M. 1974. *Juvenile hormone as a key factor regulating larval diapause of the rice stem borer, Chilo suppressalis (Lepidoptera: Pyralidae).* App. Ent. Zool. *9*: 247–255.

YIN, C.-M. & CHIPPENDALE, G.M. 1973. *Juvenile hormone regulation of the larval diapause of the southwestern corn borer, Diatraea grandiosella.* J. Insect Physiol. *19*: 2403–2420.

YIN, C.-M. & CHIPPENDALE, G.M. 1979. *Diapause of the southwestern corn borer, Diatraea grandiosella: Further evidence showing juvenile hormone to be the regulator.* J. Insect Physiol. *25*: 513–523.