otropic functions[18]. These observations suggest that some hypothalamic factor(s) are (is) needed for the integrity of the pituitary gonadotropin system[19].

LHRH is a likely candidate for such an hypothalamic factor and indeed it has been found that LHRH can stimulate the synthesis of LH (e.g. Khar and Jutisz[20], Moes et al.[21]). In the present experiments LHRH was supplied to the isolated glands at a low and constant level, that is at a level which induces no desensitization nor downregulation of the number of LHRH receptors in ovariectomized rats[12]. Still, this procedure did not normalize the pituitary LH stores in the isolated pituitary glands during the present relatively short experimental period. As it is also not known whether better results might have been obtained if LHRH had been given in a different fashion (e.g. at a higher concentration or as pulses), it still remains uncertain whether LHRH is involved in maintaining the integrity of the pituitary gland.

Infusion of a large amount of hCG did not affect the LH response to an LHRH infusion 15 h later (fig. 4). However, when a similar large amount of endogenous gonadotropins is released by the pituitary gland, due to stimulation with LHRH, the gland remains desensitized for days[15]. Apparently, a high gonadotropin concentration in the plasma for a prolonged period of time is not sufficient to induce desensitization. Also, exposure of the pituitary gland to rLH just prior to the proestrous LH surge in the female rat did not affect the gonadotropin response to a subsequent LHRH infusion[22]. Thus, it appears that neither hCG nor LH exhibit a negative feedback effect on the hypothalamo-pituitary axis of the female rat; this is in contrast with such observations in the rabbit and in man[6, 23].

Maximal plasma LH concentrations (height; ng/ml) and area under the curve (integrated LH release or AUC; in arbitrary area units, AU) during 22 h of infusion of LHRH at the constant rate of 104 ng/h and pituitary content of LH (Pit, content; µg LH) at the end of the infusion of ovariectomized rats with an isolated pituitary gland (by stalk section: St.; by autotransplantation: Transpl.) and of control rats (C). Means ± SEM

Kind of rats	Number	Height	AUC	Pit. content
C	8	2631 ± 210	208 ± 21	594 ± 136
St.	4	1172 ± 166	86 ± 2	216 ± 25
Transpl.	6	419 ± 65	36 ± 4	99 ± 14

For all columns holds: C > St. > Transpl. ($p < 0.05$).

In conclusion one might infer that the desensitization of the pituitary gland, as caused by LHRH, is not affected by the extent of the concomitant depletion of the LH stores; furthermore, there is no evidence for a hypothalamic factor influencing the kinetics of the process of desensitization, or for any autofeedback effect of secreted LH.

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Changes in patterns of ecdysteroid secretion by the ring gland of *Drosophila* in relation to the sterol composition of the diet

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Summary. Brain-ring glands from *Drosophila* larvae reared on a defined diet containing campesterol (24-methyl-cholesterol) as the major sterol, secreted – in addition to ecdysone – a compound identified previously as a 24-methyl analogue, 20-deoxy-makisterone A. Using ergosterol or cholesterol as the sterol component of the diet, only ecdysone was detectable in cultures of brain-ring glands.
Key words. Ecdysteroid; sterols; *Drosophila melanogaster;* ring gland.

20-hydroxyecdysone is widely regarded as the moulting hormone of insects; ecdysone, synthesized from cholesterol[1] and secreted by the prothoracic glands or, in Diptera, the ring gland, is hydroxylated at C20 by the fat body and other tissues to produce the active moulting hormone[2]. Recent studies on the phytophagous hemipterans *Oncopeltus fasciatus, Dysdercus cingulatus* and *Dysdercus fasciatus* indicate that the moulting hormone in these insects is a C24-methylated analogue of 20-hydroxyecdysone, makisterone A (20-hydroxy-24-methyl-ecdysone)[3–5]. Evidently, campesterol (24-methyl-cholesterol) in the diet is utilized for ecdysteroid production without removal of the methyl group at C24 of the side chain[5].

Of the Diptera, it has been shown that *Sarcophaga* ring glands secrete ecdysone in vitro[6] and in *Drosophila melanogaster*, 20-hydroxyecdysone is apparently the major ecdysteroid at around the time of puparium formation[7]. However, brain-ring glands from *D. melanogaster* larvae reared on a corn meal-yeast diet secrete, in addition to ecdysone, two less polar ecdysteroids (LP1 and LP2), one of which (LP2) is likely to be 20-deoxy-makisterone A (24-methyl-ecdysone)[8]. Here, I present evidence that

The secretion of 20-deoxy-makisterone A, relative to ecdysone, by brain-ring glands from *Drosophila* larvae reared on different diets: summary of HPLC/RIA data

Diet	Antiserum	RIA activity: pg-ecdysone-equivalents per brain-ring gland per experiment[a]			
		Ecdysone	20-deoxy-Makisterone A[b]	20-deoxy-Makisterone A as a percentage of the ecdysone peak[b]	
0.03% cholesterol	Horn I2	160	1.5[c]	1%[c]	
0.03% ergosterol	ICT-1	251	6.5[d]	2.6%[d]	
0.006% campesterol[e]	ICT-1	1225	126[f]	10.3%	Mean: 9.2%
		800	69[f]	8.6%	
		935	78[f]	8.3%	
0.03%	ICT-1	160	76	48%	Mean: 42.5%
		65	24	37%	
Corn-meal/yeast	Horn I2	361	626	173%	Mean: 123%[b]
		96	119	124%	
		128	123	96%	
		64	63	99%	

[a]Each line represents an individual experiment: the culture period varied between experiments so the amounts of ecdysteroid secreted cannot be compared directly. [b]To account for the differential sensitivity of the antisera to 20-deoxy-makisterone A, values for 20-deoxymakisterone A using the Horn I2 serum should be multiplied by 1.9 for comparison with the ICT-1 serum. [c]Indistinguishable (within 2 SD) from background values. [d]Not a discrete peak, see Redfern[8]. [e]Including 0.014% w/v sitosterol, 0.015% w/v stigmasterol and 0.01% w/v ergosterol. [f]Discrete peak, see Redfern[8].

secretion of LP2, referred to as 20-deoxy-makisterone A, results from the presence of campesterol in the diet.

Materials and methods. Drosophila melanogaster larvae (Oregon-R strain) were reared axenically on defined media at 25 °C with either cholesterol, ergosterol or campesterol (0.03% w/v) added as dietary sterol[8]. All sterols were from Sigma; the campesterol used was 98% pure by TLC (Sigma Chemical Co., personal communication). In each experiment, eggs laid on agar by flies grown on a cornmeal-yeast[8] or yeast-glucose diet[9] were dechorionated and sterilized[10] and transferred aseptically to the experimental diet. Brain-ring glands from wandering 3rd-instar larvae, selected according to salivary gland morphology, were dissected, cultured in vitro and the culture medium analyzed by high pressure liquid chromatography and radioimmunoassay (HPLC/RIA)[8,11]. HPLC and RIA conditions were as described earlier[8] except that elution from the Novapak column was at a flow rate of 1.5 ml · min^{-1}. HPLC fractions (0.5 min) from Waters Resolve or Novapak (radial compression cartridge) reversed-phase, C18 columns were analyzed with the Horn I2 (16 weeks) (a generous gift of J.D.O'Connor) or ICT-1 (a gift of H.H. Rees from material supplied by K.D. Spindler) antisera. These antisera cross-react with a variety of ecdysteroids but both display specificity towards the ecdysteroid nucleus[12]. Of the two, ICT-1 is more sensitive to 20-deoxy-makisterone A, relative to ecdysone, than the Horn I2 antiserum. For comparative purposes, a factor of 1.9 may be used to express measurements of Horn I2-RIA activity corresponding to 20-deoxy-makisterone A in terms of the ICT-1 antiserum.

Sterol determinations were done on cholesterol standards and samples of casein and agar (2 g and 1 g, respectively) after saponification and solvent extraction[13].

Results and discussion. HPLC/RIA analysis of ecdysteroids secreted by brain-ring glands from larvae grown on a defined diet with 0.03% w/v campesterol showed significant quantities of RIA activity (ICT-1 antiserum) with a retention time equivalent to 20-deoxy-makisterone A (fig. 1). In two experiments, RIA activity corresponding to 20-deoxy-makisterone A represented 37 and 48% of the RIA activity equivalent to ecdysone. In

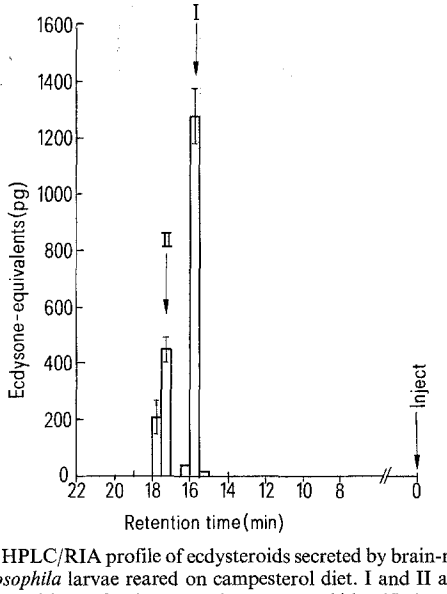

Figure 1. HPLC/RIA profile of ecdysteroids secreted by brain-ring glands from *Drosophila* larvae reared on campesterol diet. I and II are, respectively, the positions of ecdysone and a compound identified previously as 20-deoxy-makisterone A. The culture medium from 16 brain-ring glands was processed for HPLC. Half of each 0.5-min fraction from 14.5 to 19 min after injection was assayed with ICT-1 at 1:3000 dilution. Novapak C18 column. Bars are ± 1SD from duplicate assays.

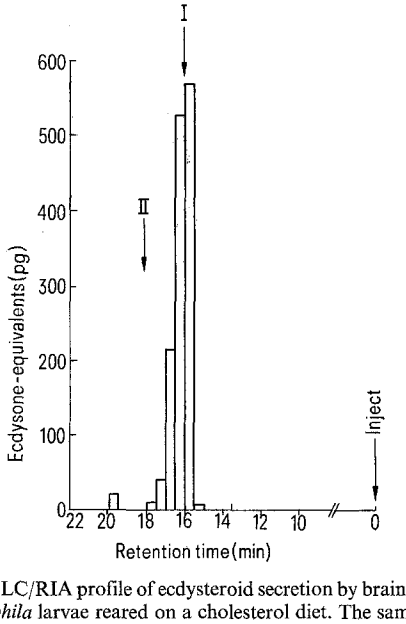

Figure 2. HPLC/RIA profile of ecdysteroid secretion by brain-ring glands from *Drosophila* larvae reared on a cholesterol diet. The sample was the culture medium from 25 brain-ring glands and one-third of each half-min fraction was assayed with the Horn I2 antiserum at 1:300 dilution. Column: Waters Resolve C18. The positions of 20-deoxy-makisterone A (II) and ecdysone (I) are marked.

contrast, there was no evidence for the secretion of 20-deoxy-makisterone A by brain-ring glands from larvae grown on a diet with cholesterol (fig. 2) or ergosterol[8]. This is clear evidence that the secretion of 20-deoxy-makisterone A results from the presence of campesterol in the diet.

These results are compared in the table in which RIA activity in the HPLC fractions corresponding to 20-deoxy-makisterone A is expressed as a percentage of the fractions comprising the ecdysone peak. Data from previous analyses[8], using brain-ring glands from larvae reared on cornmeal-yeast medium (HPLC fractionation at 1- or 0.5-min intervals) and a defined diet with a mixture of C28 and C29 sterols, including approximately 0.006% w/v campesterol (0.5-min HPLC fractions), are included for comparison. From this it is apparent that a 5-fold increase in campesterol concentration (0.006% w/v to 0.03% w/v) resulted in a comparable increase in the proportion, relative to ecdysone, of 20-deoxy-makisterone A secreted. However, the relative levels of 20-deoxy-makisterone A secreted were lower than in experiments using brain-ring glands from larvae reared on cornmeal-yeast medium.

The original identification of 20-deoxy-makisterone A in *Drosophila* was based on four criteria[8]: a) indications for side-chain modification to an ecdysteroid nucleus from experiments with different ecdysteroid antisera; b) an HPLC retention time corresponding to the major ecdysteroid secreted by *Dysdercus fasciatus* prothoracic glands; c) conversion to a compound with the characteristics of makisterone A by *Drosophila* fat body in vitro; d) the presence of makisterone A in third-instar *Drosophila* larvae. Furthermore, this ecdysteroid had similar properties on gas-chromatography/mass spectrometry to a minor ecdysteroid isolated from *Dysdercus fasciatus* and assumed to be 20-deoxy-makisterone A[5]. That the secretion of this ecdysteroid results from the presence of campesterol in the diet is additional corroboration. On this evidence, campesterol is a substrate for ecdysteroid biosynthesis and, clearly, the C24-methyl group may be retained, resulting in the synthesis and secretion of the 24-methyl analogue of ecdysone. The data suggest that ergosterol, a $\Delta 7$, $\Delta 22$, C24-methyl sterol, cannot act as a substrate for ecdysteroid biosynthesis without prior dealkylation.

Two components of the defined diet used for these experiments were present in relatively large amounts: casein (5.5% w/v) and agar (1.5% w/v). Although the casein used was extracted with petroleum ether by the manufacturer and supplied as 'fat and vitamin free', sterol estimation after saponification showed that this component of the diet contained up to 184 µg sterol per g casein. The agar used contained 48 µg per g. These two components thus contributed 0.0011% w/v sterol to the diet and on this basis the added sterol (0.03% w/v) represented 96–97% of the total. Interpretation of these experiments is therefore limited by the possible contribution of contaminating sterol, particularly from the casein component of the diet (presumably cholesterol), which may be preferentially utilized for ecdysteroid production. Thus, the ecdysone secreted by brain-ring glands from larvae reared on campesterol diet may result either from dealkylation of campesterol or (and these are not mutually exclusive) from some preferential utilization of contaminating sterol.

Two additional facts require comment. Firstly, the chemical characterization of 20-deoxy-makisterone A has not, to my knowledge, been reported and since an authentic standard of this compound was not available, it was not possible to quantify accurately the amount (in terms of moles or pg steroid) secreted. Secondly, on the basis of RIA measurements, the high proportion of 20-deoxymakisterone A secreted by brain-ring glands of larvae reared on cornmeal-yeast diet requires explanation. Assuming campesterol forms approximately 20% of the phytosterol in corn[14] and that cornmeal contains 0.27% w/w free phytosterol and 0.67% w/w saponifiable phytosterol[15], the campesterol and campesteryl ester concentration in cornmeal-yeast diet (11.8 g cornmeal per 100 ml) would be of the order of 0.02% w/v. Thus overall, the campesterol concentration in the cornmeal-yeast diet is likely to be lower than used for the campesterol diet. However, it is possible that local variation in diet constitution and/or selective feeding (on cornmeal solids, for example) by larvae could result in a higher campesterol concentration in ingested food than in the substrate as a whole.

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Effects of isaxonine phosphate and analogs on fibroblast metabolism in culture

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Summary. Human skin fibroblasts in confluent cultures were incubated for 24 h in the presence of isaxonine phosphate (Nerfactor) and several related factors. The incorporation of ^{14}C-proline into secreted proteins and the release of collagen into the medium were inhibited. When the cells were incubated for an additional period of 24 h after thorough washing, protein and collagen syntheses were