The Stimulation of Oogenesis in *Pieris brassicae* by the Juvenile Hormone Derivative Farnesenic Acid Ethyl Ester[1]

ITO[2] after observing secretory activity of the corpora allata (CA) during oogenesis in the pupal stage of *Bombyx mori* suggested that the CA may have an endocrine function related with oogenesis. However, although ITO's observations were confirmed by authors working with other lepidopterous species[3–5], the necessity of the CA for oogenesis in Lepidoptera could not be verified experimentally for a long time[5–10]. The reson for this failure was that only Lepidoptera with premetabolic egg maturation[11] had been used for the experiments. The situation changed, however, when species with postmetabolic oogenesis were investigated[12–15]. One of the species in which the necessity of the CA for oogenesis was clearly demonstrated is the large cabbage white *Pieris brassicae*[14,15].

Meanwhile it has been demonstrated in several insect orders that the gonadotropic effect of the CA is produced by the release of juvenile hormone. For Lepidoptera this has not been proved so far. The experiments reported below were made in order to obtain information on this point.

Freshly eclosed virgin females of *P. brassicae* were fed with water only and decapitated on the same day or up to 2 days later. They were then kept in moist chambers, where the insects could survive easily for at least 10 days. If the decapitated females were not further treated, they rarely produced any mature eggs (Table). However, if such females were treated topically with different concentrations of acetone solutions of farnesenic acid ethyl ester (FAEE), practically all of them matured some eggs (Table). Only 3 exceptions were found in an experiment in which the treatment was delayed up to the 7th day after eclosion.

These simple experiments prove that egg maturation in *P. brassicae* can be stimulated by FAEE in the absence of the CA. The results also suggest that high concentrations of FAEE are less favorable than lower concentrations and that early treatment is better than late treatment, though the number of individuals tested is too small to draw definitive conclusions.

Since in these experiments FAEE was substituted for the juvenile hormone, the results cannot prove, but they strongly suggest, that the CA stimulate oogenesis in *P. brassicae* by releasing juvenile hormone.

Zusammenfassung. Das synthetische Juvenilhormonderivat Farnesensäureäthylester stimuliert die Eireifung in decapitierten virginen Weibchen von *Pieris brassicae*. Da die Eireifung dieser Art normalerweise durch aktive Corpora allata stimuliert wird, darf angenommen werden, dass der gonadotrope Effekt der Corpora allata auch in Lepidopteren auf der Produktion von Juvenilhormon beruht.

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Egg maturation in virgin females of *P. brassicae* decapitated (D) at 0 to 2 days after eclosion, treated topically with 1 µl of an acetone solution of farnesenic acid ethyl ester (FAEE) of indicated concentration at day (d) after eclosion, and sacrificed (S) at indicated day after eclosion

D	FAEE		S	Females tested	Females producing eggs	Mature eggs per female	
	d	Concentration (%)				Mean	Extremes
0	–	–	8	8	1	2.2	0– 18
1	–	–	5	10	2	4.9	0– 36
2	–	–	5	7	1	5.6	0– 39
2	–	–	7	7	0	–	–
2	–	–	9	3	0	–	–
0	5	5	9	5	5	124.0	64–154
0	5	5	11	5	5	186.0	78–265
1	1	5	5	5	5	142.2	88–245
2	2	10	5	5	5	99.6	57–132
2	2	100	5	5	5	57.6	24– 75
2	7	10	10	8	5	23.6	0– 56

[1] Product of Hoffmann-La Roche Ltd., Basle. Thanks are expressed for supplying the author with a sample of this substance.

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Labelling of Steroids in Axillary Sweat After Administration of ^{3}H-Δ^{5}-Pregnenolone and ^{14}C-Progesterone to a Healthy Man

It has been suggested[1,2] that certain apocrine sweat glands may play an important role in the secretion of substances that act as pheromones. Some of these pheromones may be steroids that are sufficiently volatile to be odorous[3]. In humans, apocrine glands are particularly concentrated in the axillae[4], and dehydroepiandrosterone (DHA) and androsterone sulphates have been found in considerable amounts in armpit sweat[5]: 17-oxosteroids and other steroids are however also secreted from other parts of the body surface where apocrine glands are relatively sparse[6–8]. The actual rate of secretion of steroids by apocrine glands in isolation is of course unknown, but it is known that the growth and function of apocrine glands are under the influence of sex hormones[4], as are the sebaceous glands[9].

While studying the in vivo origin of the odorous Δ^{16}-C_{19} steroids in human urine[10], the opportunity was taken to collect sweat, not only from the general body

surface but separately from the armpits, of a healthy man (aged 33 yr) who had been given [7α-^{3}H]-Δ^5-pregnenolone (8.4 μc) and [4-^{14}C]-progesterone (9.1 μc) i.v. The proportion of the injected radioactivity that was collected in the sweat was extremely small, confirming the earlier observations of DAVIS and PLOTZ[11], but considering the relatively minute area of skin involved, the amount of injected label found in the axillary secretions was enormous (0.033% of ^{14}C, 0.112% of ^{3}H) compared with that in the general body sweat (0.026% of ^{14}C, 0.110% of ^{3}H). It was evident that steroid secretion is a particular property of axillary apocrine glands, and it seemed of interest to try to identify metabolites that were labelled.

For 36 h after the injection the subject wore pads of cotton-wool in the armpits to collect axillary sweat (A). Sweat lipids (B) from the greater part of the rest of the body, except the head and feet, accumulated over 36 h, were collected on the underclothes and by wiping arms, legs and neck with cotton-wool soaked with acetone. Nasal mucus and cerumen were also collected but were found not to be significantly radioactive. The cotton-wool and the underclothes were extracted with benzene, and subsequently with 95% and then 70% aqueous ethanol. Benzene extracted significant radioactivity only from the body (B) material. The aqueous ethanol extracts were subjected to thin-layer chromatography (TLC) on silica gel in ethyl acetate-ethanol-15N aqueous ammonia (5:5:1 by vol)[12]. Most of the armpit sweat (A) radioactivity had the mobility of steroid sulphates while the greater part of the radioactivity from the general body sweat (B) seemed to be free steroid.

Steroid conjugates were split by β-glucuronosidase hydrolysis and acid solvolysis. The total free steroids liberated thereby and the original benzene extract of B were then fractionated on columns of alumina. The proportion of the chromatographed radioactivity in the fraction (benzene-light petroleum (1:1 v/v)) which would have contained any Δ^{16}-$C_{19}O$ steroids[13] (0.8% and 1.3% of the ^{3}H in A and B respectively, and 1.3% and 5.8% of the ^{14}C in A and B respectively) was very small and was not examined further. Steroids of intermediate polarity were eluted with 0.5% and then 5% ethanol in benzene. The significant proportion of polar material (15–30% of the ^{3}H and ^{14}C applied to the columns) was not recovered. The benzene-ethanol eluates were further partially purified by TLC on silica gel in toluene-ethyl acetate (6:4 v/v) and then supplemented with carrier quantities (50 μg) of steroids considered likely to be present[9], subjected to chloromethyl-dimethylsilyl (CMDMS) ether formation[14], and chromatographed on silica gel thin-layers in toluene-ethyl acetate (8:1 v/v).

The 3 subfractions, which together contained about 60% of the total radioactivity of this last TLC separation, were then re-reacted with CMDMS reagent and subjected to gas-liquid chromatography (GLC) with gas fraction collection (GFC)[10]. As a result of this final purification procedure by GFC several carrier steroids were found to be labelled, all with both ^{3}H and ^{14}C (Table). The radioactivity associated with androstenedione, DHA and pregnenolone accounted for 45–65% of that of the three TLC subfractions referred to above. In addition to the radioactivity associated with the carrier substances listed in the Table, a considerable amount of both isotopes was found in unidentified fractions. The radioactivity in the peaks due to 5α and 5β-androsterone CMDMS ethers was negligible in both A and B: the TLC distribution showed that testosterone also was not labelled.

Considering that the area of skin surface from which extract B was derived was vastly greater than the few square centimetres of the armpits furnishing extract A, it is obvious that DHA, pregnenolone and other labelled steroids are secreted *preferentially* in the axillae and therefore probably by apocrine glands. Most of the axillary radioactivity appeared to be in the form of sulphates, but the fact that the radioactivity of the general body sweat was mainly in the form of free steroids may indicate no more than that secreted conjugates had been split by microbial enzymes on the skin surface.

The proportion of ^{3}H in pregnenolone to that in DHA was substantially higher than observed by OERTEL et al.[8] in sweat from arms and legs, after injection of pregnenolone as the sulphate. The occurrence of ^{14}C in Δ^5-3β-

Steroids labelled in sweat following injection of [7α-^{3}H]-pregnenolone and [4-^{14}C]-progesterone, after addition of carriers and purification by TLC and GLC

Armpit sweat (A)	General body sweat (B)	R.R.T.$_e$[a]
Δ^4-Androstene-3,17-dione (0.43)	Δ^4-Androstene-3,17-dione (0.54)	2.43
Dehydroepiandrosterone CMDMS ether (2.16)	Dehydroepiandrosterone CMDMS ether (8.82)	2.28
Δ^5-Pregnen-3β-ol-20-one CMDMS ether (12.4)	Δ^5-Pregnen-3β-ol-20-one CMDMS ether (140)	3.07
5β-Pregnane-3α,20α-diol di-CMDMS ether	5β-Pregnane-3α,20α-diol di-CMDMS ether	5.72
Δ^5-Pregnene-3β,20α-diol di-CMDMS ether	Δ^5-Pregnene-3β,20α-diol di-CMDMS ether	7.33
Δ^5-Androstene-3β,17β-diol di-CMDMS ether		3.87

Statistically significant ^{3}H/^{14}C ratios in parentheses. [a] Relative retention time with reference to 5α-cholestane, on 0.6% CDMS/0.75% JXR column, at 240°.

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hydroxysteroids again demonstrates the reversibility of the Δ^5-3β-hydroxysteroid dehydrogenase-isomerase enzyme system[15,16]. The absence of significant label in the Δ^{16}-C_{19} steroid fraction of sweat was not surprising in view of the small incorporation into these steroids in urine[10].

Résumé. L'administration intraveineuse du prégnène-3β-ol-20-one[7α-^{3}H]-Δ^5 avec le progestérone[4-^{14}C] à un homme sain amena la sécrétion d'autant de radioactivité dans la sueur des aiselles que dans la plupart du reste du corps. Le prégnénolone-Δ^5, l'androstène-3,17-dione-Δ^4 et le déhydroépiandrostérone furent les principaux stéroïdes marqués identifiés comme partant des deux sources de sueur.

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Adrenocortical Lipoid Hyperplasia Induced in Rats by Aniline

While studying the toxic effects of various benzene derivatives we found that in rats treated with aniline (aminobenzene), the adrenal glands were markedly enlarged and bright yellow[1]. In order to verify this observation, a detailed morphological study of the adrenals of aniline-treated rats was undertaken.

The Table shows that aniline (J. T. Baker, Canlab, Montreal, Canada), given to female Sprague-Dawley rats (100 g) at a dose of 30 mg in 0.2 ml corn oil s.c. for 7 or 14 consecutive days, markedly increases the weight of the adrenal glands.

Histologically (Figure), the adrenal cortices were strikingly enlarged and the typical zonation became indistinct. The zona glomerulosa was almost indistinguishable except for small isolated areas where a few rows of swollen glomerulosa cells were still recognizable with some round PAS-positive hyaline droplets lying free in the extracellular spaces. The cortex consisted of cords of large polyhedral cells mainly of the fasciculata-type which, in several places, extended into the fibrous capsule. Lipoid droplets of various sizes filled the cytoplasm of these hypertrophied cells where some of them coalesced and became so large that they occupied almost the entire cytoplasm thus displacing the nucleus eccentrically and endowing the cell with a signet ring appearance. At some sites where cell membranes had ruptured, the lipoid droplets merged into cyst-like structures. Occasionally, empty needle-like formations were recognized. These obviously represented sites where cholesterol crystals had dissolved during the embedding process. Although lipoid accumulation was extensive throughout the cortex, it was more pronounced in the outer zona fasciculata where, in some places, the cells were markedly vacuolated. In other areas the cells were definitely swollen and had a granular, foamy and pale eosinophilic cytoplasm without being seriously vacuolated. The nuclei were usually dark, rich in chromatin and slightly pyknotic. As a result of marked cellular hypertrophy the sinusoids were compressed. Polymorphonuclear leukocytes and mononuclear cells were scattered throughout the cortex. This inflammatory reaction was mainly localized in the inner parts of the cortex but varied considerably in degree and extent from one animal to the other. In a few adrenal glands, small, sharply demarcated foci of necrosis were seen. Some of

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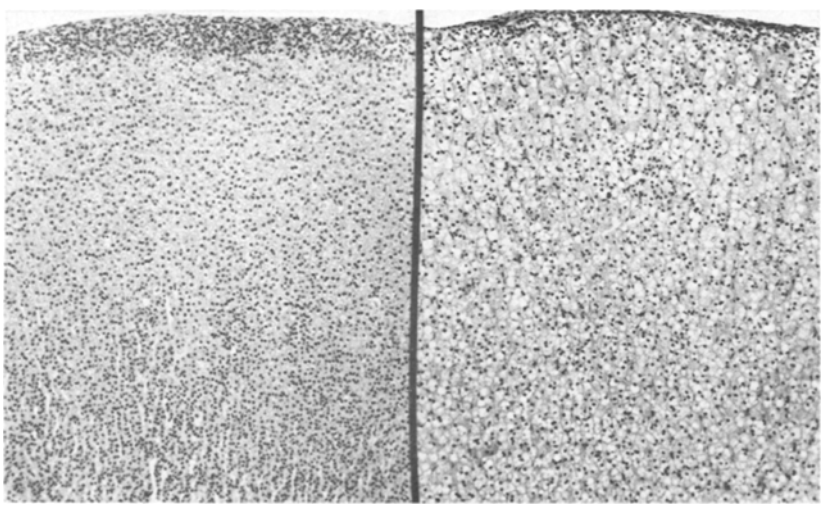

Left: Adrenal cortex of an untreated rat. Hematoxylin-Phloxine. × 120.

Right: Adrenal cortex of a rat treated for 14 days with aniline. Hematoxylin-Phloxine. × 120.