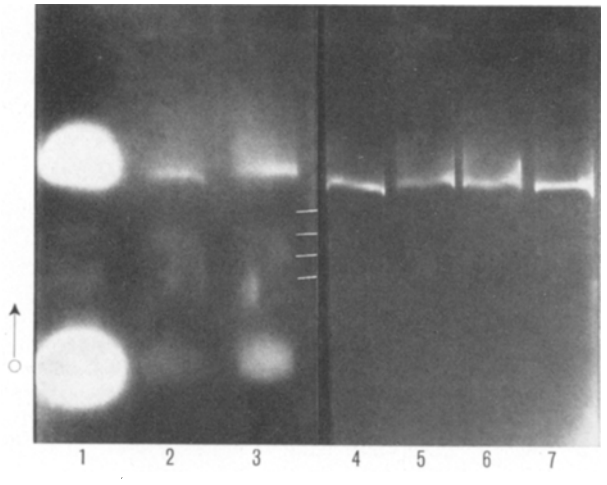
radioactivity concentration in foetal blood amounted to 21.1% of that found in the mother.

As shown in Table III, chromatoelectrophoresis of the maternal serum samples and of the infused solution showed a progressive decrease of the radioactivity at the origin corresponding to the undamaged hormone, from 56.4 to 43.3%, in the maternal circulation. Simultaneously, radioactivity in the plasma protein zone (degraded hormone) increased from 16.3 to 37.8%. Free I^{125} decreased from 29.3 to 18.9%.



Second experiment: Chromatoelectrophoresis and autoradiography: (from the left) 1st paper strip: I^{125}-ACTH and I^{125} mixture used for i.v. infusion. 2nd strip: maternal serum 30 sec after the i.v. injection and prior to infusion. 3rd strip: maternal serum after 22 min of i.v. infusion. 4th to 7th strip: foetal serum. Note the presence of free I^{125} alone.

The low radioactivity of the foetal serum required autoradiography of the chromatoelectrophoretograms. As shown in the Figure, only free I^{125} was found in the foetal circulation. The results of this study indicate that, in rabbits, I^{125}-ACTH does not cross the placental barrier and they confirm foetal autonomy of its ACTH secretion.

Résumé. Les auteurs, utilisant de l'ACTH-I^{125}, ont étudié le problème de la perméabilité placentaire à cette hormone chez la lapine. Les résultats obtenus démontrent que l'ACTH maternelle ne passe pas au-delà de la barrière placentaire et confirment l'indépendance foetale des concentrations plasmatiques d'ACTH.

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Moulting Hormone in *Locusta migratoria*: Rate of Ecdysone 20-Hydroxylation and Excretion During the Last Larval Instar

The moulting hormone of insects appears to occur in two different forms: ecdysone (α-ecdysone), supposed to be biologically inactive, and ecdysterone (20-hydroxyecdysone, crustecdysone, β-ecdysone) which exhibits hormonal activity. As yet it cannot be ruled out that ecdysone itself and related compounds, such as 3-dehydroecdysone, 3-dehydroecdysterone, 20,26-dihydroxyecdysone, could have a specific function in some particular processes regulating the moulting cycle[1-3]. Determinations of 'moulting hormone activity' in several insects has shown important and regular variations corresponding to the stage of development[4,5]. These determinations, carried out with the *Calliphora* bioassay, did not separate ecdysone from ecdysterone, both compounds being assayed together. In vivo experiments have shown that the hydroxylation rate of injected labelled ecdysone into ecdysterone is not constant, but that it also is strictly dependent on the physiological stage of the insects. In addition the excretion rate of ecdysone and ecdysterone with the faeces varies considerably during development[5].

These considerations have led us in the present study to investigate the influence of both the 20-ecdysone hydroxylase system and the rate of excretion on the titre of moulting hormone during the 5th (last) larval instar of *Locusta migratoria*.

Materials and methods. For the determination of the titre of ecdysone and ecdysterone, 15 g insects (*Locusta migratoria* in phase gregaria, reared as described in[5]) were homogenized in methanol and centrifuged. The supernatant was thin-layer chromatographed on silica gel (HF_{254}, Merck Darmstadt) with chloroform/methanol (80/20 vol/vol). Ecdysone and ecdysterone were eluted separately from the plates (identification under UV-light, reference substances being co-chromatographed in separate trails) and assayed in the *Calliphora* bioassay[6]. Hormone

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Conversion of ecdysone (α) into ecdysterone (β) and rate of excretion of total radioactivity after injection of ^{3}H-ecdysone into *Locusta migratoria* (800 ng/insect)

Day		cpm α / cpm β 120 min after injection	cpm in the insect / cpm in the faeces 8 h after injection
1	5th larval instar	1.2	0.05
3	"	0.7	0.09
5	"	0.9	0.06
7	"	0.02	0.46
9	"	1.2	0.04
15	Adult	>20	0.01

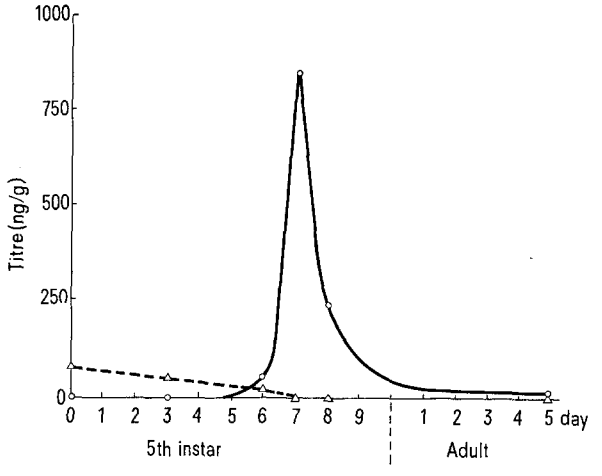

Fig. 1. Ecdysone (△---△) and ecdysterone (○—○) titres during the last (5th) larval instar and in young adults of *Locusta migratoria*.

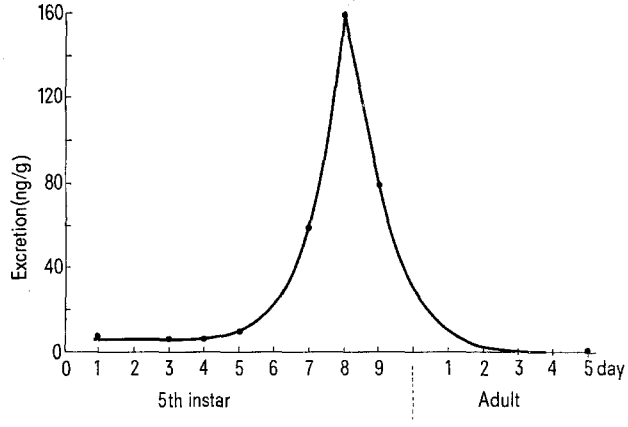

Fig. 2. Excretion rate of moulting hormones (ecdysone, ecdysterone and inactivation products being assayed together) during the last larval instar of *Locusta migratoria*.

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activity for both steroids is expressed in equivalents of crystalline ecdysone per g body weight of *Locusta*.

Moulting hormone activity of the excretory products of the insects was determined with methanol extracts from 15 g faeces collected for each assay over a 24-h-period, corresponding to days 1, 3, 4 etc. of the instar. The extracts were thin-layer chromatographed as described above. Ecdysone and ecdysterone were eluted together. Inactivation products of ecdysone and ecdysterone (Rf below 0.06) were eluted from the plate and hydrolyzed with a mixture of steroid sulphatase and β-glucuronidase (4 h, 37 °C, cf.[5]). After thin-layer chromatography of the hydrolysate, ecdysone and ecdysterone were eluted together, combined with the eluate of free ecdysones from the faeces and assayed in the *Calliphora* test. The amount of total activity recovered is expressed in equivalents of pure ecdysone per faecal material excreted by 1 g locusts within a 24-h-period.

Results and discussion. Figure 1 shows the results obtained in separate determinations of ecdysone and ecdysterone. It clearly indicates that all moulting hormone activity recorded during the first 5 days (out of a 10-day normal duration of this instar) has to be attributed to ecdysone solely. On the other hand, the very important peak of moulting hormone activity found on day 7 is due only to the presence of ecdysterone. At that time, ecdysone is not detectable in the *Calliphora* bioassay. This may be true for all insects, since for *Calliphora stygia* and *C. vicina*, GALBRAITH et al.[7] have shown ecdysterone to be the main hormone present 6 h after the onset of pupation. These results indicate that the 20-ecdysone hydroxylase system has a high activity only at the time of the moulting hormone peak (day 7 of the instar). This can also be demonstrated by injection of radiolabelled ecdysone into 5th instar larvae of *L. migratoria* of different ages (Table). The proportion of ecdysone to ecdysterone 2 h after the injection is about 1. Only at day 7 of the instar, when the moulting hormone titre rises, the hydroxylation rate is high, as indicated by a lower quotient of ecdysone/ecdysterone.

Figure 2 gives the titre of moulting hormone activity (ecdysone and ecdysterone assayed together, all inactivation products being previously hydrolyzed by enzyme solution from the snail *Helix pomatia*) in the faecal material during the normal development of 5th instar larvae of *Locusta*. It shows that little ecdysone or ecdysterone in free or conjugated form are excreted up to the time of the peak of endogenous hormone in the insects. The faecal material becomes extremely rich in ecdysones on the day following the hormone peak in the insects. Only traces of moulting hormone activity are found in the faecal material of young adults. The rate of excretion does not only depend on the amount of hormone to be excreted, but varies with the physiological age of the insects. This is shown in the Table. Locusts excrete radiolabelled material after injection of tritiated ecdysone throughout the 5th instar. Only on day 7 the excretion rate is reduced as indicated by a relatively high proportion of cpm in the insects/cpm in the faeces, which in normal insects allows the moulting hormone titre to rise. We have previously shown that this reduction of the excretion rate requires the presence of active prothoracic glands[8].

The combination of these results, and especially the comparisons between the titres of moulting hormone activity in the insects and their faeces, allow two alternative interpretations: 1. The biosynthesis of ecdysone underlies a regulation. It is low during the first half of the 5th larval instar of *L. migratoria*. On day 7 – the climax of apolysis and synthesis of a new cuticula – considerable

amounts of ecdysone are produced: each insect synthesizes up to 2 µg equivalent of pure ecdysone (average wet weight of a grasshopper at that time: 1 g).

2. The biosynthesis of ecdysone is a continuous process. Immediately after its synthesis, ecdysone is inactivated as a conjugate and possibly stored[9,10]. The rise of the moulting hormone titre on day 7 of the instar is caused by the activity of hydrolyzing enzymes. Experiments are under way to test these hypotheses.

Zusammenfassung. Der Titer von Ecdyson und Ecdysteron in Heuschrecken (*Locusta migratoria*) des V. Larvenstadiums wurde getrennt bestimmt. Ecdysteron herrscht zur Zeit des Titermaximums vor. Durch Injektion von radioaktivem Ecdyson wird gezeigt, dass dies durch ein Ansteigen der Steroid-20-Hydroxylase-Aktivität verursacht wird. Die Ausscheidung von Häutungshormonen mit dem Kot sorgt für das Sinken der Häutungshormon-Konzentration in den Tieren.

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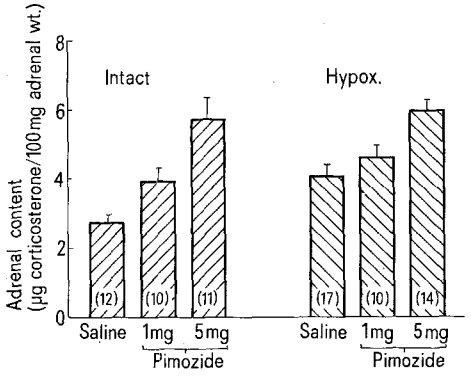
Effect of a Specific Dopaminergic Blocking Agent, Pimozide, on Hypothalamic Corticotropin-Releasing-Factor Activity in Rats: Clinical Correlates[1]

Recently attention has been focused on the effects of catecholaminergic mechanisms on the control of release and synthesis of hypophysiotrophic hormones[2,3]. This concept has become increasingly important with the widespread use of drugs affecting brain dopamine (DA) and norepinephrine (NE) and the large body of clinical evidence relating endocrine disorders to hypothalamic dysfunction[4,5].

For some years now, the neuroleptic Pimozide, a specific dopaminergic blocking agent, has been chronically administered to schizophrenic patients. Our recent clinical study with lipoatrophic diabetics (LD) has shown that the chronic administration of Pimozide (8 mg/day) causes a decrease in plasma corticotropin-releasing-factor (CRF) and a concomitant decrease in cortisol levels[6–8]. Since chronic treatment is indicated both in LD and in schizophrenia, it has become increasingly important to determine the effects of such drugs on the integrity of the hypothalamic-pituitary-adrenal axis. This report describes the effects of Pimozide on CRF activity in the median eminence of intact and hypophysectomized rats and its consequential effects on the integrity of the pituitary-adrenal axis.



ME-CRF content of intact and hypophysectomized rats treated with saline or 1 mg or 5 mg of Pimozide. CRF was estimated by changes in adrenal content in pharmacologically-blocked rats as outlined previously[9,11].

Materials and methods. 25-day-old female Sprague-Dawley rats were hypophysectomized and used 60 days later. Intact rats of the same age were used as controls. Rats (5–22/group) received saline, 1 mg or 5 mg Pimozide/rat i.p. for 7 days. On the 8th day the animals were sacrificed by decapitation and the median eminences (ME) were excised and homogenized in 0.1 *N* HCl (1 ml/ME). CRF assays were performed in pharmacologically-blocked rats by the method of Arimura et al.[9] but modifed by 12 min sampling and by estimation of adrenal content of corticosterone[10] as outlined in a previous study[11]. In this report, CRF activity is reported as increment in µg corticosterone per 100 mg adrenal weight. Plasma corticosterone was estimated using the fluorometric method of DeMoor et al.[12].

Results and discussion. CRF activity has been demonstrated to increase in the median eminence of rats after hypophysectomy[13]. In our study, administration of 1 mg or 5 mg Pimozide to hypophysectomized animals resulted in a further dose-related increase in ME-CRF (Figure).

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