The ecdysterone-specific binding protein is probably not synthesized by the prothoracic glands (which synthesize ecdysone in locust larvae[8]) or by the radiosensitive cells of the hemocytopoietic tissue, as haemolymph from permanent larvae obtained by extirpation of the prothoracic glands or by X-ray treatment of the hemocytopoietic tissue[9] still binds ecdysterone in vitro.

Earlier work on the binding of insect moulting hormones to haemolymph ('carrier') proteins has been unsuccessful so far to demonstrate a specific and saturable binding[10–15]. Apart from probable species differences, the discovery of such a binding in Locusta may be due to the use of ^{3}H-ecdysterone at the time of development when competition with endogenous ecdysterone was likely to be minimal. The physiological significance of this protein binding has now to be established. While it is probable that the 'free' hormone is the active hormone, the binding protein could possibly act as a protection against inactivation and/or excretion. In addition, an implication of the binding protein in the regulation of the hormone uptake by the target tissues has to be considered. As ecdysone is hardly bound by the ecdysterone-specific binding protein, it is necessary to reevaluate the respective roles of these 2 forms of 'the moulting hormone' in the endocrine process of moulting.

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Opposite effect of PGE_2 on cAMP levels in human adrenal medulla and pheochromocytoma[1]

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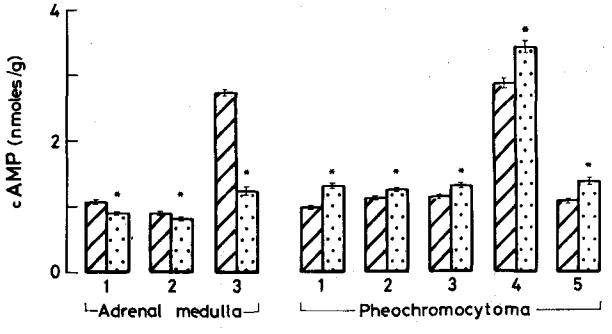
Summary. PGE_2 (10^{-7} M) caused increased cAMP accumulation in 5 pheochromocytomas, while in 3 human adrenal medullae PGE_2 caused a significant decrease of cAMP level on incubating slices in vitro. This finding is discussed in relation to the opposite effect of PGE_2 on catecholamine release from human medulla and pheochromocytoma slices in vitro.

Secretion of catecholamines (CA) from the cells of the adrenal medulla is triggered by acetylcholine released from preganglionic terminals of the splanchnic nerves. Pheochromocytomas secrete large amounts of CA into the circulation which cause the various symptoms characteristic for this tumor. However, pheochromocytomas are not innervated and, therefore,release of acetylcholine cannot be the trigger for the large secretion of CA in these tumors. We have recently reported on the inhibitory effect of α-adrenergic stimulants and of prostaglandins (PGE_1 and PGE_2) on CA secretion from rat adrenals incubated in vitro[3,4]. When PGE_2 was added to slices of human adrenal incubated in vitro, a similar inhibition of CA release was observed[5]. However, PGE_2 caused an inverse effect on CA secretion by slices of pheochromocytoma, i.e. an increased rate of CA secretion[6].

The effect of PGE could be mediated through a 'secondary messenger'. The opposite action of PGE on adrenal medulla and pheochromocytoma could, therefore, result from an opposite effect of the 'secondary messenger' in these 2 tissues. Alternatively, the primary effect of PGE could be the opposite in adrenal medulla and pheochromocytoma. Since PGE has been reported to cause activation or inhibition of adenyl-cyclase in various tissues[7], it seemed of interest to study the effect of PGE on 3′,5′-cyclic AMP (cAMP) in human adrenal medulla and in pheochromocytoma.



Effect of PGE_2 on cAMP in human adrenal medulla and in pheochromocytoma. Diagonally striped columns – cAMP in control slices. Dotted columns – cAMP in slices incubated in the presence of PGE_2 (10^{-7} M). Vertical bars – SE. Each column is the mean of 11 slices from one adrenal medulla or from one pheochromocytoma. 1, 2, 3 designate adrenals or pheochromocytomas from different patients. – *$p < 0.01$ for the difference in cAMP between slices of the same gland incubated with and without addition of PGE_2.

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Materials and methods. Human adrenals were obtained from cases of accidental death which served as kidney donors. Pheochromocytomas were obtained during surgery for removal of the tumor. The adrenals and pheochromocytomas were immediately placed on ice and transferred to the laboratory. Adrenal medulla was separated from cortex by careful dissection. Slices of 10–15 mg of adrenal medulla and of pheochromocytoma were prepared and incubated, each slice in a 50 ml Erlenmeyer containing 10 ml of medium of the following composition: NaCl–154 mM, KCl–5.6 mM, $CaCl_2$–0.5 mM, $MgCl_2$–5.5 mM, glucose–5 mM, $NaHCO_3$–1.8 mM and theophylline 10^{-2} M. 11–12 slices of each gland or tumor were incubated in control medium and an equal number of slices were incubated in the presence of PGE_2 (10^{-7} M) PGE_2 was kindly supplied by Dr J. Pike, The Upjohn Co., Kalamazoo Mi., USA

cAMP assay. cAMP in the slices was assayed using a kit of The Radiochemical Center, Amersham, with binding protein from bovine muscle. Results are expressed as nmoles cAMP/g tissue at the end of 10 min incubation.

Results and discussion. The figure shows the effect of PGE_2 on 3 different adrenal medullae and on 5 pheochromocytomas. In each of the human adrenals, addition of PGE_2 caused a reduction of cAMP in the slices. In each of the pheochromocytomas PGE_2 caused significant increase of cAMP level in the slices. Since all the incubations were carried out in the presence of theophylline, PGE_2 could not have affected cAMP level by inhibition of phosphodiesterase. Therefore, the primary effect of PGE_2 was, presumably, on adenyl-cyclase, and showed an opposite effect in human adrenal and in pheochromocytoma. cAMP causes release of CA from the adrenal gland[8] (and our own unpublished observations). A change of cAMP level could, therefore, be the 'secondary messenger' of PGE_2 action. The reason for the opposite effects of PGE_2 in normal adrenal cells and in pheochromocytoma is not clear. However, it is intriguing to suggest that a change in some characteristic of the cell membrane which accompanies the transformation of normal adrenal medullary cell into a tumor cell is reveled by this altered response to PGE_2.

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Molt induction in lobsters (Homarus americanus) by intramuscular injection of ecdysterone triacetate

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Summary. The principal component of the successful, molt-inducing ecdysterone acetate mixture was established as ecdysterone triacetate and shown to have the same activity as the acetate mixture. The triacetate induced ecdysis in male lobsters collected in winter and summer and in female lobsters collected in winter. Intramuscular injections of triacetate in ethanol were as effective as those with oil emulsions.

Perhaps due to the large adult size of the American lobster, ecdysterone (β-ecdysone, crustecdysone, 20-hydroxyecdysone) treatments have been almost entirely unsuccessful at inducing ecdysis with survival[2,3]. In this animal, ecdysterone ('molt hormone') treatment induced rapid premolt development but the lobsters died in late premolt or at ecdysis without attempting to emerge, even when the treatment was small repeated doses[2] which produced apparently perfect premolt development. In further trials of prolonged hormonal exposure, we found that treatment with ecdysterone emulsion produced the usual lethal premolt development but that the crude ecdysterone acetate emulsion was highly successful at inducing ecdysis[4] in lobsters collected in fall and winter.

To extend the above studies to lobsters collected in the summer and to obtain information on the dose-response relationship of the crude acetates, small, male, adult lobsters in early premolt (C–D_0 to not beyond stage D_0[4]) were treated with emulsions of the acetates in Freund's incomplete adjuvant (FIA; FIA: water: ethanol, 2:1:1) as before[4] by injection into the abdominal muscle at the indicated doses (see table 1). The principal constituent of the crude acetate mixture was isolated, crystallized and also injected in FIA emulsion (table 1). All lobsters were held as before[4] in individual tanks ($21 \times 29 \times 13$ cm) in flowing, unrecirculated sea water at 15 °C in a closed room with controlled lighting (16 h light/8 h dark). They were fed on live clams or beef liver chunks. Since first

Table 1. Treatment of male lobsters with emulsions containing ecdysterone and ecdysterone acetate

Treatment* (μg/g)		Number** of molts	Number** of deaths	Mean time in days from last treatment*** to molt	Mean time in days from last treatment*** to death
Control		0	0		
E	2.0	0	5		24 ± 1
EAc	2.5	4	0	38 ± 23	
	3.8	4	0	34 ± 8	
	5.0	4	1	57 ± 5	41
EAc_3	2.7	3	0	43 ± 17	

*E, ecdysterone; EAc, crude ecdysterone acetate; EAc_3, crystalline ecdysterone triacetate. **All groups were 5, intermolt animals or early premolt (stage C to D_0) randomly selected. *** Second treatment given 34 days after the first to animals which showed no significant premolt development.

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