The significance of difference between means was established by Student's t-test and unless otherwise indicated, data are expressed as the mean ± SEM.

Results. The PGF-treated rats gained 12.5 g (n=6) compared to a loss of 1.3 g (n=9), $p < 0.05$, for the control rats. Blood sugar determined 24 h prior to sacrifice was not significantly altered by PGF treatment; 492 ± 47 mg/100 ml blood for control and 425 ± 52 mg/100 ml blood for treated rats, $p > 0.3$. The data shown in table 1, however, clearly indicate that PGF treatment depressed hepatic drug metabolism in these diabetic-hypophysectomized animals.

In a similar manner, BGH injections resulted in a b.wt increase during the treatment period of 19.4 g (n=7) compared to a loss of 3.8 g (n=4), $p < 0.05$, for controls and was without significant effect on blood sugar determined 24 h prior to sacrifice; 543 ± 49 mg/100 ml blood for controls compared to 441 ± 36 mg/100 ml blood for treated rats, $p > 0.1$. Treatment with BGH also depressed the hepatic metabolism of aniline but did not significantly affect aminopyrine metabolism (table 2).

Table 2. Effect of in vivo treatment of diabetic-hypophysectomized rats with bovine growth hormone (BGH) on hepatic drug metabolism in vitro[a]

Drug substrate	Control	BGH-treated[b]	Inhibition (%)
	(μmoles/min g liver)	(μmoles/min g liver)	
Aminopyrine	62.45 ± 3.10 (4)	56.99 ± 1.66 (7)	4
Aniline	16.00 ± 1.39 (4)	10.32 ± 0.43 (7)[c]	36

[a] Results are expressed the same as for table 1. [b] Treatment conditions are described in 'methods'. [c] $p < 0.01$ versus control.

Discussion. After injection of alloxan into rats to produce diabetes, a small amount of immunoassayable insulin remains in the plasma even though the ability of beta cells to secrete insulin in response to normal stimuli is greatly diminished[8]. Although insulin is necessary for growth in some species only very small amounts of insulin are necessary for the expression of the growth response to GH in hypophysectomized-pancreatectomized rats[9]. In addition it has previously been shown that PGF stimulates the growth of alloxan diabetic rats[10] and that PGF has some 'insulin-like' activity[11]. In light of these observations it is not surprising that both BGH and PGF were observed to stimulate growth in the diabetic-hypophysectomized rats used in the present experiments.

Alloxan diabetes in rats has also been shown to decrease the in vitro metabolism of aminopyrine but increase the in vitro metabolism of aniline[4]. Insulin treatment of diabetic rats, on the other hand, decreases aniline metabolism but has no effect on aminopyrine metabolism[4]. Likewise, hypophysectomy alone has been shown to decrease hexobarbital[2] and aminopyrine[1] metabolism in rats. The present observations that PGF acted to decrease the rate of hepatic aminopyrine and aniline metabolism without altering blood sugar concentrations during a period of enhanced growth in diabetic-hypophysectomized rats indicate that PGF affects growth and drug metabolism by a mechanism that is not dependent on normal insulin levels. The observation that BGH affected only aniline metabolism under these same conditions, however, suggests that mammalian growth hormone and PGF may affect hepatic drug metabolism by independent mechanisms.

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A specific binding protein for the moulting hormone ecdysterone in locust haemolymph

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Summary. Specific binding of ^{3}H-ecdysterone to a high mol. wt protein from Locusta migratoria haemolymph was shown by gel filtration. The hormone-protein complex shows a dissociation constant $K_d \simeq 3.10^{-7}$ M, and the concentration of binding sites varies during the last larval instar.

The moulting hormones of insects, ecdysone (α-ecdysone) and ecdysterone (β-ecdysone, 20-OH-ecdysone), are polyhydroxysteroidal molecules. These hormones circulate in the haemolymph at levels which are at least a 100fold below their solubility in water. It has thus been generally agreed[1] that these hormones circulate in a 'free' form, rather than partially bound to protein carriers as steroid hormones of vertebrates. I report here the occurrence of a specific and saturable binding protein for ecdysterone in Locusta migratoria haemolymph.

Ecdysone disappears quickly from the haemolymph when injected into locust larvae[2], and chemical assay (gas chromatography-mass fragmentography) shows that ecdysterone is the principal hormone molecule circulating in larval haemolymph[3]. Moreover, after injection of ^{3}H-ecdysone, in vivo binding of ^{3}H-ecdysterone to a macromolecular fraction of the haemolymph of locust larvae has been shown by gel filtration on Sephadex G100[4].

Material and methods. In order to allow an in vitro study of this binding, ^{3}H-ecdysterone of high specific activity was first synthesized. Fat body and Malpighian tubules are the primary sites of the conversion of ecdysone into ecdysterone in locusts[2]. Malpighian tubules of 50 late fifth instar locusts were dissected and rinsed for at least 2 h at 4 °C in locust saline[5]. 15 nmoles ^{3}H-ecdysone

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(sp. act. ≃ 60 Ci/mmoles) were taken up by 5 ml of Landureau's medium (personal communication) and the Malpighian tubules were added to this medium and incubated for 6 h at 37 °C with gentle shaking. The reaction was stopped by addition of methanol and ecdysteroids were extracted and analysed by TLC[2]. About 80% of the recovered label was attributable to ecdysterone which was definitively identified by cocrystallisation. The ^{3}H-ecdysterone biosynthesized was not diluted with endogenous ecdysterone as shown by radioimmunoassay using a ^{125}I-iodinated antigen[6].

Results and discussion. Using this high specific activity ^{3}H-ecdysterone, we incubated cell-free haemolymph from 2-day-old fifth instar larvae (low endogenous hormone titre) with ^{3}H-ecdysterone and fractionnated it by Sephadex G25 gel chromatography. Figure 1 shows that 9% of the label recovered from the column is associated with the macromolecular fractions. Cell-free haemolymph does not metabolize ecdysterone, and both peaks of radioactivity are attributable to chromatographically pure ecdysterone. Assaying the hormone binding by gel filtration, it was seen that RNase does not modify the percentage of ecdysterone bound, whereas treatment with protease decreased it by 78%. Heat treatment (60 °C, 30 min) or high NEM concentrations (3 mM) also affected ecdysterone binding. These data demonstrate the existence of an ecdysterone-protein complex. This ecdysterone binding protein has a mol.wt greater than 2.5×10^5 daltons as shown by Sephadex G200 gel chromatography. When cell-free haemolymph from fifth instar locusts on day 1 was incubated with increasing amounts of ecdysterone, binding appeared to be saturable (figure 2). Only one type of binding site could be demonstrated. Their total concentration on day 1 is about 8×10^{-8} M. The dissociation constant K_d of the hormone-protein complex is about 3×10^{-7} M. ^{3}H-ecdysterone binds to haemolymph proteins from larvae as well as from adults from both sexes. The table shows that the percentage of ^{3}H-ecdysterone bound varies in reciprocal function of the level of endogenous hormone. Moreover, the concentration of bound ecdysterone on day 7 is greater than the total concentration of binding sites on day 1. Thus, the total concentration of binding sites is not constant during the last larval instar.

In our in vitro incubation and assay conditions, binding of ^{3}H-ecdysone, ^{3}H-3-dehydroecdysone and ^{3}H-3-dehydroecdysterone never exceeded 2% of the binding noted for ^{3}H-ecdysterone at the same concentration. Ecdysteroid conjugates[7] were not bound at all. It thus appears that locust haemolymph contains a specific ecdysterone binding protein with limited capacity. Our assay conditions do not exclude the existence of additional lower affinity binding sites which may release their ligand upon chromatography on Sephadex G25.

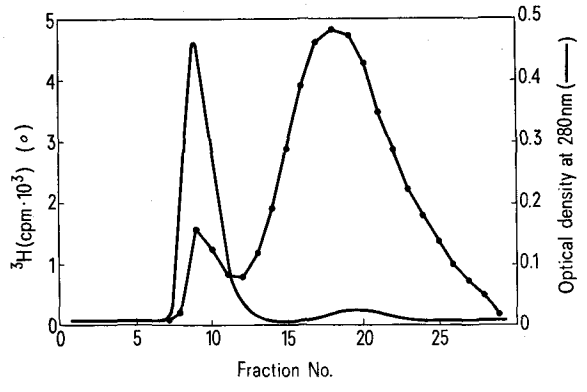

Fig. 1. Binding of ^{3}H-ecdysterone to locust haemolymph proteins: 100 µl cell-free haemolymph from 2-day-old fifth instar locusts were incubated for 60 min at 4 °C with 3.2×10^{-8} M ^{3}H-ecdysterone and fractionated by gel filtration on Sephadex G25 (15 cm column; 0.8 ml/min; 100 mM phosphate buffer, pH 6.9). Optical density was monitored with an LKB Uvicord II at 280 nm and the radioactivity of 100 µl aliquots from 450 µl fractions was counted by liquid scintillation. The first peak of radioactivity co-eluted with the macromolecular fractions and accounted for 9% of the total radioactivity recovered.

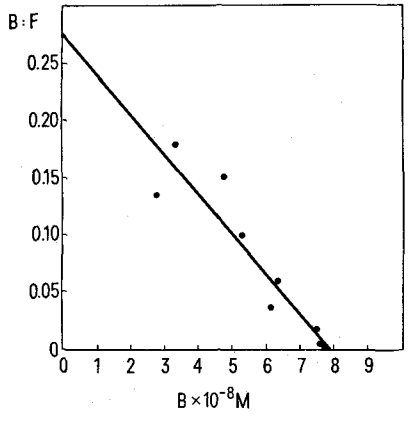

Fig. 2. Effect of increasing amounts of ecdysterone on its binding to haemolymph proteins from 1-day-old fifth instar larvae. 100 µl samples of cell-free haemolymph from 1-day-old fifth instar larvae were incubated for 60 min at 4 °C with various concentrations of ^{3}H-ecdysterone. The details for the binding assay were the same as described for figure 1. Binding was plotted according to Scatchard[16] to give the dissociation constant (Kd = 3.10^{-7} M) and number of binding sites (N = 8.10^{-8} M).

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Ecdysterone binding to locust haemolymph proteins during the last larval instar

Fifth instar, day	Endogenous hormone titre	^{3}H-ecdysterone bound (% of total radioactivity recovered)	Concentration of ecdysterone bound
2	$1 \cdot 10^{-7}$ M	9	$9 \cdot 10^{-9}$ M
5	$9.4 \cdot 10^{-7}$ M	4.2	$3.9 \cdot 10^{-8}$ M
7	$7.6 \cdot 10^{-6}$ M	1.8	$1.4 \cdot 10^{-7}$ M
9	$2 \cdot 10^{-6}$ M	2.1	$4.2 \cdot 10^{-8}$ M

100 µl cell-free haemolymph from 2-, 5-, 7- and 9-day-old fifth instar larvae (duration of the last larval instar = 10 days) were incubated for 60 min at 4 °C with 3.2×10^{-8} M ^{3}H-ecdysterone and the binding assayed as on figure 1. Values of the endogenous hormone titre in haemolymph are obtained by radioimmunoassay[6] and it is assumed that this activity is solely due to ecdysterone[3]. The concentration of ecdysterone bound to haemolymph proteins was calculated from ecdysterone titre and the percentage of ^{3}H-ecdysterone bound, assuming a normal isotopic dilution.

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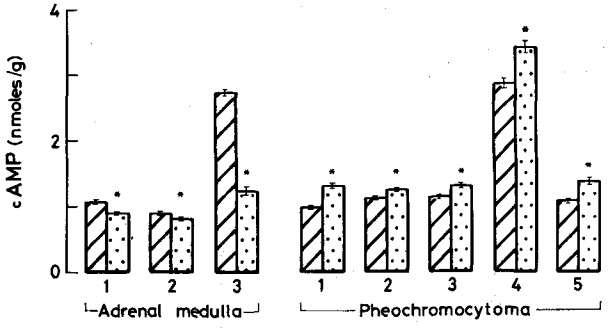
Summary. PGE_2 (10^{-7} M) caused increased cAMP accumulation in 5 pheochromocytomas, while in 3 human adrenal medullae PGE_2 caused a significant decrease of cAMP level on incubating slices in vitro. This finding is discussed in relation to the opposite effect of PGE_2 on catecholamine release from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