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 cAMP causes release of CA from the adrenal gland[8] (and our own unpublished observations).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.

1 We thank Dr A. G. McInnes and D. Smith of the Atlantic Regional Laboratory, N. R. C., Halifax for obtaining the NMR-spectrum.

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treatments were only partially successful at inducing eventual ecdysis, animals which did not respond with premolt development to stage D_1 were given a second treatment of the same amount. The results show (table 1) that in the summer, the lobsters responded to the acetates as they had in winter[4] and that the principal acetate of the mixture produced the same response as the crude acetate mixture. All deaths indicated in tables 1 and 2 were of lobsters in late premolt (approximately at ecdysis). Our crystalline acetate melted at 147 °C, which agreed with one published melting point for ecdysterone triacetate (EAc_3)[5], but we were unable to get agreement with another[6]. However, we have concluded that our crystals were ecdysterone triacetate since 100 MHz NMR clearly showed the presence of only 3 acetate methyl groups with the rest of the spectrum like that of ecdysterone.

Table 2. Treatment of male and female lobsters with graded doses of ecdysterone triacetate

Treatment* (μg EAc_3/g)	Number** of lobsters	molts	deaths	Average time from last treatment*** to molt	death
Experiment 1, males					
Em (control)	10	0	0		
0.60/Em	10	1	0	44	
1.3/Em	10	2	0	47 ± 12	
2.5/Em	10	7	0	38 ± 8.1	
4.0/Em	10	7	3	30 ± 2.6	20 ± 11
5.0/Em	10	6	4	32 ± 6.4	29 ± 6.5
Experiment 2, females					
Em (control)	8	0	0		
0.6/Em	8	1	0	64	
1.3/Em	8	1	0	62	
2.5/Em	8	5	0	51 ± 1.1	
4.0/Em	8	7	0	32 ± 3.8	
5.0/Em	8	8	0	36 ± 8.4	
Experiment 3, males					
EtOH (control)	5	0	0		
0.6/EtOH	5	1	0	63	
1.3/EtOH	5	3	0	48 ± 15	
2.5/EtOH	5	4	0	31 ± 3.4	

*Em, FIA emulsion; EtOH, solution in 95% ethanol. **All lobsters were intermolt or early premolt (stage C to D_0). Groups were selected by random numbers. ***Second treatment was 70 days after first.

The results of dose-response relationship for EAc_3 in winter caught lobsters are shown in table 2. Early premolt (C–D_0 or D_0)[4] male and female lobsters were selected. Treatments were made in the same manner as previously (and above) but with a greater dose range. Again the first treatment had little visible effect and was therefore followed by a second of the same size. The results (see table 2) show an abrupt change from an effective dose at 2.5 μg/g to a largely ineffective one at 1.3 in a manner reminiscent of results with ecdysterone[2] but unlike them in that EAc_3-treated animals molted. Male and female lobsters responded alike. Little normal premolt development occurred in the controls, therefore the ecdyses observed were clearly forced by the treatment.

While emulsions were used in our first successful treatments[4] they were undesirable as they would not allow precision in injected doses. We therefore conducted an experiment (table 2) to test for the effectiveness of intramuscular injections of EAc_3 in ethanol (0.1 μl/g). Again, a second treatment was required to induce ecdysis. It would appear that the EAc_3 was at least as effective given this way as in FIA emulsion (table 2) and probably somewhat more effective due to more accurate dosage. We conclude that at the correct dosage, pure EAc_3 can force lobsters into what appear to be normal molts, and that the emulsions we originally used were not necessary. Overdoses of EAc_3 induce lethal premolt development like ecdysterone (table 1). Since the difference in effectiveness between ecdysterone and its triacetate is so clear cut with the lobster, a re-examination of the development induced by ecdysterone in other crustaceans and insects should be done but with careful dose-response determinations.

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COGITATIONES

How to view stereoscopic pictures of crystal structures and molecular models

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Summary. Methods are presented for practicing the viewing of stereoscopic pictures of crystal structures and molecular models without optical aids.

Our world, including chemistry, is three-dimensional. Thus, any two-dimensional representation of crystal structures, molecular models and the like in books or journals is a sort of compromise. Perspective drawings for instance, can evoke the feeling of space and shape only to a certain extent. There exist, however, several procedures for the printing of stereoscopic pictures. These pictures, when properly viewed, give realistic three-dimensional impressions.

The Xograph technique was used to illustrate enzyme-substrate interaction in the case of lysozyme[1]. This procedure gives beautiful colour pictures which show full three-dimensionality with no need for optical devices or special viewing skills. However, Xographs cannot be printed on regular pages as they have to be laminated with a plastic viewing screen. Accordingly the production costs are prohibitively high.

A. Meister et al. published stereophotographs of CPK models of α-aminoadipic acid enantiomers and analogs in connection with a study on the substrate specificity of sheep brain glutamine synthetase[2]. The pictures were printed in such a way that a three-dimensional view of the models could be seen with the aid of a mirror.

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