In a reciprocal experiment, oestradiol increased corticosterone binding; the effect was maximal in presence of 60 times greater nonradioactive oestradiol than labelled corticosterone in the incubation mixture but never attained the level observed in the previous case. In all these experiments, the binding of the respective steroid was greatly diminished in presence of 100-fold excess of the homologous steroid, confirming that the observed effects are an expression of a specific binding to the cellular receptor.

Thus, activation of the heterologous steroid-receptor complex was a function of the affinity of the activator for its own receptor; maximum corticosterone stimulation (at 6×10^{-8} M) is to be compared with affinity (about K_d 10^{-10}) of the corticosteroid-receptor complex vs maximum oestradiol effect (6×10^{-7} M) and high affinity (about K_d 6×10^{-9}) of the oestradiol-receptor complex in the liver.

Data in table 2 show that glucocorticoid stimulation of liver gluconeogenesis was reversed by oestradiol; triamcinolone was used in this experiment because it is more potent than the natural glucocorticoid. Stimulation of receptor binding (table 1) was therefore associated with oestrogen-induced antagonism at the level of the physiological action of the glucocorticoid (table 2). This paradox cannot be resolved at the moment. Furthermore, there is no satisfactory biological parameter responsive to oestradiol in the liver.

What is the physiological significance of these findings? One possibility would eliminate the mediation of receptor function in the physiological action, but this seems highly unlikely. Another alternative would place recognition at the level of nuclear acceptors rather than the cytoplasmic receptor. Still another mechanism would lie in separate influences of the oestradiol-receptor and the glucocorticoid-receptor complex whose total sum is seen as the ultimate physiological response without indication of the preceding steps in the chain of events. The possibility may also be entertained for the presence of an effector site on any given R-S complex where a heterologous steroid could modulate stereospecific configuration. The fact that cortexolone, which is biologically inactive in vivo, was nevertheless able to activate in vitro is in favour of this latter hypothesis. Whatever the ultimate explanation, the fact that the presence of one steroid-receptor complex activates the receptor association of another, may have a physiological role, although the reasons for such a synergism have still not been elucidated; this would call for inhibition of receptor function for 1 set of steroid hormones by a specific inhibitor that is technically unfeasible at this time.

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Endocrine basis of wing casting and flight muscle histolysis in the fire ant *Solenopsis invicta*[1]

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Summary. In the imported fire ant *Solenopsis invicta*, wing casting and flight muscle histolysis are blocked by allatectomy. Treatment of alate allatectomized females with a synthetic mixture with high juvenile hormone activity induced wing casting and flight muscle histolysis. Apparently, wing casting and flight muscle histolysis in the fire ant are part of postemergence developmental program regulated, directly or indirectly, by the corpora allata.

Flight muscle histolysis in the Formicidae has been recognized as potentiating species survival by providing protein for oocyte development[2-4]. Alate virgin queens normally remain alate and retain the flight muscle until mated. Once the mating flight and insemination have occurred, the wings and flight muscle have served their primary function in dispersal and reproduction of the species. Subsequently, the wings are cast and the newly mated queen burrows into the soil after which ovarian development and flight muscle histolysis ensue[2,3]. Oocyte development in *S. invicta* is regulated by the corpus allatum (CA)[5] and in the following report, evidence is presented that flight muscle histolysis and wing casting in *S. invicta* require the presence of the CA.

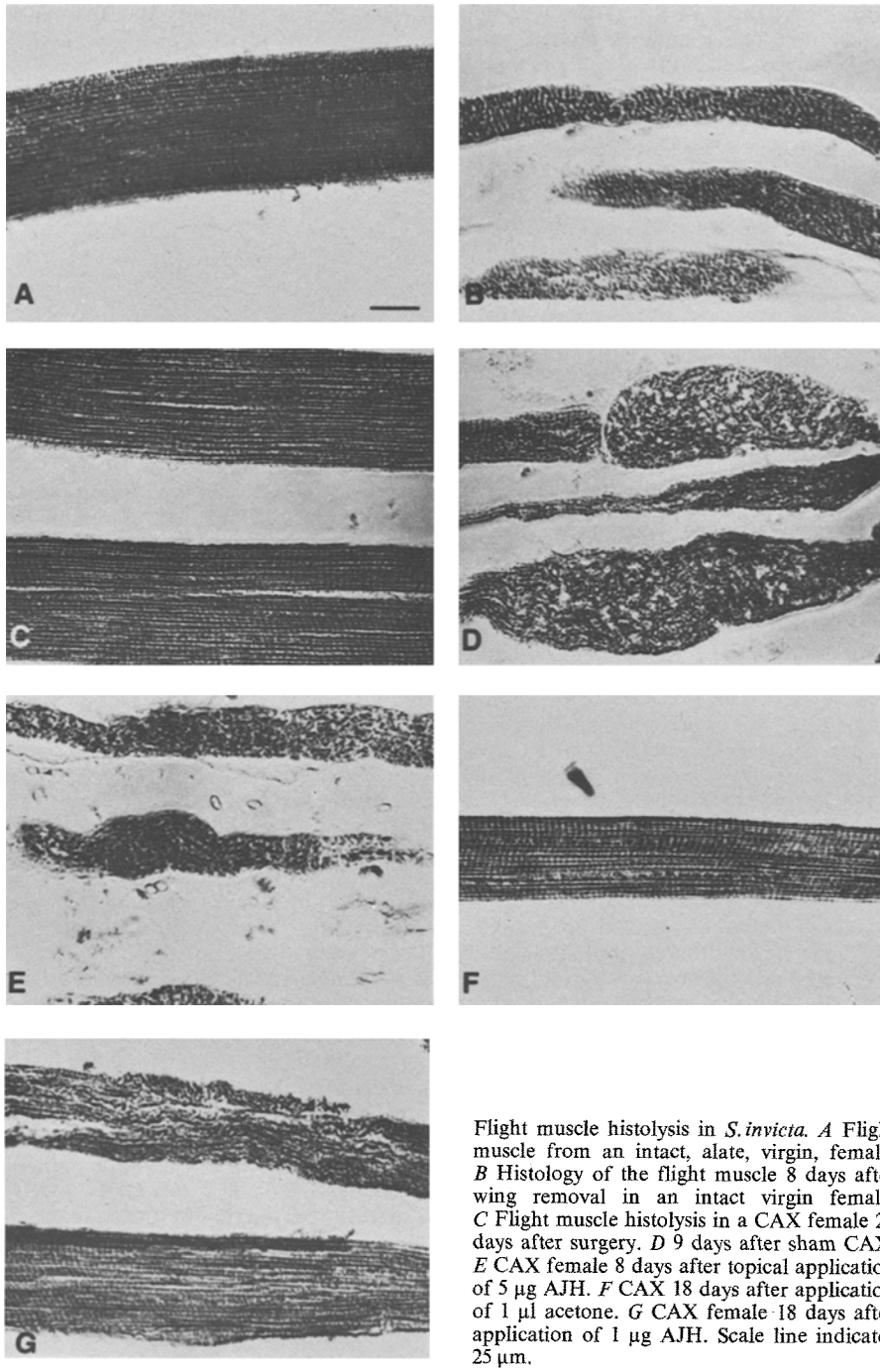
Materials and methods. Data were obtained using classical techniques of allatectomy (CAX), juvenile hormone replacement, and the subsequent effect of these procedures on flight muscle histolysis was examined histologically. Wing casting, flight muscle histolysis, and oocyte development in intact animals were induced and synchronized with CO_2. Specific details of surgical procedures, CO_2 treatment, and holding conditions have been described elsewhere[5]. The juvenile hormone used was a gift from Ayerst Laboratories Montreal, Canada and is referred to, herein, as AJH. AJH is a synthetic mixture of 8 possible geometric isomers of *Cecropia* juvenile hormone. Flight muscle was fixed in Gomori 1-2-3 fixative and stained with Heidenain Iron Hematoxylin[6]. All CAX animals and their controls were treated with CO_2 beginning on the 2nd day following surgery. A few animals which cast their wings immediately after surgery are not included, however, these animals retained their flight muscle.

Results and discussion. In contrast to intact animals, repeated treatment of 5 alate CAX females with CO_2 for 10 min on each of 15 consecutive days failed to induce casting of the wings. When alate CAX females were treated with 1.5 or 10 µg AJH 10 days after surgery, all responded by casting their wings (table). 22 of the 25 animals which responded to AJH (90%) did so in the following 12 h period after topical

Effect of AJH on wing casting of alate CAX females

	Dosage 1 µg (13)	5 µg (10)	10 µg (6)	Acetone (13)
Animals casting wings	11	8	6	0
% casting	85	80	100	0

( ) indicates the number of animals treated with the indicated dosage.



Flight muscle histolysis in *S. invicta*. *A* Flight muscle from an intact, alate, virgin, female. *B* Histology of the flight muscle 8 days after wing removal in an intact virgin female. *C* Flight muscle histolysis in a CAX female 28 days after surgery. *D* 9 days after sham CAX. *E* CAX female 8 days after topical application of 5 µg AJH. *F* CAX 18 days after application of 1 µl acetone. *G* CAX female 18 days after application of 1 µg AJH. Scale line indicates 25 µm.

application. The remaining 10% responded within 36 h. Flight muscle histolysis was examined in intact females stimulated with CO_2 in order to determine the timing of histolysis under these conditions. 3 animals were sacrificed each day for 10 days after removal of the wings and the progress of histolysis examined. Flight muscle histolysis was usually complete by the 10th day. Figure A shows the flight muscle in an alate and figure B shows flight muscle histolysis in an animal 8 days after wing removal. Fibril bundles lost integrity as histolysis progressed until the paired longitudinal flight muscles and dorso-ventral flight muscles on each side of the thorax were resorbed. Subsequently, flight muscle histolysis in 5 alate CAX females was examined 28 days after surgery, and all were found to have retained their flight muscle (figure C). 5 sham operated females resorbed their flight muscle within 10 days or less (figure D). Topical application of 5 µg AJH to 6 CAX females induced flight muscle histolysis as shown in figure E. Flight muscle resorption 8 days after application of 5 µg AJH was comparable to sham operates 8 to 10 days after removal of the wings. In contrast, 13 acetone CAX controls retained their flight muscle (figure F). 16 additional animals treated with 5 or 10 µg AJH showed complete flight muscle histolysis when examined 18 days after AJH application. Dose dependency was indicated when 13 animals treated with 1 µg AJH did not show complete flight muscle histolysis 18 days after application (figure G). Collectively, these data suggest that mating (or CO_2 narcosis) induce neuroendocrine events which stimulate secretion of JH by the CA. Directly or indirectly under the influence

of the CA, the following changes in the post-emergence reproductive development of the fire ant are initiated: wing casting, flight muscle histolysis, and oocyte development.

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Ecdysteroids in *Limulus* larvae[1]

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Summary. Free ecdysteroids were extracted from intermolt and premolt larvae of *Limulus polyphemus* in the first post-hatch stage, purified by TLC and HRLC, and assayed by RIA and the *Limulus* bioassay. 20-hydroxyecdysone appears to be a principal ecdysteroid and occurs at least 3 times higher a concentration in premolt vs intermolt animals.

Ecdysteroids have been identified in many species of crustaceans and insects but little is known about these compounds in other classes of the phylum Arthropoda. Injected ecdysteroids are effective in stimulating molting in representative members of the major classes of arthropods[3,4], yet the majority of the work on extracting and identifying these compounds has been performed on crustaceans and insects; there are, however, many references to the presumption that 20-hydroxyecdysone(ecdysterone) is a general arthropod molting hormone[5]. This compound is very effective as a molt-promoting agent in *Limulus* larvae, and here we report evidence for its occurrence in these animals.

Material and methods. Larvae were collected on Shackleford Banks near the Duke Marine Laboratory in 2 batches. 1 batch was freshly hatched and the larvae were cultured at 25 °C in large glass bowls until they were in premolt, then frozen until extraction. A 2nd batch was collected as larvae hatched in the sand substrate, cultured for 2 days at 25 °C (this produces larvae in intermolt, less than half way through the molt cycle), then frozen. Extraction was based on the method of Karlson and Shaaya[6]. These crude extracts were further purified by 1 of the following 2 methods. a) The 1st consisted of extensive clean-up by TLC on silica gel G plates (Analtech) using 3 solvent systems ($CHCl_3$:EtOH, 4:1 followed by EtOAc: MeOH:NH_4OH:H_2O, 10:2:1:1 and $CHCl_3$:MeOH: HOAc, 4:1:0.05) and 2 ecdysteroids (20-hydroxyecdysone and ponasterone A) as standards. After the 1st system 4 prominent bands in the exdysteroid zone were eluted, re-chromatographed in the 2nd system where most of the polar material was separated from the ecdysteroids. The narrow ecdysteroid zone was eluted and re-chromatographed in the 3rd solvent system. 2 bands were eluted from the plates for assay; one that co-chromatographed with 20-hydroxyecdysone (R_f 0.36) and one conspicuous band (R_f 0.52) chromatographing below ponasterone A (R_f 0.60). b) The 2nd method consisted of an initial clean-up by preparative high resolution liquid chromatography (HRLC) using a Poragel PN column and 50% MeOH:H_2O as the solvent system at a flow rate of 0.6 ml/min. Retention times for 20-hydroxyecdysone, ecdysone and the ponasterone A peak were 25–47, 47–75, and 180 min respectively. 9 fractions were collected over a period of 4 h. These were subjected to TLC using the $CHCl_3$:EtOH, 4:1 solvent system. The band co-chromatographing with pure 20-hydroxyecdysone (R_f 0.24) was eluted for assay. Ecdysteroids were detected by a radioimmune assay and the *Limulus* bioassay[4,8].

Results. The RIA and bioassay results are nearly identical for portions of the extract that, in the final purification step, co-chromatographed with 20-hydroxyecdysone (table). The results were also alike when final products from the 2 purification methods were compared. The only known ecdysteroid that at least partially co-chromatographs with 20-hydroxyecdysone in the final purification steps used is inokosterone. Inokosterone is, however, much less reactive in RIA (4×) than bioassay when compared to 20-hydroxyecdysone, and, if it were present in anything but small quantities, the results from RIA and bioassay would not be similar. Therefore we conclude that 20-hydroxyecdysone is the ecdysteroid we extracted.

The unknown band which resulted from the extract subjected to extensive TLC clean-up showed low activity in the RIA test but very high activity in the bioassay (table). Some pure ecdysteroids, such as makisterone A and cyasterone, give the same kind of effect in our detection methods. The unknown is, however, neither of these compounds. We have no details on the nature of this unknown material, except that it chromatographs between ecdysone and ponasterone A on TLC in a $CHCl_3$:MeOH, 4:1 system and is several hundred times more active in the bioassay than in the RIA. Data in the table also show approximately a 3 times greater concentration of 20-hydroxyecdysone in the premolt than during the intermolt stage in both RIA and bioassay. This finding is in agreement with data on injections of exogenous 20-hydroxyecdysone where only about 100 pg will accelerate molting in 50% of the experimental animals in late intermolt-early premolt as opposed to 500 pg or more for mid-intermolt animals. Changing cell

Quantification of free ecdysteroids in *Limulus* larvae at 2 different stages in the molt cycle

Ecdysteroid	Stage	Detection method	20-hydroxy-ecdysone equivalents pg/larva	ng/g
20-Hydroxyecdysone	Intermolt	RIA	47	9.4
		Bioassay	53	10.6
	Premolt	RIA	135	27.0
		Bioassay	179	35.8
Less polar unknown	Premolt	RIA	32	6.4
		Bioassay	946	189