ty; in fish pituitary preparations, U_I appears to be more potent than the other two peptides in ACTH release (*21*). Thus, our discovery of the sequence homology between U_I and CRF has brought to light a new potential role for CRF as a modulator of cardiovascular dynamics in mammals, as well as revealing a potential role for U_I as a pituitary releasing factor in fish.

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13. Isolation of tryptic, SAP, and CNBr fragments and their analysis were carried out by D. McMaster. In the Edman degradation, a Beckman 890C spinning cup sequencer with a Sequemat P-6 Autoconverter was used. Phenylthiohydantoin amino acids were identified by HPLC [C. L. Zimmerman, E. Appella, J. J. Pisano, *Anal. Biochem.* **77**, 569 (1977)] and by back hydrolysis [E. Mendez and C. Y. Lai, *ibid.* **68**, 47 (1975)].
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21. U_I is at least three times as potent as either the ovine CRF or sauvagine in the stimulation of ACTH release from perfused gold fish pituitaries (J. Fryer, K. Lederis, J. Rivier, unpublished observations).
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Synergistic Action of Potassium Chloride and Magnesium Sulfate on Parasitoid Wasp Oviposition

Abstract. *A dilute solution of potassium chloride and magnesium sulfate induces oviposition in artificial eggs by the egg parasitoids* Trichogramma pretiosum *and* T. minutum. *The ability to obtain large numbers of eggs through the use of this inexpensive solution is a major advance toward the development of diets and the large-scale production of* Trichogramma *spp. in vitro.*

Insecticides are not always effective or desirable for the control of many species of insects which cause enormous losses of food and fiber. Egg parasitoids of the genus *Trichogramma* are especially promising biocontrol agents because (i) they parasitize a wide range of host insects (*1*), (ii) they destroy the host in the egg so that no larval feeding damage occurs, (iii) they are readily manipulated and mass-reared in the laboratory, and (iv) host-seeking chemicals are available to enhance the effectiveness of released and indigenous parasitoids in the field (*2*). *Trichogramma* spp. are the most widely studied entomophagous insects and are used for biocontrol on a global basis both in developed and developing countries. Augmentative releases of these parasitoids are restricted by the expense associated with the production of host eggs. Economical mass production of parasitoids on artificial diets (*3*) in quantities suitable for augmentative releases requires techniques for the collection of large numbers of *Trichogramma* spp. eggs. We report the development of an artificial ovipositional stimulant as active as the host egg, which induces *Trichogramma* spp. to deposit eggs in quantities suitable for mass production.

Previous work (*4*) with solutions encapsulated in artificial wax eggs demonstrated that Neisenheimer's solution stimulated oviposition by *T. californicum* Nagaraja and Nagarkatti and "no particular salt, amino acid, or vitamin tested was seen to be essential for eliciting oviposition" (*5*). However, comparisons of the ovipositional activity of *Trichogramma* spp. in lepidopteran hemolymph (*6*) to that in Neisenheimer's solution indicated that the latter solution likely is a weak ovipositional stimulant for *T. californicum*; we observed that it was a poor ovipositional stimulant for *T. pretiosum* Riley. We found that a solution of KCl and $MgSO_4$ is several hundred times more active than Neisenheimer's solution for both *T. pretiosum* and *T. minutum* Riley.

Trichogramma pretiosum and *T. minutum* were reared on eggs of *Sitotroga cerealella* (Olivier), the Angoumois grain moth (*7, 8*). The *p*H of the aqueous test solutions was adjusted to 7.00 with either NaOH (for Neisenheimer's solution) or KOH (for all other solutions). Test solutions were encapsulated inside wax spheres (*4, 5, 9*).

Lepidopteran larval hemolymph generally is rich in potassium and magnesium and is low in sodium (*10*). Because the composition of Neisenheimer's solution (rich in Na^+, low in K^+, and devoid of Mg^{2+}) is different from that of lepidopteran hemolymph and the host's food, we hypothesized that a mixture of K^+ and Mg^{2+} is a better ovipositional stimulant.

Although elemental analyses of *He-*

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Table 1. Effects of salt solutions and water on oviposition by *Trichogramma* spp.

Solution tested	Concentration of salts (m*M*)							Eggs in wax eggs (%)*						
								T. pretiosum					*T. minutum*	
	KCl	$MgSO_4$	NaCl	$NaHCO_3$	KH_2PO_4	NaH_2PO_4	$CaCl_2$	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7
KCl-$MgSO_4$														
A	124.7	36.5			1.0			99.9a	99.8a	99.8a			99.7a	
B	83.1	24.3			1.0						98.0a	99.8a		86.4
Neisenheimer's														
$-NaH_2PO_4$	1.3		128.3	2.4			1.8	0.1b		0.0b			0.2b	
$+NaH_2PO_4$	1.3		128.3	2.4		1.0	1.8		0.2b	0.2b			0.1b	
KCl	83.1				1.0						1.9b			6.2b
$MgSO_4$		24.3			1.0						0.1b			7.4b
Water												0.2b		
								Mean number of eggs per test						
								642	568	398	3086	2436	3689	1611
								Number of tests						
								9	12	8	9	9	10	10

*Numbers followed by different letters in the same column are statistically different ($P < .01$) from each other on the basis of a multiway classification using the chi-square test (*16*).

liothis zea (Boddie) hemolymph have been made (*11*), we based the formulation of test solutions on elemental analyses of whole larvae of *H. zea* and *Heliothis virescens* (Fabricius) (*12*). We used the analyses of late last instar larvae because the potassium and magnesium composition at that stage of development probably more closely resembles the composition of the host egg where oviposition occurs. The concentration of mixture B (Table 1), based on our potassium-magnesium analyses of whole *Heliothis* larvae, was 0.083*M* KCl and 0.024*M* $MgSO_4 \cdot 7\ H_2O$. Mixture A was a 50 percent higher concentration of mixture B (*13*).

Although Neisenheimer's solution (unbuffered) was reported to be one of the better, if not the best, ovipositional stimulants for *T. californicum* (*4, 5*), we found that mixture A of KCl-$MgSO_4$ was 500 to 1000 times more active than either unbuffered or buffered Neisenheimer's solution for both *T. pretiosum* and *T. minutum* (tests 1, 2, 3, and 6 in Table 1). When tested against KCl-$MgSO_4$ mixture B, water was about as active as Neisenheimer's solution for *T. pretiosum* (test 5).

Experiments in which KCl and $MgSO_4$ were tested either separately or together demonstrated that synergism occurred when the two salts were combined (tests 4 and 7). The synergistic effect was greater with *T. pretiosum* (49-fold increase in activity over the sum of KCl and $MgSO_4$ alone, test 4) than with *T. minutum* (sixfold increase, test 7).

Fifty *T. pretiosum* females, exposed for 4 hours either to three artificial eggs containing KCl-$MgSO_4$ mixture A or to 40 *H. virescens* eggs, oviposited about ten eggs per female in each treatment. When 50 females were exposed to KCl-$MgSO_4$ mixture A (four tests, five replicates per test), newly emerged unfed *T. prestiosum* and *T. minutum* oviposited 9.8 ± 2.5 (standard error) and 13.0 ± 3.0 eggs, respectively, per female in a 4-hour period. These results were based on dissection. Thus, the KCl-$MgSO_4$ mixture, which elicits oviposition at least as well as the host egg, is a very active ovipositional stimulant (*14*).

The foregoing is unequivocal evidence that an ovipositional stimulant or any other kairomone affecting any form of behavior of an entomophagous parasitoid is composed of more than one inorganic chemical. Either $MgCl_2$ (0.025*M*) or trehalose (but not both) in combination with serine (0.5*M*), arginine (0.05*M*), and leucine (0.065*M*) elicited oviposition by *Itoplectis conquisitor* (Say) (*15*).

As far as the behavior of entomophagous parasitoids is concerned, we know of no reports implicating K^+ as an ovipositional stimulant or that synergism occurs when two inorganic salts are combined in one solution.

Potassium and magnesium may be an ovipositional stimulant for other species of entomophagous parasitoids because these elements are present in the hemolymph of most species of host insects. Because of the possibility of synergism and the presence of sizable quantities of potassium and magnesium in flowering plants, the behavior of economically important insects other than entomophagous parasitoids also may be affected by potassium and magnesium.

The KCl-$MgSO_4$ solution is a major improvement as an ovipositional stimulant. This simple and inexpensive means of collecting thousands of eggs per day now allows research to be conducted efficiently on artificial diets for *Trichogramma* spp.

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parasitoids were reared at 27°C and 80 percent relative humidity, except when the pupae were kept at 16°C. Adults used in the tests emerged soon after the temperature was changed to 27°C.

9. The preparation of the artificial eggs has been described (*4, 5*). The resulting artificial egg had a diameter of about 2.5 mm. Each glass slide held three wax eggs, and a slide for each solution tested was placed inside a petri dish (10 by 1.5 cm) that was modified by removal of the lid tabs to prevent the escape of the minute parasitoids. The sex ratio was 1:1 and we usually used about 300 to 600 of the adult female parasitoids in each dish. The number varied because counting these small insects precisely was difficult. The petri dishes containing the artificial eggs and *Trichogramma* were rotated at 1 rev/min for 16 hours. Half of each test group was held at 27°C and 80 percent relative humidity and the other half was exposed to 25°C and 40 percent relative humidity. The artificial eggs were broken open, and visual counts of the *Trichogramma* eggs were made. Because of the variable number of parasitoids in each dish, we expressed the results as percentages of the total number of eggs collected in the artificial eggs inside each petri dish. Within the range used there was no effect from the number of parasitoids on the percentage distribution of eggs between test solutions. Holding conditions (temperature and relative humidity) had no effect on percentage distribution of eggs, but the number of eggs deposited was reduced at the lower temperature and relative humidity.
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13. Mixture B is about twice as active as A, and B is about 15 times more active than a test solution of KCl-$MgSO_4$, on the basis of host egg analyses.
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Reversal of Morphine Disruption of Maternal Behavior by Concurrent Treatment with the Opiate Antagonist Naloxone

Abstract. *Rats whose pregnancies were surgically terminated on day 17 of gestation were injected with morphine, morphine plus naloxone hydrochloride, or saline, and then tested for maternal responsiveness toward foster young. Morphine treatment alone significantly disrupted the rate of onset and quality of maternal responsiveness. Concurrent administration of naloxone to morphine-injected rats reinstated the rapid onset of behavioral responsiveness toward foster young, such that the responsiveness of the rats treated with both morphine and naloxone was indistinguishable from that shown by saline-injected controls. The disruptive effects of morphine did not appear to result from a general reduction in activity levels as measured in an open-field apparatus. These findings suggest that the normal onset and maintenance of maternal behavior in the rat may be regulated by endogenous opiates.*

Endogenous opiate concentrations have recently been shown to change during the course of pregnancy and lactation. The immunoreactivity of β-endorphin increases during the second half of pregnancy in the hypothalamus of rats (*1*) and in the last trimester in the plasma of women (*2*). Changes in peptide concentrations during this reproductive period appear to be correlated with changes in sensory and behavioral processes. In rats, pain thresholds rise gradually during the second half of pregnancy, increase sharply about 2 days before parturition, and return to prepregnancy levels postpartum. A further decline in pain thresholds below that of control levels is found during lactation in the rat (*3*). These changes in pain thresholds appear to be opiate-mediated, since treatment with the opiate antagonist, naloxone hydrochloride, lowers pain thresholds during this reproductive period (*3*).

We have investigated the possibility that changes in opiate levels during pregnancy and parturition may reflect changes in central nervous system function and influence the expression of maternal behavior at the time of birth and during lactation. The onset of maternal behavior is hormonally (estradiol, progesterone) regulated in rats (*4*). Recent findings indicate that estradiol, the predominant sex steroid present in the mother at parturition (*5*), lowers brain β-endorphin concentrations in female rats (*1*). We hypothesized that the low levels of opiate activity during the postpartum period (*3*) may be involved in the regulation of maternal responsiveness in the rat. We therefore examined the effects of the opiate morphine and the opiate antagonist, naloxone hydrochloride, on the onset and quality of maternal responsiveness in rats after termination of pregnancy. This procedure stimulates the fast onset of maternal responsiveness as a result of endogenous changes in hormone secretion (*4, 6*). We focused on the period after pregnancy termination, reasoning that if lower opiate concentrations at the time of parturition are essential for maternal behavior, increasing the concentration of opiates after pregnancy termination would disrupt the behavior. Indeed, we found that morphine treatment delayed the onset and diminished the quality of maternal behavior after pregnancy-termination, and that these effects could be reversed in morphine-treated rats by the concurrent administration of the opiate antagonist naloxone hydrochloride.

Virgin female rats (Sprague-Dawley strain, Charles River Breeding Laboratories) weighing 225 to 250 g were mated in our laboratory. The morning after mating (day 1 of pregnancy) females were individually housed in polypropylene cages (25 by 45 by 20 cm). Food (Purina Rat Chow) and water were constantly available and room temperature was maintained from 21° to 24°C. In the first experiment 25 rats were ovariectomized and hysterectomized on day 17 of pregnancy as described (*6*). This procedure results in a high incidence of maternal responsiveness 22 to 24 hours later.

At the completion of surgery the females were assigned to one of three treatment groups, and received a subcutaneous injection of morphine sulfate (5 mg/kg), morphine sulfate (5 mg/kg) plus naloxone hydrochloride (0.5 mg/kg), or 0.9 percent saline (0.5 ml). All animals received the same dosages again 19 to 22 hours later, 1 hour before they were tested for maternal behavior.

To test for maternal behavior, we placed three recently fed rat pups (2 to 8 days old) in the home cage of the female and monitored her behavior continuously for 15 minutes. We checked her behavior again at 30, 45, and 60 minutes. Latencies to carry a pup, retrieve pups to the nest site, and crouch over the young were recorded. The pups and the females remained together until the following morning when their positions were again recorded; this checkpoint was referred to as the post-test rating. Pups were then removed from the home cages and the females were injected with freshly prepared solutions of morphine sulfate, morphine sulfate plus naloxone, or saline, at the same dosages as before. An hour later the test for maternal behavior was repeated with another set of recently fed pups. Each female was tested for 11 days or until she responded fully (that is, retrieved, grouped, and crouched over all three pups) during the 1-hour test on two consecutive test days. Animals that responded fully in the home cage were then tested for the intensity of maternal responsiveness in a T-maze apparatus for 4 days (*7*).

Morphine treatment significantly dis-

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