acyl chains were degraded and resynthesized, as this would result in a specific loss of tritium. Tritium was not lost by a direct mechanism such as Δ^9 desaturation since [1-^{14}C]myristate-labeled TDA consisted only of the Δ^{11} isomer. The findings indicate that most radioactivity is not directly incorporated into TDA from an exogenously added fatty acid, but results instead from de novo synthesis after complete β-oxidation.

The inability to detect direct desaturation of myristate could reflect differences in the rates or intracellular sites of desaturation and degradation processes that greatly favor β-oxidation. Alternatively, the CoA derivatives used in β-oxidation and as substrates for mammalian Δ^9-desaturases (*12*) may not be the preferred form for desaturation in insects. The possibility that desaturation occurs during fatty acid biosynthesis, as in bacterial systems (*13*), cannot be ruled out, although this process has not yet been observed in eukaryotic cells. It is of interest, in this regard, that the only Δ^{11}-desaturases reported have been in bacteria (*14*) and in insect tissue (*1*). The observation of direct Δ^{11}-desaturase activity in the bacterial system was facilitated by the lack of de novo fatty acid biosynthesis, and it may be necessary to reduce the level of biosynthesis or degradation of fatty acids before direct Δ^{11}-desaturation can be demonstrated in the spruce budworm.

Although it has not been possible to identify the exact route of TDA biosynthesis, our findings suggest that this compound is synthesized de novo from acetate even in the presence of exogenously added fatty acids. This observation may be relevant to the biosynthesis of the many other lepidopteran sex pheromones that contain acetate ester functional groups. In the budworm, however, the acetate ester appears to act as a pheromone precursor, as it is synthesized specifically in the pheromone gland and is degraded concurrently with pheromone release. The conversion of an acetate ester precursor into an alcohol or aldehyde pheromone could easily have arisen in evolution to provide an increase in sex pheromone specificity within a group of closely related species. The increase in specificity provided by modification of the functional group can be achieved without causing a major change in the biosynthesis or regulation of pheromone production.

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8. This technique is based on that described by L. B. Bjostad and W. L. Roelofs [*J. Biol. Chem.* **256**, 7936 (1981)], except that a 1-μl solution containing 20 nmol of labeled compound in DMSO was applied to 20 female glands. No excess DMSO was left on the gland surface.
9. Saponification was performed by dissolving the lipid in 0.5 ml of 0.5*N* KOH in 90 percent methanol, followed by a 60-minute incubation at 60°C. Neutral lipids were extracted from the saponification mixture with 2 × 1.0 ml of hexane after addition of 0.5 ml of water. In control experiments with tetradecanyl acetate labeled in the tetradecanol and acetate moieties, respectively, 98 percent of the alcohol and less than 1 percent of the acetate was extracted after saponification.
10. The procedure used was modified from E. von Rudloff [*Can. J. Chem.* **34**, 1413 (1956)]. Only 1 μg of labeled lipid was redissolved in 0.5 ml of *t*-butanol, to which was added 0.5 ml of oxidant (97.5 m*M* $NaIO_4$ and 2.5 m*M* $KMnO_4$) and 0.5 ml of 25 m*M* $KHCO_3$. After a 60-minute reaction at room temperature, the mixture was acidified with one to two drops of concentrated H_2SO_4 and extracted with 2 × 1.0 ml of hexane.
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Facilitated Sexual Behavior Reversed and Serotonin Restored by Raphe Nuclei Transplanted into Denervated Hypothalamus

Abstract. *Fetal raphe cells transplanted into the hypothalamus reversed facilitation of feminine sexual behavior in rats with brain lesions induced by 5,7-dihydroxytryptamine. Immunocytochemical and chemical analyses of serotonin indicate that reinnervation of the ventromedial nucleus of the hypothalamus by the transplants is associated with behavioral recovery. The findings suggest that transplanted fetal tissue can exert functional regulation over an innate, complex, hormone-dependent behavior in adult rats.*

The hypothalamus is an obligatory site for the mediation of gonadal hormone–dependent sexual behavior in the female rat. Within the hypothalamus, the ventromedial nucleus is believed to be critical for transmitting signals that regulate neuroendocrine circuits for reproductive behavior, since hormonal stimulation of this nucleus alone is sufficient to activate such behavior (*1*). Serotonergic systems are believed to play a major role in the hormonal regulation of sexual behavior through tonic inhibitory inputs to the hypothalamus (*2*). Consistent with this hypothesis, selective lesioning of serotonin-containing fibers in the hypothalamus by treatment with 5,7-dihydroxytryptamine (5,7-DHT) results in enhanced sexual behavior after administration of estradiol (*3*). Facilitated behavior is shown for up to 8 weeks and disappears coincident with reinnervation of the hypothalamus by serotonin-containing fibers 9 to 10 weeks after 5,7-DHT treatment (*4*).

Previous studies established that fetal brain cells can survive transplantation into neonatal, adult, and aged brains (*5*). Such transplants reverse stereotypical drug-induced behaviors seen after specific chemical lesioning of the adult host brain (*6*) and ameliorate age-related motor and learning deficits in the aged host (*7*). In addition, transplantation of preoptic tissue from a neonatal rat into the preoptic area of another neonatal rat increases the sexual behavior of the hosts in adulthood (*8*). We now report the ability of fetal cells transplanted into an adult brain to regulate an innate, complex, hormonally dependent behavior of the host. We transplanted fetal tissue rich in serotonergic raphe cell bodies into the hypothalamus of females with 5,7-DHT–induced lesions. The raphe transplants caused a rapid reversal of 5,7-DHT–facilitated sexual behavior and increased serotonin concentrations in the ventromedial nucleus.

One week after ovariectomy adult female rats (Charles River) received desmethylimipramine followed by bilateral stereotaxic application of 5,7-DHT creatinine sulfate (5 μg in 400 nl of ascorbic acid and saline) into the hypothalamus just dorsal to the ventromedial nuclei (*3*). Other females (group S) received sham injections of the ascorbic acid vehicle. One week later fetal tissue was stereotaxically placed into the hypothalamic

area of 5,7-DHT–injected females (*9*). Thirteen females received raphe nuclei (group R); the brains of six of these were analyzed for monoamine content. In the remaining animals serotonin was localized immunocytochemically. Four females received transplants of parietal cortex (group C), and their brains were analyzed for monoamine content also. All rats were housed singly on a reversed day-night cycle and received unlimited food and water.

A female sexual behavior—lordosis—was tested in mating arenas with a stud male during the dark portion of the cycle (*10*). Serotonin was localized immunochemically with an antibody to serotonin and the peroxidase-antiperoxidase method (*4*). Monoamine content in brain nuclei was measured by the Palkovits sampling technique and high-performance liquid chromatography with electrochemical detection (*11*).

Figure 1 shows lordosis quotients for experimental groups up to 7 weeks after transplantation. As previously observed (*3*), treatment with 5,7-DHT resulted in facilitated sexual behavior throughout the experiment. In contrast, transplantation of fetal raphe tissue into 5,7-DHT–treated females (group R rats) resulted in a reversal of facilitated behavior 4 weeks after transplantation. The sexual behavior of these females did not differ from that of the group given sham injections (group S) from 4 weeks until the end of behavioral testing at 7 weeks. Transplants of cerebral cortex into the hypothalamus of 5,7-DHT–treated females (group C rats) did not reverse the facilitated sexual behavior.

The brains of group R females were examined immunocytochemically or chemically to determine the extent and nature of transplant growth and to ascertain the concentrations of serotonin and catecholamines in the hypothalamus. Photomicrographs of serotonin-immunoreactive cell bodies and axons in transverse sections through the hypothalamus of females bearing transplants are shown in Fig. 2. The raphe cells show considerable maturation from the fetal state and are large, multipolar neurons sending processes into the host brain.

Monoamine concentrations in brain nuclei of groups R and C were measured 7 weeks after transplantation (8 weeks after 5,7-DHT) and compared to those of group S (Table 1). At this time lordosis quotients were low in groups S and R and did not differ between them, while group C still showed facilitated behavior. The content of norepinephrine and dopamine was not different between groups in any nuclei examined. Concentrations of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) in the dorsomedial nucleus of both transplant groups had returned to group S levels. In the ventromedial nucleus serotonin was significantly lower in group C than in groups S or R. In contrast, in the vertical nucleus of the diagonal band, a brain region distant from the site of the implant, serotonin and 5-HIAA concentrations were lower in group C than in group S but did not differ from those in group R. In other hypothalamic nuclei, such as the anterior hypothalamic and medial preoptic nuclei, serotonin levels were similar in groups R and C but lower than in group S.

Table 1. Monoamine content in hypothalamic nuclei in groups S, R, and C. Values (picograms per microgram of protein) are means ± standard errors for seven group S rats, six group R rats, and four group C rats. The animals were killed 57 days after being injected with 5,7-DHT (50 days after transplantation surgery). Lordosis quotients were 12 ± 6, 15 ± 6, and 70 ± 16 for groups S, R, and C, respectively.

Nucleus	Group	Serotonin	5-HIAA	Norepinephrine	Dopamine
Ventromedial	S	2.9 ± 0.4	2.5 ± 0.2	10.0 ± 0.8	1.5 ± 0.2
	R	2.7 ± 0.7	2.7 ± 0.6	9.1 ± 0.1	1.4 ± 0.1
	C	0.7 ± 0.3*	1.5 ± 0.3	10.4 ± 0.6	1.2 ± 0.1
Dorsomedial	S	5.2 ± 0.2	4.8 ± 0.5	25.5 ± 1.7	3.0 ± 0.3
	R	6.0 ± 0.7	6.2 ± 0.8	25.2 ± 3.5	3.1 ± 0.3
	C	4.4 ± 0.9	3.9 ± 0.5	29.8 ± 2.3	2.7 ± 0.2
Diagonal band (vertical)	S	4.7 ± 0.4	3.9 ± 0.4	10.0 ± 1.2	1.3 ± 0.2
	R	3.6 ± 0.5	3.2 ± 0.5	10.8 ± 1.1	1.5 ± 0.1
	C	1.9 ± 0.4†	1.8 ± 0.3‡	11.5 ± 2.7	1.8 ± 0.2

*Significantly different from corresponding values for groups S and R ($P < 0.05$). †Significantly different from corresponding value for group S ($P < 0.01$). ‡Significantly different from corresponding value for group S ($P < 0.05$).

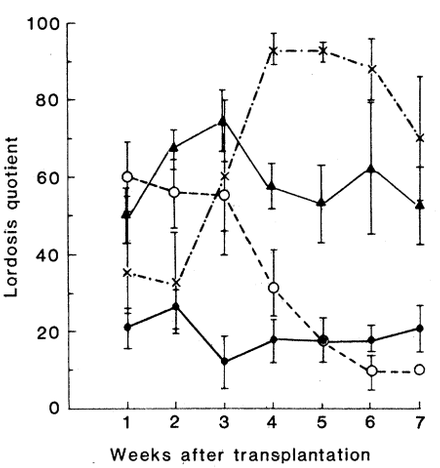

Fig. 1. Effect of raphe transplants on feminine sexual behavior. The hypothalamus of ovariectomized rats was injected with 5,7-DHT or with vehicle alone (●). Seven days later 5,7-DHT–treated rats were given transplants of fetal raphe (○) or cortex (x) or did not receive any further treatment (▲). Estradiol benzoate (2.5 μg) was given and sexual behavior was tested at least once weekly beginning 1 week after transplantation surgery (*10*). Values are mean lordosis quotients ± standard errors for 7 to 28 group S rats, 6 to 21 rats receiving 5,7-DHT only (group D), 4 to 13 group R rats, and 4 group C rats. Data for each week were analyzed by analysis of variance and differences between groups were tested by the Newman-Keuls procedure. Group R differed significantly ($P < 0.05$) from group S at 1, 2, and 3 weeks but not thereafter; group D from group S from weeks 2 to 7; and group C from group S from weeks 3 to 7. Groups D, R, and C were not different from each other at 3 weeks. At 4 weeks groups R and S were not different from each other but were different from the other two groups; and groups D and C were different from each other at 4 and 5 weeks but not at 6 and 7 weeks.

The concentration of serotonin in adult raphe tissue, approximately 20 pg per microgram of protein, was not affected by the presence of raphe transplants in the hypothalamus. Transplanted raphe tissue was identified and assayed in five of the six females, and the mean serotonin concentration was 17.5 ± 6.8 pg/μg. Cortical transplants were identified in only two females, and serotonin levels were 1.7 and 6 pg/μg.

It appears that a complex, hormonally dependent behavior can be disrupted by selective removal of serotonin-containing hypothalamic fibers and can be reinstated by microinjection of minced fetal raphe tissue. Fetal raphe neurons may have the capacity to assume the function of the endogenous neuronal system since the reversal of hormone-dependent behavior is associated with viable serotonin-containing immunoreactive cells and fibers in specific nuclei of the adult hypothalamus. Chemical measurement of serotonin supports the immunocytochemical results showing survival of the fetal cells and indicates that the concentration of serotonin in the transplanted cells is the same as in raphe neurons from normal adult rats.

Measurement of monoamines in hypothalamic nuclei suggests that restoration of the normal behavioral response to estrogen may be dependent on increasing serotonin concentrations in the ventromedial nucleus of 5,7-DHT–treated females. The ventromedial nucleus was

the only hypothalamic nucleus where serotonin was correlated with the behavioral response (Table 1). Previous studies have shown that the ventromedial nucleus is critical in the hormonal regulation of lordotic behavior (*1*). In addition, when serotonin is increased above normal in the ventromedial nucleus, lordosis is inhibited (*12*). The low concentrations of serotonin in the ventromedial nucleus of 5,7-DHT–treated females receiving cortical transplants are consistent with results of previous immunocytochemical and chemical studies of serotonin distribution after intrahypothalamic administration of 5,7-DHT. The ventral hypothalamus is among the last areas to be reinnervated by newly growing serotonin-containing fibers after uni- or bilateral application of 5,7-DHT to the dorsal hypothalamus (*4*). The placement of fetal raphe tissue directly above the ventromedial nucleus appears to be very effective in increasing serotonin in the ventral hypothalamus. Furthermore, these results suggest that hormonal regulation of serotonin in relation to behavior normally occurs at neuronal endings in the hypothalamus rather than at cell bodies in the raphe nucleus. Thus the implants may be supplying the ventromedial nucleus with "permissive" input that is then integrated into reproductive networks by the ventromedial neurons themselves.

Many studies have shown that fetal transplants are effective in reversing stereotypical drug-induced behaviors seen after specific chemical lesioning of the host brain (*6*). While these studies have been valuable for describing interrelations between neural components in defined central pathways, they leave unanswered whether fetal transplants can function in restoring innate, complex behavioral patterns in an adult. From our research it appears that a normal pattern of gonadal hormone–dependent sexual behavior can be reinstated after lesions by transplanting fetal cells containing a specific neurotransmitter into the hypothalamus. Although the hypothalamus integrates many complex neuroendocrine functions, it appears that some of these functions can be modulated differentially by fetal transplants containing specified cells. Transplantation of vasopressin-rich cells into the third ventricle of Brattleboro rats alleviates polydipsia and polyuria in many hosts (*13*). Transplantation of preoptic cells containing GnRH into the third ventricle of GnRH-deficient mice (*hpg* mutant) increases hypothalamic, pituitary, and plasma gonadotropin and initiates spermatogenesis in the testes (*14*). Thus, grafting of fetal cells into the hypothalamus may provide an excellent system for studying neural connectivity and functional regulation in the central nervous system.

In conclusion, our results indicate that the serotonergic system in the ventromedial nucleus of the hypothalamus plays a prominent role in the regulation of reproductive behavior in the female rat and that transplanted fetal tissue can exert functional regulation over such behavior. Transplantation techniques may ultimately prove effective in reversing deficits in other complex, neurally mediated behaviors caused by disease or degeneration.

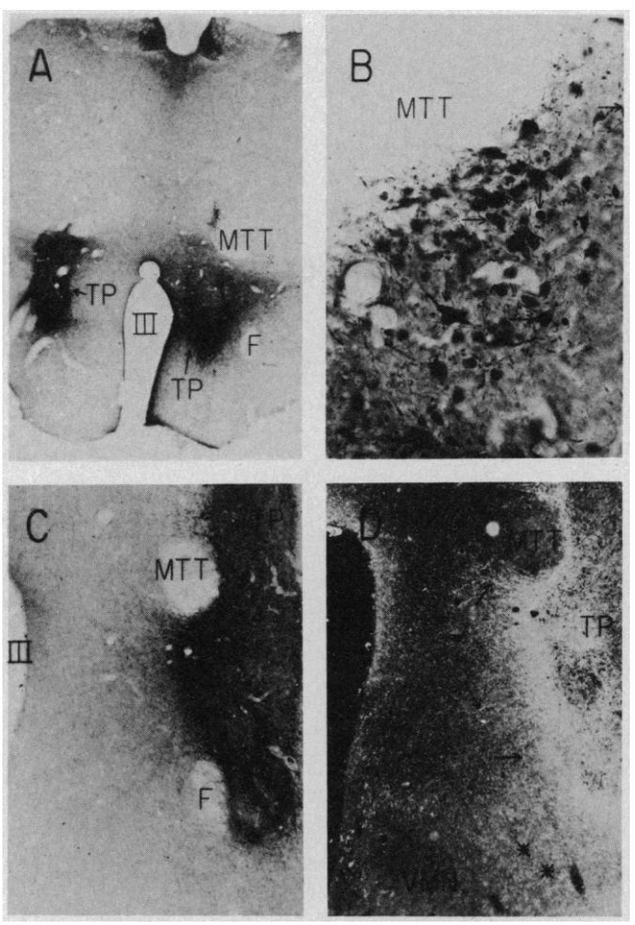

Fig. 2. Photomicrographs showing serotonin-immunoreactive cell bodies and axons in transverse sections of the rat hypothalamus (not counterstained). (A) Low-power view of transplanted fetal raphe tissue (TP) located bilaterally on either side of the third ventricle. The sites where minced fetal tissue (~750 nl) was injected are medial to the fornix (F) and ventral to the mamillothalamic tract (MTT). (B) Higher magnification photograph of serotonin - immunoreactive cell bodies and processes in the transplant just ventral to the mamillothalamic tract. The cytoplasm and nuclei are heavily stained with diaminobenzidine reaction product (arrows) while the nucleoli remain unstained. This staining pattern is characteristic of serotonergic cell bodies in adults (*15*). The size and shape of the cells are not similar to those of serotonin-immunoreactive neurons previously described in the hypothalamus (*16*). (C) Light-field photograph of another transplant and adjacent hypothalamic tissue of the host. The immunoreactive somata have migrated toward the medial edge of the transplant, but they do not usually enter the host tissue (*17*). The transplant surrounds both the fornix and mamillothalamic tract. (D) Dark-field photograph of the same section shown in (C). The immunoreactive fibers are exiting toward the medial hypothalamus (arrows). A few fibers (asterisks) have reached the edge of the ventromedial hypothalamic nucleus (VMN), pars lateralis. The pattern of serotonergic innervation in the VMN surround is similar to the normal pattern (*3*).

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10. Males were allowed to mount each female ten times and the lordosis quotient (number of lordotic responses/number of mounts × 100) was calculated. Estradiol benzoate (2.5 μg, subcutaneously; Sigma) was administered each day for 2 days before testing. All groups were tested at least once per week. The seven females who received raphe tissue and were examined immunocytochemically also received progesterone and an additional behavior test before being killed. Lordosis scores were maximal (87 ± 13), indicating that the transplants had not interfered with brain centers responsible for lordotic responding.
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Induction of Interleukin 2 Messenger RNA Inhibited by Cyclosporin A

Abstract. *Cyclosporin A blocked production of the lymphokine interleukin 2 by activated T lymphocytes. In a human and a murine cell line this inhibition reflected an absence of interleukin 2 messenger RNA. Under conditions in which these cells are normally stimulated to secrete high levels of interleukin 2, they failed to do so in the presence of cyclosporin A. In both cell lines this failure was accompanied by an absence of interleukin 2 messenger accumulation.*

Cyclosporin A (CsA) is an effective immunosuppressant that has proved useful in organ transplantation. Its mode of suppression may be based, at least in part, on its ability to inhibit production of the lymphokine interleukin 2 (IL-2) by T lymphocytes (*1, 2*). Stimulation of T-lymphocyte growth by IL-2, on the other hand, is not sensitive to CsA (*2–4*). Failure to detect IL-2 synthesis in leukocyte populations in the presence of CsA, however, is not definitive evidence that IL-2 is not being generated. Rapid utilization or destruction of the secreted lymphokine could account for its absence. Furthermore, even if it can be shown that IL-2 is not being produced, it is desirable to determine whether the block created by CsA is at the level of transcription, translation, or post-translational processing. We therefore examined the effects of CsA at the level of IL-2 messenger RNA (mRNA). Several T-lymphocyte lines produce IL-2 in response to stimulation, including mouse T-lymphoma line EL4 (*5*) and human T-cell leukemia line Jurkat (*6*). We found that lines EL4 and Jurkat are both inhibited from producing IL-2 by CsA and that this inhibition is correlated with an absence of IL-2 mRNA measured biologically or chemically.

Subclone EL4.E1 of line EL4 (*5*) was selected for its high level of IL-2 secretion on stimulation with phorbol myristate acetate (PMA). Subclone JEH.3B of the Jurkat line generates somewhat lower levels of IL-2 on stimulation with PMA plus concanavalin A (Con A) than EL4.E1 does. To determine whether synthesis of IL-2 by EL4.E1 and JEH.3B cells is sensitive to CsA, we stimulated both lines in the presence of various concentrations of CsA. CsA blocked secretion of IL-2 by both cell lines (Fig. 1). The concentration of CsA required for 50 percent inhibition, 5 to 10 ng/ml, was similar to concentrations previously found to be effective (*2*). Nearly complete inhibition was observed at 50 ng/ml, and this concentration was used in subsequent experiments. As others have found (*1, 2*), CsA had no effect on DNA synthesis in the IL-2–dependent mouse T-cell line used for assaying IL-2. Furthermore, CsA had no apparent effects on the growth or morphology of line EL4.E1 or JEH.3B.

To determine whether the lack of IL-2 secretion in the presence of CsA was due to a lack of mRNA coding for this lymphokine, we isolated mRNA from cells and translated it in *Xenopus laevis* oocytes (*7, 9*). The biologically active translation products were assayed on the cloned murine cytotoxic T-lymphocyte line MTL2.8.2 (*8*). Stimulation of EL4.E1 cells with PMA induced generation of IL-2 mRNA, as detected by translation in oocytes (Table 1). When CsA was also present in the cell cultures at 50 ng/ml, PMA did not lead to accumulation of translatable IL-2 mRNA. As a control, mRNA was prepared from cells stimulated in the presence or absence of CsA and mixed at harvest. The mixed mRNA preparation gave the expected yield of translated IL-2, demonstrating that the lack of mRNA in the CsA-treated cells was not due to inhibitory material (Table 1). These results indicate that CsA reduced the level of IL-2 mRNA by a factor of at least 10.

To confirm that lack of translation was not due to a peculiarity of the oocyte system, we also translated some mRNA samples in the cell-free wheat germ extract system. This system is much less efficient than microinjected oocytes, but the result was the same, namely that no biologically active IL-2 mRNA could be isolated from EL4.E1 cells stimulated in the presence of CsA.

Similar results were obtained with line

Table 1. Effect of CsA on the level of translatable IL-2 mRNA in EL4.E1 cells. Subclone EL4.E1 of the IL-2–producing T-lymphoma line EL4 (*5*) was grown in RHFM (*7*) supplemented with 10 percent fetal calf serum (FCS). Cells in logarithmic-phase growth were collected and suspended at a density of 1×10^6 per milliliter in RHFM supplemented by 2 percent FCS. A 1.5-liter culture was treated with PMA (group A), a second 1.5-liter culture was treated with PMA plus CsA (group B), and a 1-liter culture was incubated without stimulation (group C). After 16 hours the cells were harvested and 3.5×10^8 cells from each of groups A and B were mixed to form group D, which served as a control to show that CsA-treated samples do not inactivate IL-2 mRNA or prevent its translation. Groups A, B, and D each contained about 7×10^8 cells (PMA stops proliferation, even in the presence of CsA). Group C, which had continued to proliferate, contained 1.8×10^9 cells. Cells were homogenized in 7.4*M* guanidine HCl (*11*), and polyadenylated RNA was isolated as described earlier (*9*). The mRNA was injected (50 nl at 2 mg/ml, 20 oocytes per group) into *Xenopus laevis* oocytes, and these were incubated at 25°C for 63 hours. Media from the groups were pooled and the oocytes were pooled and homogenized. Supernatants and homogenates were assayed on the mouse cytotoxic T-lymphocyte line MTL2.8.2 at various dilutions in triplicate (see legend to Fig. 1). Data from the activity-dilution curves were converted to 30 percent effective dose (ED_{30}) units per milliliter. One ED_{30} unit stimulates 30 percent of the maximum proliferative response under the assay conditions.

Group	Addition*	IL-2 synthesized by oocytes (mean ED_{30} units per milliliter ± standard deviation)
A	PMA (20 ng/ml)	128 ± 7
B	PMA (20 ng/ml) plus CsA (50 ng/ml)	1.4 ± 0.2
C	Medium only	0.4 ± 0.3
D	Cells from groups A and B	60.0 ± 1.1

*Addition to culture of EL4.E1 cells from which polyadenylated RNA was extracted.