Table 2. Nuclear spin-lattice relaxation time T_1 in tails of tumorous mice and of normal mice. Observations on normal mice 1 and 2 were made during the period when tumor mice 1 to 5 were under study.

Test mouse	T_1	Time (days)
	Frequency, 18 Mhz	
Tumor 3 ♀	730(50)	32
Tumor 4 ♀	700(90)‡	32
Tumor 5 ♀	720(60)	37
Normal 1 ♂	285(15)*	
	320(20)*	
	330(20)	
Normal 2 ♂	330(10)*	
	365(25)	
	Frequency, 8 Mhz	
Tumor 6 ♂	500(70)*†, linear fit	28
	527(60)*†, linear fit	28
Normal 3 ♂	195(15)*	
	230(10)*	
Normal 4 ♂	235(25)*	
Normal 5 ♀	250(20)*	

*, †, ‡ See definitions of corresponding symbols in Table 1.

0.7 second. Reasonable agreement is obtained between the data of Fig. 2b and a synthesized curve, Fig. 2e, if we assume a ratio of tumorous to normal tissue protons of 2 : 1.

A potential source of error is due to the pulse sequence repetition rate. If too rapid a rate is used, relative to the rate at which the nuclear magnetization recovers, the observed relaxation time may be shorter than the true value. When relaxation consists of two superimposed exponentials, then the data corresponding to the longer relaxation are more distorted. It should be emphasized that the so-called "null method" used by Damadian (*1*) to obtain T_1 cannot be applied to our composite data. The null method T_1 obtained would be a weighted average of the two T_1's, with the weighting depending on the relative amounts of tissue.

The limit of detectability for tumorous tissue in our work was not less than 10 percent by volume. However this limit could be lowered substantially by taking advantage of digital signal averaging, automatic amplitude calibration (to compensate for instrumental drift), and higher magnetic fields to improve signal to noise.

This detection of an increase in vivo of T_1 in tumorous tissue does not differentiate between the model of tissue-water behavior suggested by Damadian (*1*), in which the intracellular water has less structure in tumorous than in normal cells, and another model in which cancerous tissue contains relatively more intercellular water, as in an edema. More detailed measurements of T_1 in vivo and in vitro and NMR amplitude as a function of magnetic field, temperature, and volume of the tumorous and normal tissue (within the radio frequency coil) might distinguish between models.

We have been able to detect and monitor the growth of a cancer (a transplanted S91 melanoma) in a live animal, using pulsed NMR. Our results suggest that it would be worthwhile to attempt to develop this technique for the detection and monitoring of tumors in humans. Perhaps NMR could take its place beside thermography or radiography as a nonsurgical technique for cancer detection and analysis of cancer growth rate.

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Evidence for Active Immunity to Morphine in Mice

Abstract. *The serum from mice actively immunized with a morphine immunogen contained antibodies that could bind dihydromorphine. Morphine effects were diminished in these "immunized" mice, and the concentration of morphine in their plasma was altered.*

The development of tolerance is associated with the chronic use of many drugs. Two of the most common mechanisms invoked to explain tolerance are adaptation of the cells of the central nervous system and increased peripheral metabolism of the drug. Central nervous system adaptation is believed to account for tolerance to the narcotic analgesics. However, a number of observations indicate that an immune-like mechanism may also be implicated in narcotic tolerance (*1*): (i) some types of narcotic tolerance persist for many months, (ii) factors from the serum and tissues of tolerant animals contain material that can be passively transferred to a second animal and influence narcotic action in that animal, and (iii) tolerance to narcotics can be decreased by drugs that inhibit protein synthesis.

If such an immune mechanism for tolerance to a drug exists, it should be possible to demonstrate (i) proteins or antibodies which can specifically bind the drug and (ii) a modification of the pharmacologic activity or concentration of the drug in the immunized animal. We report here the production of antibodies that bind morphine in mice that have been actively immunized with a conjugate of morphine and protein and the production of an altered effect and biologic disposition of morphine in these mice.

The morphine immunogen, 3-carboxymethylmorphine coupled to bovine serum albumin, was previously shown to be effective in producing antibodies specific for morphine in the rabbit (*2*). Mice are also able to develop antibodies to morphine. Weanling male mice were injected subcutaneously once a week for 16 weeks with 0.1 ml of either buffered saline, 50 percent complete Freund's adjuvant emulsified in buffered saline, or 1 μg of morphine immunogen in 50 percent complete Freund's adjuvant emulsion (*2*). Six mice treated with adjuvant, or six treated with morphine immunogen plus adjuvant, were killed; the serums were pooled within each group, and a portion of the pooled serum was diluted with nine parts of buffered saline. This diluted serum (0.1 ml) was incubated for 20 minutes with 0.4 ml of buffered saline (Grand Island Biological) and tritiated dihydromorphine [15,000 disintegrations per minute (dpm), specific activity 680 mc/12.5 mg (New England Nuclear)] and was assayed for specific narcotic binding by an ammonium sulfate precipitation technique (*2*). The serums from mice injected with the morphine immunogen in Freund's adjuvant bound [^{3}H]dihydromorphine (245,000 dpm per milliliter of serum). The serums from those mice that received only Freund's adjuvant or saline bound no dihydromorphine in this test, an indica-

tion that control serums do not bind the narcotic with significant affinity.

The binding of the dihydromorphine was specific, as another amine, [^{3}H]-serotonin, was not bound by the serums of mice treated with immunogen. Moreover, morphine displaced [^{3}H]dihydromorphine from the antibody, whereas serotonin or naloxone did not (*3*).

The in vitro binding of dihydromorphine suggests the presence of antibodies that can bind narcotics in actively immunized mice. Additional evidence supporting this hypothesis was obtained in an in vivo study. Mice were immunized for 16 weeks, or were injected with saline or adjuvant, and then were injected intravenously with 50 μc of [^{3}H]dihydromorphine. They were killed 1 or 5 hours later, and their serums were assayed for dihydromorphine (*4*). Mice immunized with the morphine immunogen had 20 times more dihydromorphine present in their serum after 1 hour than mice injected with saline (control mice) and had more than 50 times more dihydromorphine after 5 hours than did control mice (Table 1). The half-life of dihydromorphine in the serum thus appears to be prolonged in immunized mice, as the narcotic is sequestered there.

In the same experiment, we found that 1 hour after dihydromorphine administration there is more than 30 times more dihydromorphine bound to protein in the serum of immunized mice than in the serum of control mice (*3*), as determined by the ammonium sulfate precipitation test. The data from the in vivo and in vitro experiments show that the serum from mice actively immunized with a morphine immunogen can bind dihydromorphine to a greater extent than the serum of control mice.

As the narcotics are usually given clinically for the relief of pain, we used analgesia as an index of pharmacologic activity. Morphine analgesia in mice was determined by measuring the ability of morphine to decrease writhing induced by *p*-phenylquinone (*5*). The pharmacologic effect of morphine was diminished in mice that had been injected with morphine immunogen in Freund's adjuvant for 6 or 16 weeks (Table 2). For example in experiment 2, 0.5 mg of morphine per kilogram of body weight caused a 75 percent reduction in writhes in mice used as controls. In contrast only a 7 percent reduction in writhes occurred in mice treated with immunogen. If the dose of morphine administered to the mice was increased to 0.75 mg/kg, there was a greater protection against the *p*-phenylquinone irritant in all the mice. However, morphine was again least effective in those mice actively immunized against the drug. Moreover, mice that were treated with immunogen averaged significantly more writhes per mouse than did control mice. Other results also indicate that morphine-induced motor incoordination and lethality can be decreased in immunized mice as compared to adjuvant-treated controls.

It is unlikely that the decreased effects of morphine in actively immunized mice were the result of morphine dissociating from the antigen and thus causing tolerance.

Table 1. Disposition of [^{3}H]dihydromorphine in serum of actively immunized mice. Number in parentheses represents the number of mice used for each determination. Results are expressed as the average ± standard error of the mean.

Treatment of mice (injection)	[^{3}H]Dihydromorphine in serum (dpm/ml) after:	
	1 hour	5 hours
Saline, control (7)	12,357 ± 487	1,529 ± 181
Adjuvant, control (6)	19,116 ± 3,088	8,941 ± 3,558
Morphine immunogen (6)	284,689 ± 57,462*	782,919 ± 90,870*

* Statistically significant difference, $P < .001$, compared to saline or adjuvant controls.

Table 2. Analgesic effect of morphine in immunized mice. In experiment 1, weanling mice were injected subcutaneously with 0.1 ml of a 50 percent emulsion of complete Freund's adjuvant (control), or with 1 μg of morphine immunogen in 50 percent complete Freund's adjuvant (morphine immunogen) twice the 1st week, and then every 7 to 10 days thereafter for 6 weeks. Fifteen mice were tested for each treatment (five for each of the three doses of morphine), and compared to five control mice who received no morphine. In experiment 2, mice were injected in a similar fashion for 16 to 20 weeks with one exception: from the 3rd to 10th week, saline was substituted for the Freund's adjuvant in the control group, and in the group receiving morphine immunogen plus adjuvant. When the mice were examined for effects of morphine during the 10th week, no difference occurred among the groups. Adjuvant was then reinstated in the group receiving adjuvant and in the group receiving morphine immunogen plus adjuvant for each subsequent weekly injection. Four days after the last injection ten mice from each group (five for each of the two doses of morphine) were tested and were compared to five control mice who received no morphine. Analgesic response was estimated by the *p*-phenylquinone (PPQ) writhing test. Morphine sulfate (experiment 1) and morphine hydrochloride (experiment 2) were injected subcutaneously, in the doses indicated, 15 minutes before PPQ administration, and writhes were counted 10 minutes after PPQ administration for 5 minutes (*3*). Numbers in parentheses indicate the average number of writhes for each mouse ± standard error of the mean. Mice not given morphine writhe an average of 23 ± 3 times each. The observer of analgesic tests did not know prior treatment of mice.

1) The 1 μg of antigen protein injected weekly had less than 0.5 μg of morphine bound covalently, hardly enough to produce tolerance (*6*).

2) Injection of the narcotic antagonist, nalorphine (10 mg/kg), into actively immunized mice produced no signs of physical dependence such as jumping, agitation, or diarrhea (*6*).

3) Narcotic tolerance was noted consistently only when the immunogen was administered with the complete Freund's adjuvant. Apparently, an immunologic stimulant is required to obtain sufficient antibody activity to diminish the morphine effect. The partial tolerance observed is therefore not related to the usual type of narcotic tolerance.

The mice that were least affected by morphine were those that received the morphine immunogen, and had produced antibodies that bound morphine. We therefore suggest that these antibodies reduced the concentration of morphine at critical sites in the brain, thus decreasing the pharmacologic effect of the drug. However, this interpretation requires caution as direct

Experimental animal	Analgesic response* to morphine doses (mg/kg) of:			
	0	0.5	0.75	1.0
	Experiment 1			
Control	0	28	87	91
Injected with morphine immunogen	0	26	4	58
	Experiment 2			
Control	0 (0)	75 (6 ± 2)	82 (4 ± 2)	
Injected with morphine immunogen	0 (0)	7 (21 ± 6)†	24 (17 ± 3)†	

* The decrease in total writhes for five mice for each dose of morphine are expressed as a percentage of a group of controls treated with saline and receiving only PPQ. † Indicates significantly different from controls $P < .05$ (Student's *t*-test).

evidence of a decrease in narcotic concentration in the brain has not as yet been obtained. It also remains to be determined if active immunity can modify analgesia, as measured by other procedures. Moreover, there is a possibility that the morphine-hapten-antibody complex may not only act as a source of inactivation of the drug but, in contrast, as a depot for morphine. This situation could arise if there is a significant amount of dissociation of morphine from the antibody-hapten complex.

These studies do not establish an immune component for any aspect of narcotic addiction in man. The major contribution of this report is to show that the mouse can be made to develop antibodies that recognize a narcotic both in vitro and in vivo, and thus the mice may be a useful model for the study of immune theories of narcotic tolerance. However, in light of these findings, we must consider somewhat more actively that morphine, or its congener heroin, can bind to human proteins and potentially act as an immunogen-like material in man. Proteins with a strong affinity for narcotics have already been found in the serum of some heroin addicts (*7*). It also has been shown (*8*) that after the administration of a single dose of morphine to normal subjects, low concentrations of the alkaloid were present in plasma for as long as 48 hours. Our data provides the first evidence that antibodies to narcotic analgesics can potentially modify the pharmacologic effects or biologic disposition of these drugs. Further studies with antibodies against specific drugs to modify pharmacologic or physiologic responses seem indicated. There is a possibility that narcotic tolerance and dependence may be influenced by active or passive immunity.

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A New Approach in Integrated Control: Insect Juvenile Hormone plus a Hymenopteran Parasite against the Stable Fly

Abstract. *Two insect juvenile hormone analogs, 4[(6,7-epoxy-3-ethyl-7-methyl-2-nonenyl)oxy] benzene and 6,7-epoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene, when applied topically to pupae of the stable fly,* Stomoxys calcitrans *(L.), were morphogenetically effective against the metamorphosing pupae but did not affect oviposition and development of the hymenopteran parasite,* Muscidifurax raptor *Girault and Sanders, in the treated pupae. Also, reproductivity of the parent generation of parasites was not affected.*

The realization of the part played by beneficial insects (*1*) in controlling pest insects has resulted in a variety of efforts to increase the impact of these parasites and predators (*2*). At the same time, insect juvenile hormone (JH) analogs (*3*) are being considered as replacements for broad-spectrum toxicants because they are more specific for the target insect (*4*). For example, pupae of the stable fly, *Stomoxys calcitrans* (L.), an economically important, blood-sucking pest of both man and his domestic livestock, are sensitive to nanogram quantities of selected JH analogs (*5*). This hormonal activity is expressed by cellular differentiation of the head and thorax of the developing adult but not the abdomen. The result is a pupal-adultoid intermediate that dies within the puparium.

We report herein than topical treatment of stable fly pupae with the JH analogs 4[(6,7-epoxy-3-ethyl-7-methyl-2-nonenyl)oxy] benzene (JHA1) (*6*) and 6,7-epoxy-1-(*p*-ethylphenoxy)-3,7-dimethyl-2-octene (JHA2) (*7*) did not interfere with the development of the parasitic insect *Muscidifurax raptor* Girault and Sanders (Hymenoptera: Pteromalidae) in the pupae. Also, we observed no alterations in the reproductivity of these *M. raptor* parasites. Thus, this report introduces a new approach to integrated control, that is, the use of a JH analog in conjunction with an effective insect parasite for control of a pest insect.

In 1971, we applied two JH analogs to the surface of the breeding medium of stable flies in Nebraska and Florida (*8*) since nanogram levels had earlier produced morphogenetic effects in pupae of the stable fly which prevented the emergence of adults (*5*). Both analogs proved to be potential third-generation pesticides against stable flies (*8*). Moreover, when samples of these stable fly pupae were taken into the laboratory, two species of parasites, *M. raptor* and *Spalangia endius* Walker (*9*), emerged from puparia containing pupal-adultoid intermediates. The biology and worldwide distribution of these species are well documented, and they are known to parasitize both stable fly

Table 1. Development of *M. raptor* in stable fly pupae treated with two juvenile hormone analogs (six tests with 25 stable fly pupae per test) as expressed by eclosion of adult *M. raptor*. Values are numbers of adult *M. raptor* eclosing from stable fly pupae. (No stable flies eclosed when exposed to *M. raptor* or to either of the analogs.)

Treated with JH analog 1*	Treated with JH analog 2*	Untreated
15	20	23
22	21	22
19	15	24
21	12	14
16	13	25
15	18	24

* See Fig. 1 for the structures.

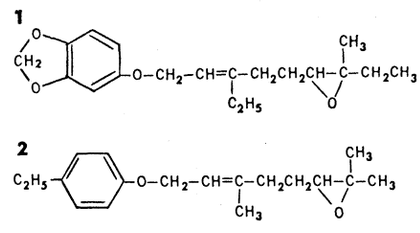

Fig. 1. Structures of JH analogs 1 and 2.