power-generating plants; however, the daily exposure duration and frequency used for at least the two highest concentrations, 0.12 and 0.30 ppm, are more extreme than is likely to occur at present in most industrial areas. The linear relationship between SO_2 concentration and mean yield loss for the three cultivars (Fig. 1) suggests that in seasons with favorable growing conditions, as in 1979 at Beltsville, SO_2 doses even lower than our minimum could reduce the productivity of susceptible crops when present in combination with ambient oxidants now present in the mid-Atlantic seacoast region. The yield data indicate that, of the three cultivars tested, BBL 274 has the most tolerance to mixtures of O_3 and SO_2.

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Morphine in Cow and Human Milk: Could Dietary Morphine Constitute a Ligand for Specific Morphine (μ) Receptors?

Abstract. *Morphine has been found in cow and human milk at concentrations of 200 to 500 nanograms per liter. Multistep purification yields a material that has immunological, biological, pharmacological, and chemical properties identical to those of morphine. Similar morphine-like material, which has been tentatively identified in some common plant sources, may be a ubiquitous dietary constituent and a possible source for the material in milk. Since morphine (μ) receptors have a low affinity for enkephalins, and since morphine-like materials have been described in brain and intestine, it is possible that morphine in food may be the source of this material and a normal ligand specific for μ receptors.*

Several hormones are present in the milk of human and other mammals (*1–3*). Recently, compounds displaying opioid activity in the receptor binding assay and guinea pig ileum were reported in chloroform-methanol extracts of lyophilized bovine milk (*4*). Further purification resulted in several active compounds, and the major one displayed opioid activity in the guinea pig ileum, a tissue that contains predominantly μ or morphine receptors (*5, 6*), but not in the mouse vas deferens, which contains predominantly δ or enkephalin receptors (*5, 6*). Because the amounts of these opioid activities were highly variable in milk (*4, 7*), a substance with opioid activity was isolated from an enzymatic digest of casein (*7*) in the belief that it represented a similar, peptic material (*8*). This compound, a heptapeptide named β-casomorphin (*7*), exhibited very low opioid activity (250 times less potent than normorphine in the ileum assay), whereas the NH_2-terminal pentapeptide exhibited higher activity (22 times less potent than normorphine). We recently reported (*9*) that the synthetic peptide NH_2-Tyr-Pro-Phe-Pro-NH_2 (morphiceptin), the tetrapeptide amide of β-casomorphin, also found in enzymic digests of β-casein, has morphine-like activities and is specific for μ receptors but not for δ receptors. We now report the presence of the alkaloid morphine in cow and human milk.

Morphine was first detected in lyophilized cow's milk that was homogenized (Polytron) with 2*N* glacial acetic acid in methanol and then filtered. Amounts measured by a radioimmunoassay (RIA), which is specific for morphine (*10*), ranged between 0.3 and 5 ng per gram. In a typical experiment to further identify the morphine, 100 g of powdered milk or lyophilized skim milk (1 liter) was homogenized in 1 liter of 2*N* glacial acetic acid in methanol for 10 minutes at room temperature, filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure. Recovery at this step, with [^{3}H]dihydromorphine as a marker, is around 90 percent. Although morphine is very soluble in chloroform and methanol, these solvents were much less effective for extraction, which suggests strong interaction with the proteins in milk. After extraction, additional protein was precipitated by the addition of acetonitrile (10 percent by volume). After centrifugation, evaporation of acetonitrile, and filtration the sample was applied on a preparative high-performance liquid chromatography (HPLC) column (*11*); the active fractions (H_2O and acetonitrile, 1:1 by volume) were lyophilized and further purified as described in Fig. 1. Although purification resulted in very low (about 4 percent) yields, the material was a single compound that had the same retention time as morphine in four different HPLC systems. (In addition to the procedures described in the legend to Fig. 1, the following were used: Whatman Partisil ODS and a mobile phase with 8 percent acetonitrile and 92 percent 10 m*M* am-

Table 1. Characteristics of morphine present in milk.

Property	Morphine in milk*	Morphine
Cross-reactivity with antiserum to morphine	Positive	Positive
Cross-reactivity with antiserum to codeine	Negative	Negative
IC_{50}† for δ receptors‡	33 n*M*	35 n*M*
IC_{50} for μ receptors§	0.7 n*M*	0.5 n*M*
ED_{50}‖ in guinea pig ileum	0.9 μ*M*	0.9 μ*M*
Retention time in four HPLC systems	Identical to morphine	
Molecular ion	285.1365	285.1365

*Concentrations were calculated as equivalent to morphine by RIA. †Concentration of ligand causing 50 percent inhibition of specific binding. ‡Binding assays with ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin (*14*). §Binding assays (*14*) with [^{3}H]naloxone (0.38 n*M*). ‖The concentration causing 50 percent depression of smooth muscle contractions.

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monium acetate, *p*H 4.2, and a mobile phase 70 percent acetonitrile and 30 percent 10 m*M* ammonium phosphate, *p*H 7.4.) The compound cross-reacted well (equivalent in amount to its pharmacological activity in the ileum) with antiserum specific for morphine (*10*) and its activity correlated with that of morphine on the basis of the intensity of the ultraviolet peak (Fig. 1C), but did not cross-react with highly specific antiserum to codeine. Like morphine, the compound was resistant to degradation by Pronase, aminopeptidases, trypsin, chymotrypsin, and brain membranes, indicating its nonpeptide nature. Finally, mass spectrometry showed that the compound had a molecular ion at 285.1365, identical to the molecular ion of morphine (*12*). Morphine was also found in human milk (200 to 400 ng/liter) that was subjected to similar purification procedures.

The possibility still exists that the substance may not be chemically identical to morphine, since despite the physical data obtained on a compound, the assigned structure is never guaranteed (*13*). Each physical test reduces the possibility of an error in structural assignment. In seven separate systems the unknown isolated from milk behaved identically to morphine. Three of the systems used reverse-phase HPLC with different *p*H's and different mobile phases. In all these systems, the amino group would likely be protonated with a pK_a of 9.85 while the analogous phenolic derivative would exist in one case as an anion and another case as an OH neutral-anion equilibrium with a pKa of 8.04. The fourth system involved gel permeation chromatography. The mean effective dose (ED_{50}) was identical to that of morphine. The RIA specific to morphine (*10*) was also positive; the only significant interference known for this RIA is normorphine, which has a molecular weight that is 14 mass units less than morphine. The only interferences expected for this RIA should be morphine-like structures that have differences on the nitrogen atom of the molecule. Finally, this material produced a molecular ion and accurate mass measurements identical to those of morphine. All the evidence yields a high probability that the unknown compound in milk is morphine. Further experiments were not carried out because of the impracticality of scaling up these experiments to yield more product.

We have shown (*6*, *14*) that brain membranes have two classes of opiate receptors, μ receptors with a higher affinity for morphine and δ receptors with a higher affinity for enkephalins. Binding

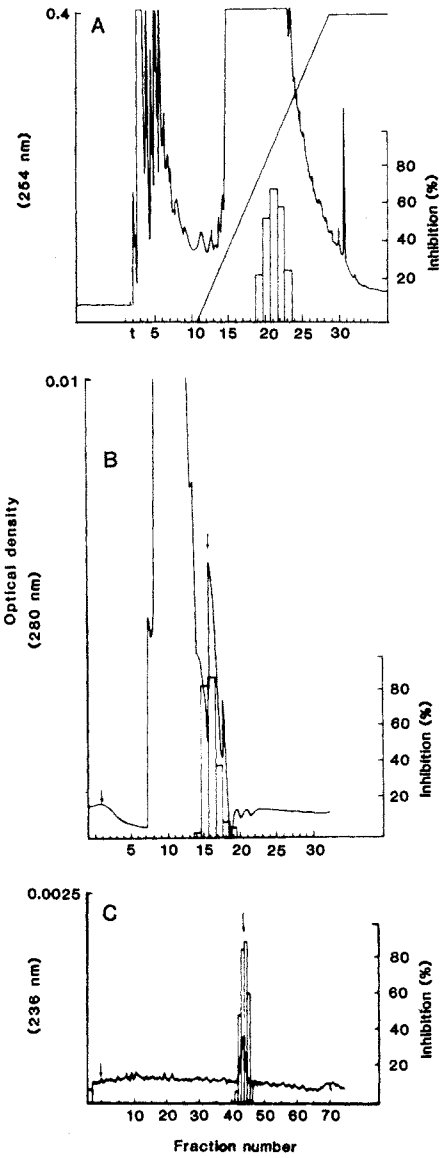

Fig. 1. Purification of morphine from milk. (A) The compound was applied on Waters μBondapak C-18 column (3.9 mm by 30 cm); mobile phase and gradient: H_2O (15 minutes), 0 to 100 percent linear gradient of acetonitrile (30 minutes) and 100 percent acetonitrile (15 minutes); flow rate, 2 ml/min; each fraction, 3 ml. (B) The active fractions from (A) (by RIA and binding assays, open bars) were applied on a Waters 100-Å μStyragel column (7.8 mm by 30 cm) and run with 60 percent tetrahydrofuran and 40 percent dimethylformamide; flow rate, 1 ml/min; each fraction, 0.5 ml. (C) Active fractions (open bars) were applied on μBondapak C-18 column (3.9 mm by 30 cm); mobile phase: 9 percent acetonitrile, 91 percent ammonium acetate buffer (10 m*M*, *p*H = 4.2); flow rate, 1 ml/min; each fraction, 0.5 ml. Arrows indicate position of authentic morphine samples. Radioimmunoassay was performed as described in (*10*). The antiserum to morphine was usable at a dilution of 1:1000 and covered the range 0.03 to 3 ng of morphine per milliliter. These antibodies have negligible cross-reactivity with codeine (*10*) and opioid peptides; β-casomorphin and its NH_2-terminal pentapeptide and tetrapeptide do not cross-react at $10^{-5}M$. Binding assays were performed as described in (*14*) with ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin.

studies (Table 1) indicated that the morphine of milk had a high affinity toward μ receptors (apparent dissociation constant, K_d, ~ 0.7 n*M*, competition against [^{3}H]naloxone) and low affinity toward δ receptors (apparent K_d ~ 33 n*M*, competition against ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin); morphine has apparent K_d values of 0.5 and 35 n*M*, respectively.

The pharmacological activity of the morphine of milk was measured in the guinea pig ileum, which is sensitive to morphine in a specific fashion and has predominantly μ receptors (*5*, *15*). The electrically stimulated twitches were depressed in a dose-dependent manner. The activity of the morphine of milk was the same as that of morphine (ED_{50} = $9 \times 10^{-7}M$), and it was blocked by naloxone; the concentration in milk was calculated as equivalent to morphine by RIA and by binding assays.

These data suggest that the morphine material in milk is identical in chemical, biochemical, immunological, and pharmacological properties to authentic morphine (Table 1). However, the origin of morphine in milk is enigmatic. Studies (*16*) indicate that morphine is also present in various plants such as hay and lettuce (2 to 10 ng per gram, dry weight). The compound purified from lettuce also has the same retention time as morphine in two different HPLC systems. We postulate that morphine may be a ubiquitous constituent of plant-derived foods and that the substance may gain entrance into the body through the gastrointestinal tract. Perhaps, in addition, an active concentrating mechanism exists in the mammary gland. Studies (*17*, *18*) have shown that after experimental morphine or codeine administration, morphine can be detected in milk in significant amounts over 12 hours (*18*), and weaning infants of addicted mothers often show opiate withdrawal symptoms (*17*).

The presence of significant quantities of morphine in common dietary plants and in milk suggests that morphine may be a common and important dietary constituent and may be distributed widely through body tissues. This might have broad biological significance. Certainly the possibility exists that dietary morphine could have pharmacologic significance by interacting with opiate receptors, even though the quantities consumed are relatively small. Such small quantities of exogenous morphine may play important but as yet unknown physiological roles. Because of the low affinity of enkephalins for μ receptors in the brain, we predicted (*6*, *14*) that morphine-like substances that have ready

access to such receptors should exist endogenously. A morphine-like, nonpeptide compound with immunological cross-reactivity to morphine has been reported in mammalian brain (*19*) and in samples of human cerebrospinal fluid (*20*). This compound exhibited specific binding to opiate receptors (*21*) and was active in both guinea pig ileum and mouse vas deferens, but its activity was not reversed by naloxone or naltrexone (*19*). Nevertheless, immunocytochemical studies (*22*) with specific morphine antibodies indicated its localization within neurons. To date, the chemical identity of this substance has not been elucidated. Killian *et al.* (*23*) confirmed the existence in brain of an immunologically morphine-like substance, whose pharmacological activity could be readily reversed by naloxone. These results have been confirmed in our laboratory, where similar activity has also been identified in extracts of the intestinal tract of rodents (*24*). It is possible that this substance is morphine, that it is derived from dietary sources, and that it is a natural ligand for the specific μ receptors of the brain and gastrointestinal tract. Under certain circumstances (for example, during breast feeding) other exogenous compounds, such as morphiceptin (*9*), may also interact physiologically with μ receptors. Other precedents exist for the occurrence of receptors or enzymes for substances elaborated exclusively outside the body, such as vitamins, essential fatty acids, and essential amino acids.

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12. The active compound (Fig. 1C) was lyophilized and, after the addition of 1 ml of 10 m*M* sodium carbonate (*p*H 9), extracted three times with 1 ml of chloroform containing 5 percent ethanol. After the solution was evaporated under a nitrogen stream, mass spectrometry was performed. A Varian MAT 731 double focusing mass spectrometer coupled to a Varian 1024 time-averaging computer was tuned to 8000 resolution with 10 percent valley definition. The sample was introduced by direct probe and a glass crucible was used; the sample evaporated over a temperature range of ambient to 350°C. The accurate mass of the molecular ion of morphine was monitored according to the procedures described by G. L. Peele and D. A. Brent [*Biomed. Mass Spectrom.* **5**, 180 (1978)].
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Group Living, Competition, and the Evolution of Cooperation in a Sessile Invertebrate

Abstract. *Competition and cooperation are thought to represent the opposite extremes of organism interactions. I here show that the formation of aggregations in a sessile organism requires cooperation between individuals and that the gregarious pattern of habitat selection generating these aggregations is a response to a density dependence in the outcome of interference competition.*

One of the oldest concerns of ecologists, evolutionary biologists, and ethologists is why organisms live in groups (*1*). As group living often intensifies competition among members of the group, its occurrence appears anomalous. If group living is adaptive, its benefit to members of the group must outweigh its cost (*1*). These benefits have been thought to be threefold: an increased protection from inclement weather, a reduced risk of predation, or an increased feeding efficiency (or some combination thereof) (*1, 2*). I now report the existence of a fourth type of benefit—increased interspecific interference competitive ability—and show that this benefit accounts for an observed pattern of gregarious settlement in a sessile, colonial invertebrate.

Virtually all cost-benefit analyses of group living involve the study of behaviorally sophisticated mobile organisms (*1, 2*). In mobile organisms, the formation of groups often varies in a complex fashion with environmental conditions, age, sex, and other factors. Hence, quantification of the costs and benefits of group living is difficult. Sessile invertebrates, in contrast, have only one opportunity to choose whether or not to live in a group. Thus, direct field measurements of the relative costs and benefits of group living are not only feasible, but also lead to new insights into the conditions favoring the evolution of group living.

The bryozoan *Bugula turrita* commonly occurs in dense stands on pilings and rocks at shallow depths along North American coastlines. It grows as a single arborescent colony with a flexible stalk, lightly calcified skeleton, and loosely articulated zooids. By mid- to late summer at Woods Hole, Massachusetts, virtually all colonies (> 90 percent) of *B. turrita* on pilings below a depth of 1 m are fertile and are found in contact with colonies of another bryozoan, *Schizoporella errata*. Unlike *B. turrita*, *S. errata* is an encrusting organism composed of heavily calcified zooids. This rigid skeleton enables colonies of *S. errata* to push down and overgrow the more flexible colonies of *B. turrita*. As such overgrowth inevitably leads to the death of the *B. turrita* colony, this form of interference compe-

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