The depth profile of foraminiferal samples according to their states of preservation (Fig. 2C) suggests that in the tropical region there is a zone between depths of 3 and 4 km below which foraminiferal shells dissolve markedly (*14*). The occurrence of well-preserved samples below this zone is believed to reflect postdepositional displacement of sediments. A similar level of change in preservation ("lysocline"), has been found in the central Atlantic at somewhat greater depth (*8*). In the Pacific, this lysocline appears to coincide with a drastic change in the rate of calcite solution in the water column, as found by experiment (*15*). The lysocline is less distinct and relatively shallow in fertile areas, probably owing to the large amount of organic carbon and the concomitant carbon dioxide production in the sediment (*8*). Thus, dissolution of Foraminifera appears more pronounced in the fertile equatorial areas than in adjacent tropical regions (*9*), which accounts for the equatorial minimum in species diversity on the ocean floor.

If some species dissolve faster than others, it is immediately clear why dissolution decreases the number of species encountered in a sample (Fig. 2D). The occurrence of maximum values of compound diversity in assemblages at intermediate stages of preservation, with corresponding minimum values of dominance (Fig. 2, E and F), needs some comment, however.

In tropical areas, low diversities occur in samples that are well preserved, where *Globigerinoides ruber*, *Globigerinita glutinata*, and, to some extent, *Globigerinoides sacculifer* dominate because of their high shell output. Low diversities also occur in poorly preserved samples, where *Globorotalia tumida*, *Pulleniatina obliquiloculata*, and *Globoquadrina dutertrei* dominate because of their great resistance to dissolution. These resistant species are relatively rare in well-preserved samples. The compound diversity maximum at intermediate stages of dissolution is, therefore, contingent on the fact that species with high shell output tend to dissolve easily, so that, upon dissolution, dominance first decreases, as the abundant species are removed, and then increases again as certain resistant species begin to dominate the assemblage (*16*). Contouring of dominance patterns without regard to this mechanism (*4*) is likely to lead to unwarranted conclusions when such patterns are related only to biological processes in surface waters.

Why should the initially dominant species of the tropics dissolve most rapidly? These highly productive species live in surface water (*17*) and build relatively light, open-structured, porous shells, possibly in response to rapid growth or to the low viscosity of warm surface waters, or to both, and these thin shells are able to stay in the euphotic zone. On the other hand, living representatives of resistant species of the more common thick-shelled types encountered in sediment assemblages tend to live below the photic zone (*18*), where growth is slow and may manifest itself chiefly as an increase in shell thickness and density (*19*). Thus, the previously noted correlation between depth distribution and solution ranking (*8*) is not a coincidence. This correlation is the cause of the observed compound diversity maximum (and dominance minimum) in foraminiferal assemblages showing intermediate preservation. Such assemblages tend to occur near the lysocline.

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Morphine: Radioimmunoassay

Abstract. *The development of a radioimmunoassay for morphine is described. The hapten morphine is made antigenic by coupling it to a protein at the phenolic group of the molecule. Extremely low concentrations of morphine (0.5 nanogram) can be measured by this assay procedure.*

Despite the fact that extraction procedures and thin-layer chromatographic methods for assaying morphine have been used successfully (*1*), these techniques are relatively time-consuming, laborious, and lack sensitivity with respect to analysis of unlabeled morphine. We now describe procedures for the conjugation of morphine to protein, and for the radioimmunoprecipitation of this conjugate with specific antiserum, which can be used to measure nanogram amounts of morphine in serum.

Morphine was converted to 3-*O*-carboxymethylmorphine by reaction of the free base with sodium-β-chloroacetate in absolute ethanol (*2*, *3*). The product after recrystallization from hot absolute ethanol had a melting point of 292° to 293°C. The 3-*O*-carboxymethylmorphine acid was positive by the Dragendorff test and negative by the Pauly test (*4*) and had an R_F of 0.6 on thin-layer silica-gel chromatography, with glacial acetic acid and methanol (1:1) as the solvent system. In the same solvent system

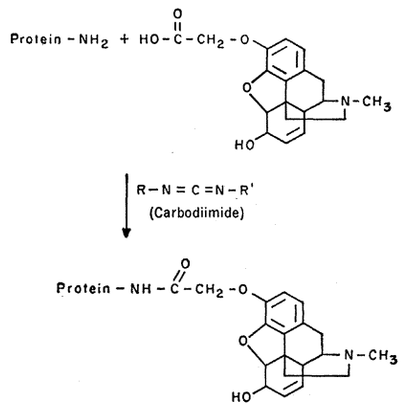

Fig. 1. Probable structure of protein-morphine complex.

morphine had an R_F of 1.0. The carboxymethylmorphine was coupled to bovine serum albumin (BSA) in aqueous solution, in the presence of a water-soluble carbodiimide. Carboxymethylmorphine (8 mg) was dissolved in 2 ml of distilled water containing 10 mg of BSA. The *p*H of the mixture was adjusted to 5.5, and 8 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (*5*) was added. The mixture was incubated overnight at room temperature, and then dialyzed for 7 days against distilled water, with four to five changes per day. Conjugates analyzed by the spectrofluorometric procedure of Balatre *et al.* (*6*) contained three to four carboxymethylmorphine groups per molecule of BSA (if we assume a molecular weight of 70,000 for the protein). Rabbits (New Zealand albino) were immunized with 1 mg of carboxymethylmorphine–BSA (Fig. 1). The immunogen was dissolved in phosphate-buffered saline *p*H 7.4 and emulsified with an equal volume of complete Freund's adjuvant. The initial dose was 1.6 ml, 0.4 ml being injected into each footpad. A booster injection of 100 μg of antigen in adjuvant was given every 6 to 8 weeks, 25 μg in each of the footpads or 50 μg in each of the thighs.

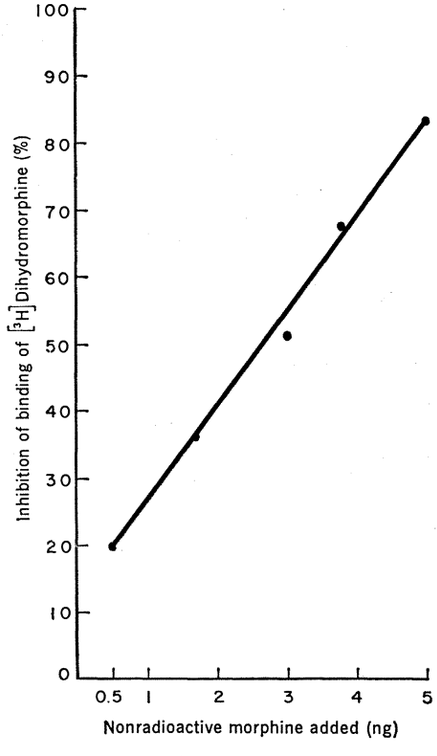

Fig. 2. Inhibition by nonradioactive morphine of binding of [^{3}H]dihydromorphine by antiserum to a morphine-bovine serum albumin (BSA) complex (0.1 ml of a 1:500 dilution). The assay contained approximately 1000 moles (4000 count/min) of [^{3}H]dihydromorphine. In the absence of nonradioactive morphine, 1400 count/min were precipitated.

Blood was collected 5 to 7 days after booster injections. The antiserum was collected and subjected to radioimmunoassay as follows.

Various dilutions of antiserums were incubated in the presence of 100 pmole of [^{3}H]dihydromorphine (New England Nuclear, 388 mc/mmole), 4000 count/min at 4°C overnight. After incubation, a neutral saturated ammonium sulfate solution (volume equal to incubation medium) was added to all tubes. The precipitate, sedimented by centrifugation at 5000 rev/min for 15 minutes at 4°C was washed twice in 50 percent ammonium sulfate. The washed precipitate, containing antibody-bound morphine, was dissolved in 0.5 ml of NCS solubilizer (*7*), and the radioactivity was counted in a Packard Tri-Carb liquid-scintillation spectrometer. The tube that contained radioactive dihydromorphine and antiserum, but no unlabeled morphine, served as a measure of maximum antibody-bound radioactivity. The addition of increasing amounts of unlabeled morphine to a fixed amount of [^{3}H]dihydromorphine and antiserum resulted in a competitive inhibition of the labeled dihydromorphine for the formation of the antibody-hapten complex (Fig. 2). The data demonstrate the sensitivity of the method. With the addition of 0.5 ng of unlabeled morphine per tube [a concentration of 1 ng/ml before addition of $(NH_4)_2SO_4$], 20 percent of the labeled dihydromorphine was displaced from the antibody. The specificity of the antiserum for morphine was demonstrated by incubating the labeled hapten with normal rabbit serum. The radioactivity remaining in the precipitate from normal rabbit serums after washing was slightly above background and was subtracted from values obtained on all other tubes.

The inhibitory capacity of codeine, which is morphine 3-methyl ether, was compared with that of morphine. Codeine was even more effective than morphine (on a molar basis) in producing 50 percent inhibition of precipitation of labeled hapten-protein complex. This is not surprising since codeine has greater structural similarity to the immunizing carboxymethylmorphine group than does morphine itself. Nalorphine (500 ng) decreased precipitation of radioactivity by about 35 percent, whereas the same amount of methadon produced little or no inhibition.

The morphine-containing antigen used in these studies was effective in eliciting morphine-specific antibodies, and, to our knowledge, this is the first description of the experimental production of antibodies with specificity for the morphine alkaloid. Antibodies directed against steroid haptens have been reported to interfere with the physiological actions of the steroid (*8*), but it is not yet known whether morphine antibodies may interfere with the pharmacological effects of morphine.

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