possibility. Nevertheless, the results of studies both in vitro and in vivo strongly support the view that bilirubin toxicity in vivo starts with a selective action on protein phosphorylation, a pathway central to an array of biochemical events linking bilirubin to bilirubin-induced impairment of energy-dependent cerebral metabolism.

Cyclic AMP–dependent phosphorylation of target brain cell proteins is a central pathway to diverse cellular functions such as neurotransmitter-mediated transmission of the nervous impulse at the synaptic junction (*6–8*) and selective nuclear activation through histone phosphorylation (*8*). As these functions have different duration times (*8*), from a few milliseconds (neurotransmission) to years (memory), influence exerted on them externally may induce correspondingly short time effects or long-lasting modifications.

Even though this report does not provide direct evidence for an effect of bilirubin on synaptic membrane–associated protein kinase, such an effect may not be excluded in view of the symptoms developed in the bilirubin-treated animals. The immediate toxic effect of bilirubin, induced by moderate hyperbilirubinemia, usually reversible and expressed by sleepiness, sluggishness, and disturbances of respiratory and cardiac function, may be due to impairment of synaptic protein phosphorylation. The clinically opposite symptoms arising from the intravenous administration of aminophylline (alertness, irritability, and hyperactivity) support this view. The long-lasting toxicity related to high and lasting hyperbilirubinemia may be due to impairment of phosphorylation on nuclear histones in the brain cell.

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Calanoid Copepods, Feeding Currents, and the Role of Gravity

Abstract. *Feeding currents of free-swimming calanoid copepods, observed through an expanded krypton laser beam and a back-focus dark-field optical system, show that these planktonic animals generate a double shear field to help in detecting food. The interrelation between flow field, perception of food items, and body orientation explains why these animals are generally negatively buoyant.*

Calanoid copepods are an integral part of the plankton of seas, estuaries, and lakes. Herbivorous calanoids (Fig. 1) graze on algae; carnivorous ones prey upon fellow zooplankters. Algae are sized from 2 to 100 μm and are dispersed at low concentrations in open pelagic waters. The planktonic world is therefore best described as a nutritionally dilute environment (*1*). Because of the pivotal role of the algae-zooplankton interface in the aquatic food web (*2*), this link has received considerable attention. Most research and textbook descriptions (*3*), however, have followed Cannon's (*4*) early interpretations about the copepod filtering mechanism and the feeding currents. Observations with high-speed microcinematography (*5*) does not support the idea that these animals are filter-feeders (*6*). Calanoids set up flow fields whose properties enhance the chance of detecting food items. The forces which determine the animal's velocity through the water column while feeding have to be balanced to provide a uniform flow field. One of these forces is gravity. The question then arises as to why these animals are not neutrally buoyant so that they can remain at the same depth without having to swim constantly.

Pelagic suspension feeders such as calanoids, salps, and appendicularians must scan large amounts of water for their daily rations because only one part per 10^5 to 10^7 parts of water is of nutritional value. Herbivorous calanoids generate a feeding current with their mouthparts (see cover). These currents were thought to pass through a mesh of setae and setules on the second maxillae. Algae were thought to be retained passively on this filter so that the spacing of setules and setae on this appendage determined the size of particles captured (*7*). However, observations of tethered calanoids (*5*), placed in a dye stream to visualize the flow (*8*), showed that they use second maxillae to capture and separate algae from the feeding current. Just before capture by second maxillae, other mouthparts direct algae into the capturing area through the "clap and fling" mechanism (*9*) and changes in shear fields (*10*). These observations suggested that calanoids perceive the approximate locations of nearby algae, and chemoreception probably assists in this recognition (*11*). Koehl and I observed that the feeding current is governed by viscous forces (low Reynolds number) and therefore has laminar flow (*8*). Any chemical diffusing from an alga sets up an active space (*12*) which will be deformed in the flow field in a predictable way. To take advantage of this deformation the animal must generate a feeding current with a stable flow structure.

Cannon (*4*) observed the feeding current in a drop of water under a microscope and described a relatively powerful pumping mechanism which circulated water against the walls of the drop, thereby creating eddies, counter-eddies, and an unnatural flow. To observe a natural flow field, I filmed feeding behavior with a collimated red light beam of low power (*13*). All light which passed through a 5-liter vessel of seawater undisturbed by particles was blocked in the back focus of the collector lens by a black dot on a glass surface (*14*). Algae and free-swimming calanoids scattered some light (Fig. 1), allowing filming at a speed of 100 frames per second (exposure time, 0.5 msec per frame) (*15*). Except for the laser light, the room was dark. The animals and their food items were at natural densities.

Fifteen films were evaluated, and only the paths of algae which were affected by the presence of the calanoid were drawn (Fig. 2). The structure of the feeding current (Fig. 2A) of *Eucalanus crassus* was constant during feeding bouts of 10 to 30 seconds. The flow field in front of the mouthparts shows a double shear field, one extending laterally from the

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median plane and another parallel to the median plane. Once an alga enters this flow field its path through it is determined. The active space of the chemical around the alga is elongated (*16*), increasing its detection time by *Eucalanus* (*17*). Numerous chemoreceptors on the first antennae (*18*) and on the mouthparts (their setae) (*19*) scan the flow and perceive not only the presence but also the trajectory of the alga. Slight changes of the flow field near the mouthparts ensure that the alga comes close to the second maxillae (*5, 8*). *Eucalanus pileatus* executed such changes 430 msec before the alga reached the capture area or when the alga was approximately 1.25 mm away. The animal cannot perceive the exact location of the alga because of insufficient information within the active space along the path (*20*). To trap the alga, the calanoid must literally capture a volume of water and squeeze it through the setae and setules of the second maxillae (*21*).

After feeding bouts the animals change direction or position in the water column, or both. They sink passively, or they ascend actively using their mouthparts. This cruising mode (Fig. 2B) is different from the feeding mode (Fig. 2A): no anterior double shear field is generated since the intake for propulsion is from the side. I also observed that carnivorous calanoids such as *Euchaeta russelli* and *Epischura lacustris* swim exclusively in the cruising mode. Because mechanoreception plays the major role in predator-prey interactions (*22*), the absence of a shear field is advantageous. Omnivorous calanoids can switch from one mode to the other; true carnivorous ones do not display the feeding mode.

While filming *Eucalanus crassus* I observed that, during a feeding bout, all females swim at a constant speed of 0.175 cm/sec (*23*). Males and copepodites swim, according to their sizes, at lower speeds (*24*). The animals always swim backwards with the body axis vertical. They have to move in this way to create a flow field to match their distribution of sensors, the morphology of their mouthparts, and the range of phytoplankton concentrations that they will encounter. Other species, however, swim with different body orientations and velocities. For example, in the feeding mode, the urosome can be held perpendicular to the body axis, either ventrally or dorsally oriented, or it can be along the body axis. The animal can beat it at the frequency of the mouthparts, use it as a rudder, or keep it motionless. The body can be oriented ventral up, down, against, or with the swimming direction, or at a particular angle.

Body orientation and swimming velocity are given by the combined action of five forces (Fig. 1): gravity, buoyancy, drag, pressure gradient, and torque. The centers of gravity and buoyancy are not the same since calanoids store fat in different parts of their bodies (*25*). The pressure gradient is a result of the ventrally positioned feeding current. The torque balances all other forces to maintain constant body orientation. Each species I have observed has a different body orientation and velocity, and each has a different configuration of these five forces. Whether there are as many vec-

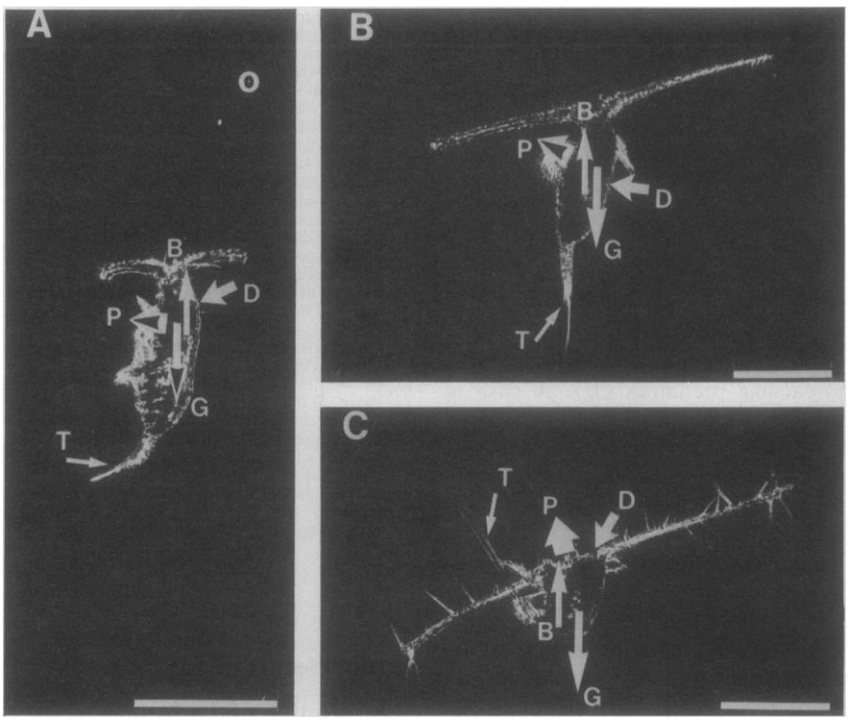

Fig. 1. Natural body orientation of (A) *Paracalanus parvus*, (B) *Eucalanus crassus*, and (C) *Eucalanus monachus*. Back-focus dark-field pictures (35 mm, Tri-X Pan, 1-msec exposure) with horizontal optical axis. Note: *Eucalanus monachus* oriented with horizontal body axis. Scale bars, 1 mm, and white spots, such as that circled in (A), are single *Dunaliella tertiolecta* (7 to 10 μm in diameter). Suggested configurations of the five forces determining body orientation are shown: *G*, gravity; *B*, buoyancy; *D*, drag; *P*, pressure gradient; and *T*, torque.

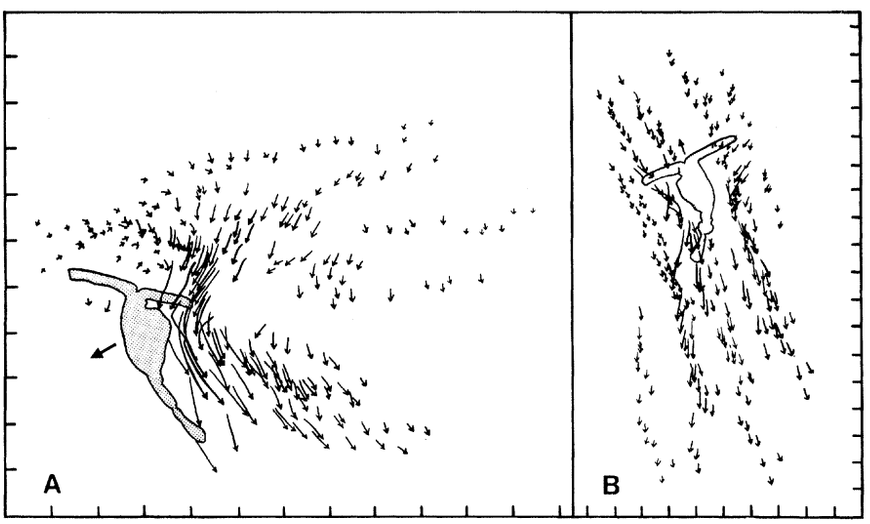

Fig. 2. (A) Flow field in feeding mode of *Eucalanus crassus*. Time interval, 0.4 second; scale units, 1 mm. Flow through capture area, 345 ml/day. Reynolds number of flow, 0.75 (*16*). (B) Flow field in cruising mode. Same animal and parameters as in (A).

tor configurations as there are calanoid species, or whether some species share the same configuration—species which do or do not inhabit the same environment—is not known.

Gravity serves two functions: (i) it gives calanoids orientational direction at night within unlimited uniform space, and (ii) it helps to set up a large feeding current. Calanoids do not possess internal gravity receptors (*26*). Their mechanoreceptors on the first antennae function, during sinking, as gravity receptors (*27*). A neutrally buoyant animal (*28*), unable to orient itself, persistently executes escape movements, uses up all its energy (*29*), and dies. During a feeding bout the animal does not sink. It uses its relative weight to create a strong feeding current and takes advantage of the double shear field to perceive its food. The extended first antennae serve, therefore, not only as parachutes (*30*) but also as sophisticated arrays of sensors, perceiving trajectories of algae, gravity, and approaching predators (*31*). A case in point is the report (*32*) that Arctic calanoids, which store large amounts of wax esters and are, therefore, positively buoyant, behave in an upside-down fashion (*33*).

In summary, negative buoyancy helps calanoid copepods to orient and to create a large laminar feeding current in which the active space around an alga is deformed predictably. Arrays of chemosensors perceive the trajectory of an alga in the feeding current. In selecting the flow through the capture area (Fig. 2A) from the feeding current, calanoid copepods maximize encounter rate with algae. This allows them to survive in nutritionally dilute environments (*1*).

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Suppression of Ovulation in the Rat by an Orally Active Antagonist of Luteinizing Hormone–Releasing Hormone

Abstract. *A synthetic antagonist of luteinizing hormone–releasing hormone blocked ovulation in rats in a dose-dependent manner when given by gavage on the afternoon of proestrus. Ovulation was delayed for at least 1 day in all animals given 2 milligrams of antagonist and in some of the animals treated with 1 or 0.5 milligram. Oral administration of 2 milligrams also blocked the preovulatory surge of luteinizing hormone. This demonstration that antagonists of luteinizing hormone–releasing hormone can have oral antiovulatory activity clearly enhances their therapeutic potential.*

The isolation, structural elucidation, and synthesis of luteinizing hormone–releasing hormone (LH-RH), which controls the secretion of luteinizing hormone (LH) and follicle-stimulating hormone from the pituitary, have opened new approaches to contraception (*1*). These approaches are based on antagonistic as well as agonistic analogs of LH-RH (*1*). Several laboratories have reported the synthesis of antagonistic analogs of LH-RH that inhibit ovulation in several species by suppressing the preovulatory surge of gonadotropins (*2*). Some of these antagonists are active in humans (*3*). However, the necessity of administering these peptides parenterally has hindered their clinical use for contraception. We now report a potent new antagonist of LH-RH that has antiovulatory effects when given orally.

The LH-RH antagonist [N-acetyl-D-*p*-chloro-Phe^{1,2},D-Trp^3,D-Arg^6,D-Ala^{10}]-LH-RH (Phe, phenylalanine; Trp, tryptophan; Arg, arginine; Ala, alanine) is synthesized by solid-phase methods and has potent antiovulatory effects in the rat (*4*). As little as 5 μg of the peptide completely inhibited ovulation in 4-day-cycling Sprague-Dawley rats when injected subcutaneously in 40 percent propylene glycol and saline at noon on the day of proestrus (*5*). All five animals injected with vehicle ovulated. We studied the analog further by determining its ability to suppress LH levels in ovariectomized rats (Fig. 1). The animals were ovariectomized 14 days before receiving

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