Detergents: Effects on the Chemical Senses of the Fish Ictalurus natalis (le Sueur)

Abstract. *Two types of detergents, one easily oxidized and the other not, damage the chemoreceptors of yellow bullheads,* Ictalurus natalis *(le Sueur) exposed to concentrations of 0.5 parts per million in the surrounding water. This concentration is considerably lower than that at which sublethal damage has been detected previously. Histological examination reveals erosion of the taste buds; electrophysiological methods and observations of swimming and feeding behavior reveal impairment of receptor function. Affected fish do not fully recover after 6 weeks in detergent-free water.*

Since 1930, synthetic detergents have virtually replaced soap for household washing purposes. Because a residue of 1 mg per liter (one part per million) of household detergents in sewage outflows still causes foaming in rivers (*1*), attempts have been made, though not always successfully, to keep the detergent loads of sewage effluents lower than this. Higher concentrations frequently occur because the so-called "hard" detergents with branched-chain alkyl benzene sulfonates (ABS) as their surfactant ingredient are not readily amenable to degradation or biological oxidation (*2*). Following an earlier trend in Europe, American detergent manufacturers are now changing over completely to the production of "soft" detergents which have linear alkyl benzene sulfonates with unbranched sidechains (LAS) as their main ingredient and can be decomposed biologically. By the end of 1965 all household detergents manufactured in the United States will be of the "soft" type (*3*).

There have been few investigations of either the lethal (*4*) or sublethal (*2*) effects of detergents on aquatic animals. Since both hard and soft detergents reduce surface tension and act as fat solvents, the cell membranes of fish that come into direct contact with water containing detergents are susceptible to damage. For example, the gills of sunfish (*Lepomis gibbosus* and *L. macrochirus*) are damaged by 3 ppm ABS and by lower concentrations of LAS (*4, 5*).

The functioning of olfactory and gustatory receptors also depends on direct contact with water. In certain fish, such as bullheads, taste buds are found on the barbels of the head, on the body surface, and in the mouth (*6*). We have studied the effects of low concentrations of ABS and LAS on the chemoreceptors of yellow bullheads, a fish generally considered hardy and tolerant of low oxygen concentrations and other stresses.

The fish, weighing between 200 and 400 g each, were trapped in detergent-free lakes in the vicinity of Ann Arbor. They were kept in the laboratory and exposed to graded concentrations of 10 to 0.5 parts of ABS and LAS per million in the water (*7*), for up to 4 weeks, depending on the concentrations of the detergent. The dechlorinated tapwater used for these exposures had a *p*H of 9.1; the alkalinity was 16 mg of $CaCO_3$ per liter when tested with phenolphtalein and 65 mg of $CaCo_3$ per liter when tested with methyl orange; there are no residual chlorides. The water temperature in the aquariums fluctuated between 19° and 22°C.

Histological preparation of olfactory rosettes, barbels, and taste buds on oral surfaces from 21 experimental fish were compared with similar preparations of tissues from control fish kept in detergent-free water. Sections were stained with Bodian silver and by acid alizarin-blue anilin-blue staining methods (*8*). Damage to taste receptors became visible when the bulge of the taste bud disappeared and a pore-like depression formed in its stead. Later, the central portion of the taste bud disintegrated and lacunae appeared within it. The rate of disintegration varied with the concentration of ABS or LAS to which the fish were exposed and with the time of exposure (Table 1; Fig. 1, *B* and *D*).

Histological preparations of olfactory epithelium indicated that this tissue may be more resistant to detergents than are taste buds; only at concentrations between 4 and 5 ppm could one detect a thickening of the borders of sensory cells in the folds of the olfactory rosette, and fewer bipolar cells than in sections from control animals.

Progressive damage of receptor func-

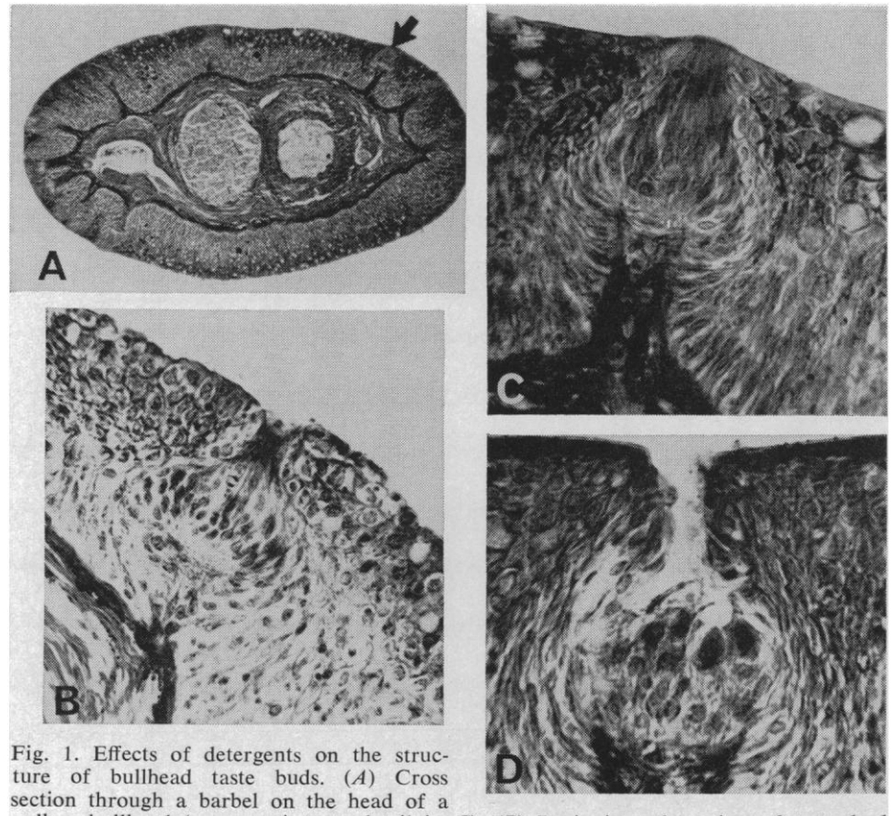

Fig. 1. Effects of detergents on the structure of bullhead taste buds. (*A*) Cross section through a barbel on the head of a yellow bullhead (arrow points to detail in *C*). (*B*) Beginning of erosion of taste bud with a pore forming in place of the bulge. (*C*) Typical taste bud from a control fish (note the apical bulge where the sense cells come in contact with the water. (*D*) Disintegrated taste bud with lacuna in center.

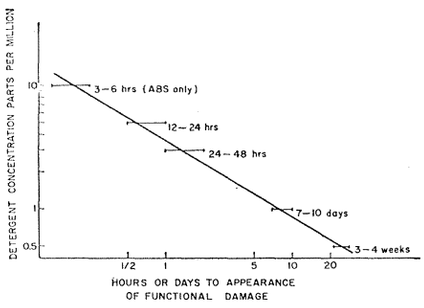

Fig. 2. Effects (on a log-log scale) of decreasing concentrations and increasing exposure times to detergents on the onset of functional damage to chemoreceptors in the barbel of yellow bullheads.

tion was ascertained by recording action potentials from afferent nerve bundles at the base of the barbels which were severed from the fish and prepared as in an earlier investigation of chemoreceptors of hakes and sea robins (*9*). Cystein was used for assay after its efficacy of stimulating normal taste buds had been established.

Visible signs of histological damage lagged behind the functional impairment of chemoreceptors; action potentials from affected taste buds diminished after only a few hours of exposure to a 10-ppm solution of ABS. At lower concentrations of the detergents electrophysiological tests also demonstrated damage to the receptors after shorter exposure times than histological methods (Figs. 2 and 3).

Observations of food-finding behavior in one-way viewing chambers were made with two pairs of fishes: one member of each pair served as a control in detergent-free tap-water and the other was exposed to a 1-ppm solution of either detergent. Exposure to ABS lasted 28 and to LAS 25 days, with daily renewal of the soft detergent to avoid lowering of the concentration, before the observations were begun. The detergent concentration, monitored with the methylene-blue technique (*10*), was maintained in the experimental tanks throughout the observations. Sublethal effects on the gill tissues were presumably minimal at this level of concentration, so that the difference in the feeding behavior between experimental and control fish was most probably due to the effects of detergents on the sensory organs.

Single food pellets (*11*) were presented to the bullheads at random through one of ten equally spaced, opaque feeding tubes that protruded deeply into the 30-gallon (113-kiloliter) test aquariums. The control fish of the pairs always rested on the bottom, as healthy bullheads in aquariums are wont to do during the day; the treated fish cruised with much greater frequency. Shortly after a pellet had gently dropped to the bottom the control fish spread their fins and stiffened their barbels, with a delay roughly proportional to their distance, at the time, from the respective feeding tube. Then they swam to the pellet and picked it up. The experimental fish found the pellets only when they accidentally swam over them or touched them with their lower jaw or lip. Their unexpected heightened activity, as a result of having been exposed to the detergent in the water, made meaningless any comparison of the time the two fish of each pair took to find the food; in some observations the treated fish took a much longer time, in others a shorter time, because they swam around and would accidentally encounter the pellet, almost as it fell to the bottom.

Table 1. Histological effects of graded series of detergent solutions on the barbels of bullheads. Fish exposed to 10 ppm LAS died; the effect of the two detergents, LAS and ABS, were otherwise similar.

Concentration of detergent (ppm)	Time after which damage appears (days)	Degree of damage
10	1	Lacunae and disintegration
5	3	Lacunae and disintegration
1	7–10	Lacunae and some disintegration
0.5	24 (approx.)	50 percent of tips of taste buds eroded

After 75 paired observations the experimental and control fish were reversed; the former was placed in clean tap water and the latter in a 1-ppm detergent solution. During the 6 weeks following the reversal an additional 150 paired observations showed that the formerly hyperactive experimental fish had quieted down while the previously quiet control fish had become increasingly active. The experimental fish had only partially recovered however, because after 6 weeks in clean water they could sense the pellets only from a distance of 12 cm or closer; the control fish had initially reacted to substances that emanated from pellets that were at least 50 cm away.

Since fish locate distant chemical stimuli by smell (*12*), the impaired food-finding behavior suggests that the olfactory epithelium may have been affected even by detergent solutions of 1 ppm, though histological examination did not reveal damage at this concentration. The almost continuous cruising of the treated animals may further

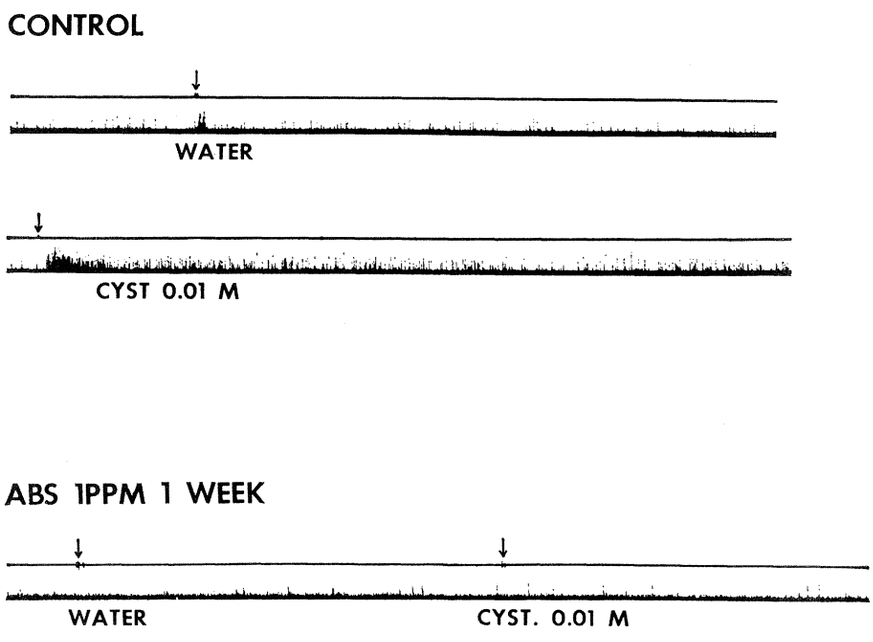

Fig. 3. Oscilloscope records of discharge characteristics of normal taste fibers of a yellow bullhead and of those from similar fibers after exposure of the fish to a soluton of 1-ppm ABS for 1 week. Arrows on upper trace indicate application time in relation to response (camera speed, 10 mm/sec).

point to general physiological effects, perhaps caused by minor gill damage or effects on other organs. While regeneration of taste buds could be expected after the treated fish was placed in clean tap water—analogous to taste bud renewal after the healing of a cut barbel nerve (*13*)—we have no information on corresponding repair processes in the olfactory epithelium; 4 to 6 weeks in clean water are apparently not sufficient to permit recovery from the effects of detergents on the chemical senses.

The chemical senses of fishes, especially olfaction, are important in many aspects of their behavior (*14*), and in bullheads, barbels with intact gustatory structures are required for efficient feeding (*15*). Fishes that rely mainly on their chemical senses for finding food will feed less efficiently in waters persistently carrying around 0.5 ppm of detergents. They may also become weakened and more susceptible to disease than normal fish.

The destruction of the sense of taste in fishes by detergents may also have immediate deleterious effects; certain substances in the water are only smelled while others are only tasted. Among the latter are acids, alkalies, and some metal ions that are often present under conditions of industrial pollution (*16*). These substances are poisonous individually and synergistically with each other and with different pollutants. Their continued presence in natural waters sooner or later leads to an exclusion of fishes, at least near the zone of such pollution; however, fishes can survive beyond such zones. Fishes affected by detergents will be less able than normal ones to avoid certain industrial pollution zones and are more likely to be doomed.

Many major rivers now have detergent levels between 0.1 and 0.2 ppm (*17*) though some, like the Illinois River below Chicago, remain above 0.5 ppm for 150 miles (240 km) and many smaller streams in highly populated areas also are bound to carry regionally a substantially higher detergent load than 0.1 ppm (*18*). The impending change-over to LAS in all commercial detergents should assure detergent concentrations well below 0.5 ppm in most natural waters, under conditions of adequate sewage treatment (*19*). Even if peak detergent loads occurred only temporarily, leaving the fish sufficient time to regenerate the damaged sensory cells, effects will be small and our findings of minor importance. However, whether or not detergent concentrations will increase in natural waters will largely depend on the rate of improvement in submarginal sewage facilities. If this improvement does not keep pace with the increasing pressure on flowing waters, engendered by the rise in the population and by urbanization, many, especially the smaller rivers, will carry detergent concentrations that do not kill fishes outright, but produce the sublethal effects which we described.

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Sample No.	617256 ABS	620570 LAS
Active, type	Branched-chain sodium alkyl benzene sulfonate	Straight-chain sodium alkyl benzene sulfonate
Mol. wt.	ca. 345	ca. 345
Wt. (%)	58.0	57.2
Sod. sulfate, wt. (%)	2.4	1.6
Total solids, wt. (%)	60.4	58.8
Water, wt. (%)	39.6	41.2

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Lung Surfactants, Counterions, and Hysteresis

Abstract. *The wide hysteresis and low surface tension of lung extracts, as studied on a modified Langmuir-Wilhelmy surface balance, are dependent on the presence of subphase electrolytes. A possible mechanism for the hysteresis and its importance to the exchange of surfactants between the alveolar cell and the alveolar surface film are discussed.*

Crude lung extracts (*1*) produce surface films which are characterized by a marked lowering of surface tension on compression and a rapid increase on reexpansion. When surface tension is recorded simultaneously with surface area during compression and expansion of the surface film of a crude lung extract in 0.85 percent NaCl, a wide separation of the compression and expansion isotherms appears; that is, there is marked hysteresis of the surface film (Fig. 1, unshaded loop). Analogous hysteresis in the pressure-volume diagram of air-filled lungs has been attributed to surface forces (*2*), but the mechanism of hysteresis is unknown. The role of electrolytes in the medium used for preparing lung extracts had not been considered previously. We have found a striking difference in surface activity between distilled-water extracts of the lung (Fig. 1, shaded loop) and saline extracts (Fig. 1, unshaded loop), and have carried out experiments to define the nature of the dependency of surface activity on electrolyte content. We then formulated a working hypothesis to explain the mechanism of hysteresis and its importance to the exchange of surfactants (*3*) between the lung alveoli and the alveolar surface film.

The lungs of normal New Zealand white rabbits (3 to 4 kg, aged about 4 months) were used, and all procedures were carried out at room temperature. The rabbits were killed by exsanguination from an abdominal aortic transection. The lungs were removed immediately and samples (3 g), minced finely with scissors, were stirred in 50 ml of the given medium (either water or salt solution) for 30 minutes, and filtered through four-layer gauze; the filtrate was centrifuged (Sorvall RC-2) at 5000 rev/min for 10 minutes and then at 18,000 rev/min for 20 minutes. The supernatant was placed in the