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

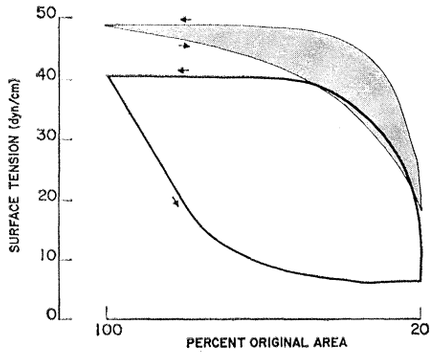

Fig. 1. Shaded loop: lung extract in distilled water. Unshaded loop: lung extract in 0.85 percent NaCl.

trough of a modified Langmuir-Wilhelmy surface balance (*1*), and cyclic compression and expansion of the surface was begun after 5 minutes. The area was 68 cm^2 at full expansion and 13 cm^2 at the end of compression. Compression and expansion times were 12 minutes each; the compression barrier was driven by a constant-speed motor (linear drive). Surface tension and area were recorded simultaneously on a Houston X-Y recorder.

Lung samples from each of 11 rabbits were paired; one of the pair was extracted in 0.85 percent NaCl and the other in distilled water. The extracts in 0.85 percent NaCl gave the usual surface tension–area diagrams (Fig. 1, unshaded loop); the mean (and range) of the maximum surface tension (γ_{max}) was 40.8 (34.0 to 48.0) dyne/cm, the minimum surface tension (γ_{min}) was 6.3 (0.5 to 14.0) dyne/cm, and there was marked hysteresis. The variation in surface tension is probably related to the ratio of the amount of alveolar tissue to that of extra-alveolar tissue, including blood, in the lung sample used, that is, the larger the alveolar tissue component, the greater the quantity of extractable surfactants. The amount of extra-alveolar tissue in any 3.0-g lung sample is related to several factors, including the pulmonary blood volume and the region of the lung from which the sample was taken. Other experiments in which lung samples from the same animal, treated in the same manner, were compared showed that variations in γ_{max} and γ_{min} lie within a smaller range than that given above. However, differences in blood volume may be greater between two different animals than between two randomly selected regions of the lung of the same animal. The distilled-water extracts gave the diagram shown in Fig. 1 (shaded loop): γ_{max} was 46.5 (41.5 to 56.5) dyne/cm; γ_{min} was 19.1 (14.0 to 26.5) dyne/cm; and there was poor hysteresis, which by some criteria (*4*) could be interpreted as a loss or absence of normal lung surfactants.

Lung extracts were also prepared in distilled water, and after centrifugation, the supernatant was brought to 0.85 percent NaCl by the addition of NaCl. These extracts gave a γ_{max} of 44.8 (37.0 to 47.0) dyne/cm, γ_{min} of 7.3 (0.5 to 12.5) dyne/cm and wide hysteresis, values essentially the same as those of the samples extracted directly in 0.85 percent NaCl. These experiments indicated that the normal lung surfactants were indeed extractable in distilled water but required electrolytes in solution to manifest normal behavior in the surface balance.

In another series of experiments, lungs were extracted in distilled water, the supernatant was placed in the trough, and cyclic compression and expansion was carried out until the γ_{min} was reproduced in two consecutive cycles (generally after 5 to 6 cycles). Midway during the subsequent expansion the electrolyte solution was added to the subphase, that is, the aqueous medium below the film, without interruption of the movement of the barrier. The electrolyte solution (1 to 2 ml) was injected into the subphase through a 20 to 22-gauge needle placed immediately behind the barrier. The needle tip was pointed toward the under edge of the barrier, and the solution was injected quickly to obtain good mixing in the subphase. Injections of distilled water changed neither the surface tension nor the shape of the surface tension–area diagram during subsequent cycles.

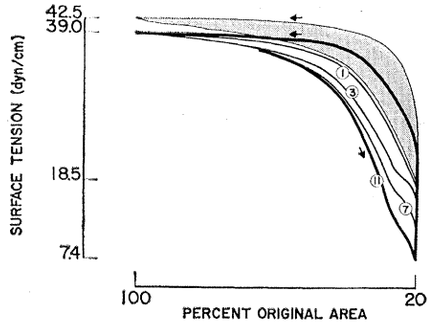

Fig. 2. Shaded loop: lung extract in distilled water. Cycles 1, 3, and 7: compression isotherms after injection of NaCl into the subphase to bring the concentration to 0.10 percent NaCl. The maximum effect of the added NaCl was obtained in the 11th cycle.

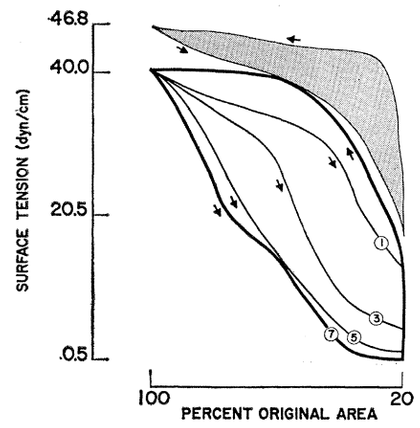

Fig. 3. Shaded loop: lung extract in distilled water. Cycles 1, 3, and 5: compression isotherms after injection of NaCl into the subphase to bring the subphase concentration to 0.15 percent NaCl. The maximum effect of the added NaCl was obtained in the 7th cycle.

Injection of NaCl to bring the concentration of the subphase to 0.1 percent NaCl produced a gradual lowering of the mean γ_{max} from 42.5 to 39.0 dyne/cm and of the mean γ_{min} from 18.5 to 7.4 dyne/cm without change in hysteresis (Fig. 2). Nine to 13 cycles were required to change the surface tension after injection of NaCl. Raising the NaCl concentration of the subphase to 0.15 to 0.2 percent resulted in a lowering of both the mean γ_{max} from 46.8 to 40.0 dyne/cm and γ_{min} from 20.5 to 0.5 dyne/cm and in the restoration of normal hysteresis after six to eight cycles (Fig. 3). Increase of the subphase concentration to 1.4 percent NaCl yielded essentially the same results as those obtained with 0.15 to 0.2 percent NaCl. Other experiments in which lung extracts were prepared di-

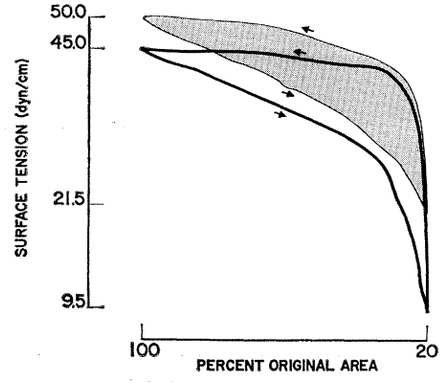

Fig. 4. Shaded loop: lung extract in distilled water. Unshaded loop: maximum effect after addition of KCl to the subphase to bring the subphase concentration to 0.042 percent KCl.

rectly in various concentrations of NaCl gave the same diagram as when NaCl was added to the distilled-water subphase.

Similar experiments were conducted with KCl being injected. Bringing the distilled water to 0.042 percent KCl (equivalent to the K^+ concentration in extracellular fluid) resulted in a drop of the mean γ_{max} from 50 to 45 dyne/cm and γ_{min} from 21.5 to 9.5 dyne/cm, but there was no appreciable change in hysteresis (Fig. 4). Reconstitution of the distilled-water subphase to 0.15 to 0.2 percent KCl or higher gave results similar to those obtained with equivalent concentrations of NaCl.

Our experiments demonstrate the importance of subphase electrolytes to the formation and behavior of surface films of lung extracts wherein the electrolyte solution provides the counterions for the surface film. Also, whereas the lung surfactants are extractable in distilled water, both the lowering of surface tension and the marked hysteresis of normal lung extracts are dependent on the ionic composition of the subphase. A high value for γ_{min} of distilled-water extracts of the lung has been reported (*5*) but the mechanism was not defined.

Electrolytes are known to lower the surface tension of monolayers of collagen (*6*) and long-chain ions (*7*). In the absence of electrolytes in the subphase of lung surfactants not only is the surface tension higher, but the compression isotherm follows the expansion isotherm more closely. A similar phenomenon was observed when 0.85 percent NaCl extracts of the lung were serially diluted (*8*). Therefore, the compression isotherm reflects conditions of greater surface concentration; that is, the greater the surface concentration, the steeper the slope of the compression isotherm, the greater the fall in surface tension for a standard reduction in area, and the wider the separation between compression and expansion isotherms. In the presence of subphase electrolyte the surface concentration of surfactants increases as indicated by the lower surface tension and the greater slope of the compression isotherm (Figs. 1 and 3). During the early phase of compression intermolecular distance decreases, and surface tension falls rapidly. With marked compression and collapse of the film (late phase) the surfactant molecules leave the surface and may enter the subphase. Upon high compression of the film, the more electrolyte present (to an optimal concentration), the more surfactant will dissolve or disperse in a flux from the surface into the subphase. Ellis and Pankhurst (*6*), working with collagen films, found that the electrolyte in the subphase was most effective in lowering the surface tension of the film when the film was highly compressed; they concluded that compression caused the surface molecules to move from the surface to the subphase. During the early phase of reexpansion, water or hydrated ions or both must move into the surface, effectively lowering the surface concentration of surfactants and increasing surface tension. Surfactants return to the surface more slowly throughout reexpansion, and gradual restoration of the original surface concentration (per square meter of surface area) takes place over the plateau of the expansion isotherm. The circulation of surfactant molecules between the surface and subphase in this way would tend to facilitate the exchange of surfactants between the alveolar cell and the alveolar surface film in a similar system in the lungs. Lung alveoli probably have a surface lining of surfactant film and subphase similar to that of lung extracts (*9*). Such films could exhibit the characteristic surface behavior of lung extracts in the presence of Na^+ concentrations found in the extracellular fluid, as simulated by the studies with 0.85 percent NaCl. The Cl^- concentration in extracellular fluid (equivalent to 0.6 percent NaCl) could also account for normal surface activity. However, the K^+ concentration of extracellular fluid (equivalent to 0.042 percent KCl) is too low to account for the normal surface activity of lung extracts.

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Pineal Gland: Influence on Gonads of Male Hamsters

Abstract. *Exposure of male hamsters to cycles of 1 hour of light and 23 hours of darkness causes atrophy of the gonads. Pinealectomy prevents this atrophy, but has no effect on animals exposed to light-dark cycles of 16 : 8. Likewise, removal of both eyes induces gonad atrophy which is prevented by pinealectomy. These data emphasize the importance of the pineal gland in the regulation of photoperiodic influences on the gonads.*

Recent investigations (*1*) show that exposure of male hamsters to low temperatures (6°C) and long periods of light [light:darkness (LD) 14:10, in hours] or room temperatures (22°C) and short periods of light (LD 2:22) induce testicular atrophy. The combination of low temperatures and short periods of light results in additive effects. While the effects induced by cold are not surprising, since hamsters are hibernators and normally show reduced reproductive activity in preparation for and during hibernation (*2*), the changes induced by short periods of light were of particular interest since so little information on mammalian responses to photoperiods is available for comparison. In view of the increasing awareness that the pineal gland is a mediator in the effects of light on reproductive functions (*3*), studies were initiated to determine the influence of this gland on the gonads of hamsters exposed to various amounts of light and darkness.

Forty-nine male hamsters weighing 84 to 135 g were used. Twenty-nine animals were pinealectomized by techniques described elsewhere (*4*) and 20 animals were subjected to comparable bleeding and trauma in the same area, but the pineal gland was left intact.