apparatus (fig. 3). The synaptic terminals are filled with clear synaptic vesicles and characterized by synaptic ribbons (fig. 4). The pineal photoreceptors do not form a distinct, stratified, structure as is typical of vertebrate lateral retinae, and the number of photoreceptors in a single lumen is small compared to the large population of supporting cells.

The pineal organ is known to function as a photoreceptor in a variety of amphibians[3,4], and has been reported to be a major influence on circadian rhythms in birds[5-8]. The locomotor activity of the Japanese common newt under a 12:12 light-dark schedule shows a circadian rhythm which cannot be entrained after ablation of the pineal organ. In constant darkness (12:12 dark-dark) the rhythms persist. The locomotor activity rhythm is entrained by light-dark cycles in newts whose lateral eyes have been enucleated but whose pineal organs are intact. This indicates that the cone-like cells are functional photoreceptors, and that the pineal organ does have the function of extraocular perception of light. It seems that one role of the pineal organ in the newt is to effect entrainment and/or oscillation of the circadian locomotor rhythms.

Further studies are in progress to analyze the mechanisms of the synapse of the photoreceptor, and of the neuronal network of the pineal organ.

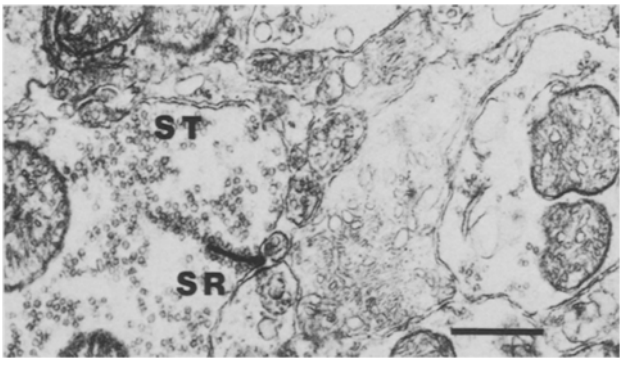

Figure 4. Electron micrograph showing synapse. The synaptic terminals (ST) are filled with clear synaptic vesicle. The synaptic ribbons (SR) are surrounded by vesicles. Bar indicates 0.5 µm.

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Fluid fluxes in the ferret trachea[1]

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Summary. A net reabsorption of fluid was observed in isolated ferret trachea under control conditions. Cholinergic stimulation resulted in net secretion of fluid while atropine blocked this response without any effect on the basal process of fluid reabsorption.

Mechanisms regulating water and ion movements across mammalian tracheal epithelia are essential to maintain effective mucociliary transport[2]. While ion transport across this tissue has been well-studied[3-5], data on the fluid movements which accompany these ionic fluxes are limited[6,7]. This report describes in vitro studies of fluid fluxes across the ferret tracheal epithelium.

Materials and methods. Male ferrets *(Mustela putorius furo)* weighing between 800 and 1000 g were used in these experiments. The ferret was sacrificed by exsanguination and a tracheal segment was excised and rinsed in a modified Krebs-Henseleit solution. The trachea was trimmed of connective tissue and then mounted in a plexiglass perfusion chamber. The chamber was then filled with the modified Krebs-Henseleit solution containing inulin. The pH of the solution was maintained between 7.35 and 7.40 by continuous bubbling with 95% O_2 and 5% CO_2. This produced a partial pressure of oxygen in the bath and lumen greater than 100 torr. The temperature of the bath was held at 37 °C by means of a circulating water bath. Stationary perfusion of the tracheal lumen, using a modification of the split oil column technique, similar to that employed by Gertz et al.[8] was used in these studies. The perfusate bubble occupied approximately 2.0 cm of the excised tracheal segment. Water fluxes were measured by determining changes in the concentration of inulin in the perfusate. Preliminary studies demonstrated that inulin did not cross the ferret tracheal epithelium. Inulin concentration was measured by a spectrophotometric assay adapted from the fluorometric technique developed by Vurek and Pegram[9].

The net water movement at each interval was calculated by the equation:

$$\frac{BIA - SIA}{BIA} \times Vp = \text{Net fluid secreted or reabsorbed}$$

where BIA was the baseline inulin absorbance level, SIA was the sample absorbance level, and Vp was the volume of the perfusate. At each interval, BIA was adjusted not only for the volumes removed as samples but also for calculated water fluxes from previous intervals. The flux or flow rate per trachea was expressed as µl/h × trachea. The viability of the preparation was checked at the end of each experiment by exposing the luminal surface to trypan blue to see whether or not the epithelial cells took up the dye.

Results. Over the 2-h baseline period in the 7 tracheas a net reabsorption of fluid at a flux of 30 ± 3 µl/h × trachea ($\bar{x} \pm SE$) was observed. Cholinergic stimulation by addition of 10^{-6} M carbamylcholine to the submucosal bathing solution in these same tracheas resulted in a net secretion of

fluid at a mean flux of 46 ± 15 µl/h × trachea (fig. 1). This mean flux as well as the individual flux from each of the 7 tracheas post-carbamylcholine, was significantly different from not only the last baseline flux but also from all of the baseline fluxes.

In 2 preparations significant trypan blue staining of the mucosa was observed. No net fluid flux was detected in these tissues. This data was not included in the analysis.

In the study on the effect of cholinergic blockade on fluid movement (fig. 2), the baseline reabsorption flux of 31 ± 3 µl/h × trachea was again demonstrated. This value was not statistically different from the mean baseline flux of the other 7 tracheas. Atropine (10^{-5} M) added to the submucosal bathing solution, did not significantly alter the baseline flux but completely blocked the response to cholinergic stimulation.

Discussion. These data demonstrate that under control conditions (fig. 1) a net reabsorption of fluid occurs across the epithelium of the ferret trachea. This represents the first time that fluid reabsorption has been demonstrated in mammalian trachea. This observation, however, is in contrast to the recent reports of Welsh et al.[6] and Durand et al.[7]. Under control conditions in the dog[6] and bovine[7] trachea, little or no net secretion of fluid was observed. Species differences may account for the differences between our study and those of Welsh et al.[6] and Durand et al.[7]. Previous work on the dog trachea has demonstrated minimal or no net flux of ions under open-circuited conditions[2]. Since fluid fluxes occur in response to local osmotic gradients established by active ion transport, little if any fluid flux should be seen in the dog trachea model of Welch et al.[6]. Unfortunately, no ion flux data was reported for the bovine trachea; thus it is not possible to determine if the ion fluxes in this specie are compatible with zero net fluid flux. In contrast to this work, Davis et al.[10] have shown that in the rabbit trachea a net reabsorptive ion transport occurs under basal open-circuited conditions. While we did not measure ion fluxes in these studies, the presence of a similar system in the ferret, as exists in the rabbit, would be compatible with our fluid flux data.

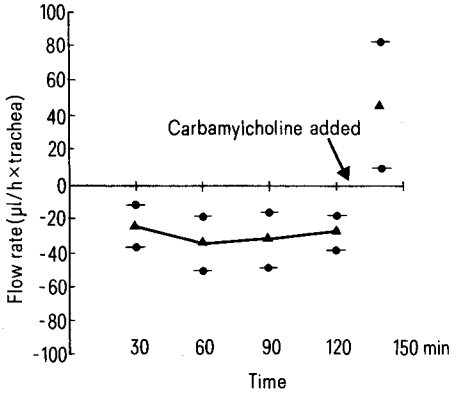

Figure 1. Baseline fluid fluxes and the effects of cholinergic stimulation. Mean fluxes from 7 tracheas (△) and the 95% confidence interval are shown. In this and in figure 2, negative numbers denote reabsorption of fluid while positive values indicate fluid secretion.

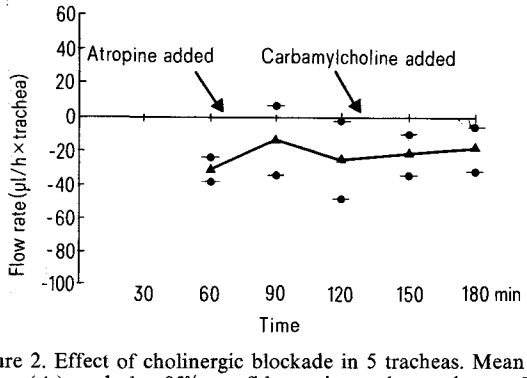

Figure 2. Effect of cholinergic blockade in 5 tracheas. Mean fluid fluxes (△) and the 95% confidence interval are shown. These values are not statistically different from each other or from the baseline values in figure 1.

Cholinergic stimulation in this study resulted in secretion or fluid movement into the tracheal lumen (fig. 1). Since the ferret trachea is well-endowed with submucosal glands[11], this fluid could have arisen from increased secretory activity of these glands[12]. However, fluid fluxes across the epithelium may have also contributed to this net fluid secretion. Again, ion transport data[13,14] from the literature are compatible with this thinking, since net fluxes of ions from the submucosa to the lumen have been observed in several species following cholinergic stimulation. Cholinergic blockade completely inhibits this fluid secretion but apparently has no effect on fluid reabsorption (fig. 2).

The perfusion technique is limited by the fact that it is not currently possible to measure accurately the surface area participating in this transport process. If we assume that the surface area covered with the heavy mineral oil does not participate in fluid absorption then we can estimate the surface area involved as that of a right cylinder approximately 2 cm long and 0.4 cm in diameter. Using the equation for surface area of a right cylinder, $2\pi rh$, the minimum surface area which produced the observed fluid flux was 2.5 cm^2. However, it could be larger. Consequently, the whole trachea was used as the unit of reference for calculating fluid fluxes. This was justified since the ferrets were fairly constant in size and thus the tracheal lumens were of reasonably constant diameter.

In conclusion, this study has demonstrated for the first time that fluid reabsorption can occur in the mammalian trachea. It is postulated that this may represent an important system for regulating the volume of respiratory tract fluid in certain mammals.

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