Twenty-two birds were subjected to 5 experimental procedures: a qualitative and a quantitative test of the effectiveness of an olive oil solution subcutaneously injected, a test of a saline solution, a more prolonged injection of oil solution, and finally an injection of saline solution in divided doses.

Results. Injection of an olive oil solution of theelin in a dosage as low as 20 rat units daily is sufficient to cause a change of new-growing feathers from male back to female type, as Juhn and Gastavson described for the capon. Alternate bands may be produced in these feathers by alternating injection periods with non-injection periods. A saline solution of theelin in similar dosage is without effect in altering this feather reaction. A dosage of 150 rat units per day, given as 3 injections per day (one every 8 hours) is barely sufficient to change the color of the mid-portion of the feather back to female type. With more prolonged injection (3 weeks) of the oil solution, no change in head furnishings was noted, but autopsy revealed a decided stimulation of oviducts, which average 6 times those of controls in weight, and show gland and muscle development.

Conclusions. 1. The crystalline product, theelin, is quite effective in restoring to female type those secondary sex characters which have been altered by ovariectomy—namely, feathers and oviduct. 2. With 3 weeks' injection, theelin does not interfere with the stimulus to comb-growth afforded by the compensatory right gonad in this species. 3. Olive oil solution is far more effective in producing these results in the fowl than is saline solution, possibly because of the too rapid absorption and elimination of the saline solution.

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Comparison of Ciliary Activity under In Vitro and In Vivo Conditions.*

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Normal ciliated epithelia removed from the animal body and kept under optimal conditions vibrate vigorously for long periods of time.

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Epithelium from the frog's pharynx is a classical example of the unceasing vibratility and indefatigability of cilia; it has been kept viable in tissue culture for as long as 56 days.[1] It has therefore been generally assumed from results of methods available for study that cilia under *in vivo* conditions show a similar unceasing activity. McDonald, Leisure, and Lenneman,[2] Seo,[3] and Pohle[4] in their experiments on frog's epithelium *in vivo,* regard the normal state of the cilia as active. The need of methods which will permit direct observation of ciliary activity has been emphasized,[5] and the present report deals with the application of a method by which cilia arranged in a field, as they are in the pharynx of the frog and the nasal cavity and sinuses of higher animals, may be kept under direct observation, thus eliminating the indirect evaluation of movement of inert particles applied to the surface, which method is of value in a study of *direction* of ciliary movement but not in the determination of *extent* or *continuity* of movement. The use of such a direct method reveals that when no stimulating factors are present the cilia are not active.

A strong concentrated beam of light passed through a water filter is thrown on the ciliated surface at as an acute angle as possible. The microscope is focused upon one of the many "high-lights" reflected. A microscope tube fastened to a sliding rod with adjustable joints permits focusing the microscope at any angle and thus quickly and easily aligning the optical axis with the reflected beam from the "high-light".

Magnification of 78 times with a Spencer 5.4× objective and 20× ocular is ample. The long working distance of 29 mm. permitted with this objective is a decided advantage. The low magnification is adequate because the light directed across the surface of the epithelium causes the ciliary waves to cast shadows and thus appear larger than when they are viewed with transmitted illumination.

A frog† is fastened to an adjustable platform and its lower jaw retracted. Most of the animals were pithed, but for comparison a few were not. The cilia of the pharynx showed no activity a few minutes after the jaw was retracted and frequently this state was

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1 Umeda, T., *Acta Dermat.*, 1926, **8**, 95.

2 McDonald, J. F., Leisure, C. E., and Lenneman, E. E., *Am. Acad. Ophthal. and Otolaryngol.*, 1928, 318.

3 Seo, A., *Jap. J. Med. Sc.*, III, *Biophysics*, 1931, **2**, 47.

4 Pohle, K., *Arch. Exp. Path. u. Pharm.*, 1931, **159**, 452.

5 Lucas, A. M., *J. Morph.*, 1932, **53**, 243.

† Winter frogs only have thus far been employed.

reached immediately after the mouth was opened. A mucous layer covered the ciliated surface and it also was inert. The light was not responsible for this quiescent state, because, if it has any effect at all it acts as a stimulus. Occasionally when the lamp has been turned on after the frog has rested in the dim diffuse light of the room there occurs after a few seconds scattered, weak vibratory movements of cilia which formerly were quiescent. Cilia *in vitro,* likewise, according to Umeda[6] are stimulated by light of long wave lengths. Wharton[7] finds, however, on excised trachea that light has an inhibitory effect. Evaporation of water with its consequent drying of the surface is not a factor responsible for the quiescent state of the cilia for many reasons brought out in the following experiments.

Fine dry carbon particles of lamp black were delivered from an atomizer upon the quiescent cilia of the pharynx. No reaction was apparent for several seconds, after which a rapid acceleration in activity took place followed by a diminution to a more or less constant rate. The activity sometimes endured until the carbon from regions anterior to the point of observation passed across the field, but more often isolated groups of cilia became temporarily quiescent; soon again resuming an active part in the propulsion of mucus. The mucous film being a viscid elastic sheet as pointed out by Hilding[8] continued movement across the groups of quiescent cilia. The mucous cells likewise appeared to react to the presence of carbon, it seemed by direct observation. The cilia, however, had become active upon the application of carbon before changes in the thickness of the mucous layer became apparent. A rough estimate of the thickness of the mucous layer may be determined by the fact that the ciliary waves are distinct when the layer is thin and disappear from view when the layer becomes sufficiently thick; also particles upon the surface of the mucus cast shadows on the epithelium, which criterion also may be used as a measure of thickness.

The vibratory movements of the cilia soon ceased following the passage of the mucus with its overlying particles. The diminution of activity is not as rapid nor as uniformly distributed as the acceleration and it is possible that following the passage of the carbon laden mucus certain readjustments of the new sheet are involved before the cilia come to complete rest.

Air as well as dry carbon was blown on the pharynx when the

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[6] Umeda, T., *Acta Dermat.*, 1927, **10**, 603.

[7] Wharton, D. R. A., *Am. J. Hyg.*, 1931, **14**, 109.

[8] Hilding, A., *Arch. Otolaryng.*, 1932, **15**, 92.

atomizer was used. A current of air containing no carbon, therefore, was blown vigorously against the surface; it produced a marked decrease in the thickness of the mucous layer. It occurred too rapidly to be a drying effect and was undoubtedly due to a pushing of the semifluid material outward in all directions. The cilia underlying the thinned area of mucus became active several seconds later, showing the same reactions that they did when carbon was included except that this activity lasted for a much shorter period of time—less than one minute to several minutes, dependent upon the strength of stimulus, the length of time the animal has been under observation, et cetera. When the cilia again assumed a quiescent state the mucous layer was apparently as thick as it was before.

The same frog was subjected to carbon or blasts of air repeatedly over a period up to an hour with the same sequence of events in each trial. Thus the cessation of activity is not the result of treatment nor can the absence of movement apparent after the mouth is opened, be ascribed to drying effects.

Any possible mechanical effect due to currents of air was eliminated by dusting on the carbon through a cloth bag. Relatively large flakes fell upon the surface instead of fine particles obtained with the atomizer. The cilia became active as before after an interval of about 5 to 10 seconds and ceased activity when the carbon had passed. It is emphasized that cilia were seldom continuously active, but irregular areas and groups during the removal of the mucous coat repeatedly alternated from active to quiescent conditions, nor was the evidence clear that there was any marked predominance of activity immediately underlying the separate flakes of carbon. The irregularities in activity of groups of cilia are in agreement with the variable results of Galtsoff[9] in which the total work accomplished by the cilia under *in vivo* conditions is measured.

Evidence that response may be localized was obtained when a crystal of camphor was placed on the surface of the pharynx anterior to the field of view. The cilia became active in front of the crystal for a distance of several millimeters and their movements ceased a short distance behind the passing object. The stoppage of the cilia was not due to the camphor because the events could be repeated when the crystal was lifted from the posterior end of the pharynx and again placed at the anterior end.

The reaction to air and carbon in the unpithed frog were the same

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[9] Galtsoff, P. S., *J. Gen. Physiol.*, 1928, **11**, 415.

as those found in the pithed animal, except that respiratory movements added a complicating stimulus to the cilia. The slight tension exerted on the pharynx mucosa which accompanies the opening and closing of the glottis had no effect on the cilia, but depression of the eyes which bulged the mucosa quickly stimulated the cilia to activity, followed by a gradual slowing and if the interval between movements of the eyes were long enough the cilia ceased their movements completely. It remains to be determined whether this effect is entirely due to the mechanical factors of the stimulus.

Prodding the surface of the mucosa with an instrument will elicit a localized response. The spread of activity from the point touched is related to the strength of stimulus and the sensitivity of the epithelium.

Cessation of the heart beat and absence of blood flow through the mucosa is soon followed by failure of the cilia to respond to the stimuli applied. The same results come about more gradually during the progress of experimentation, probably because the lungs cease to function when the mouth is held open. Although the frog respires through its skin, at room temperature (25-26°C.) gaseous exchange by this means is probably insufficient.

The manipulations already described were repeated under *in vitro* conditions. The excised mucosa of the pharynx spread on a thin piece of glass was examined in a moist chamber. Care was taken that no fluid came in contact with the ciliated surface and that none of the ciliated edges were curled under against the glass. The cilia ceased several minutes after the handling. The surface was subjected at different times to carbon from the atomizer, air from the atomizer and carbon dusted from the cloth bag. The responses were practically the same as they were *in vivo*. When the cilia again assumed a quiescent state after stimulation the mucous layer was as thick as it was originally. Minor differences between ciliary behavior and mucous flow *in vivo* and *in vitro* may be attributed perhaps to the absence of blood circulation in the latter condition.

If the mucous membrane is removed from the pharynx after the experimental manipulations no longer produce the characteristic reactions, and placed on a slide with Ringer's solution and a cover slip, the cilia become vigorously active. Blood corpuscles and debris are rapidly swept along the surface. The cilia beat metachronously and the whole picture is the same as that described for studies on ciliated epithelium in an extraneous fluid medium. Again it is evident that cessation of activity is not due to any impotency in the ciliated cells themselves. The details of the effects produced by

Ringer's *in vivo* are difficult to determine because the "high-light" is reflected from the surface of the added fluid and not from the cilia. When particles are present, however, they can be followed. Their movement becomes more rapid after the aqueous solution is added, which agrees with Hill's observation[10] that the removal of mucus and addition of Ringer's solution greatly increases the rate of movement of particles in the excised trachea.

The results have demonstrated that the cilia of the frog's pharynx in the absence of extraneous factors maintain an inactive quiescent state, that with mechanical stimulation due to movements of the eyes or prodding with an instrument or addition of inert particles or derangement of the mucous layer, the cilia are stimulated to activity which ceases when the cause is removed. Therefore, the base line for physiological studies of ciliary behavior *in vivo* and even *in vitro* is zero for the frog's pharynx and not some value obtained from the rate of particle movement over the surface, nor after the surface has been washed with Ringer's solution. Thus it is evident that the effects on ciliary activity reported for many drugs, solutions, gases, et cetera, have been learned from an epithelium which was already acting under the influence of one or more stimuli and it may be that some of those agents which are said to have no effect on ciliary movement may act as a stimulant when the normal base line of activity is taken at zero.

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Nature and Function of Certain Fibers of the Vagus—A New Concept in Peripheral Nerve Organization.

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An analysis of the vagus nerves of the cat and turtle (Heinbecker[1]) demonstrated 3 distinguishable potential complexes. The first had physiological properties characteristic of somatic nerve fibers, the other 2 had properties of a much slower order, properties which our subsequent investigations have associated with autonomic motor functions. A correlation between potential form and

[10] Hill, L., *Lancet*, 1928, **215**, 802.

[1] Heinbecker, P., *Am. J. Physiol.*, 1930, **93**, 284.