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The influence of the pH and osmotic pressure upon tracheal ciliary beat frequency as determined with a new photo-electric registration device

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SUMMARY

A method for measurement of tracheal ciliary beat frequency in vitro is described. Light transmitted through the cilia is detected by a phototransistor mounted in a microscope, while the frequency is measured instantaneously and the waveform is displayed by an oscilloscope, connected to a transient recorder. Due to the magnification and the method of illumination, the movement of approximately 30 cilia is projected on the phototransistor. In Locke-Ringer solution the waveform shows a very constant amplitude. Interference arises after a noxious influence and is dependent on the frequency of the ciliary movement. The effect of pH and osmotic pressure on chicken embryo and rat tracheal ciliary beat frequency is assessed. The frequency is not influenced between pH=7 and pH=10, but higher and lower pH values decrease the frequency. Hypertonic NaCl solutions decrease the frequency of chicken embryo cilia as much as hypotonic NaCl solutions. Rat colia turned out to be less sensitive for hypotonic NaCl solutions.

INTRODUCTION

Nasal drops have been used during the last decennia, however only little information is available about their influence upon the ciliary function of the mucosa. Cilia were described by De Heide and Leeuwenhoek in the seventeenth century, but it took untill the thirties before Proetz emphasized the importance of studying the ciliary function and physiology (Proetz, 1932). The ability of the mucosal epithelium to remove foreign particles is essential to human health. The nasal cilia are covered by a blanket of mucus. The aerodynamic characteristics of the nose force a great deal of the particles in the inspired air to precipitate on the mucus. Mucus and particles are transported together by the cilia to the throat. Nasal medications can change and even destroy the epithelial cells and dependent on the agent the recovery takes a few hours to a few months. In order to improve the pharmaceutical formula of drugs administered in nose and respiratory tract, a model and method is needed to investigate the influence of these drugs and additives on ciliary motion.

Dalhamn (1955) estimated the frequency of ciliary waves with high speed cinematography. A motion picture is recorded at high speed and afterwards projected at low speed. The method is accurate, but expensive and laborious. Gallay (1960) assessed the activity of cilia of guinea pigs by determining the time, necessary to provoke a cessation of movement. Mirimanoff and Paley (1966) modified the method so that a rough indication could be obtained about the time, needed for recovery. Andersen (1971) illuminated cilia with stroboscopic light, which consisted of a variable number of flashes per second. When this number equals the frequency of the ciliary movement, the cilia are perceived stationary. This method is simple, but is not applicable for frequencies lower than 10 Hz and prolonged observations are extremely strenuous. Dalhamn and Rylander (1962) used a CdS cell and Mercke et al. (1974) used a photomultiplier to transform light variations, resulting from mucociliary waves into voltage variations. After suitable amplification the frequency can be assessed. This method is sensitive to vibrations, but accurate and quickly performable. Lee and Verdugo (1976, 1977) illuminated cilia from the rabbit oviduct with a laser beam. The spectrum of the scattered light can be analysed and gives information about the frequency of the ciliary movement. This method is sophisticated, and highly accurate, but very expensive.

All these methods involve more or less complicated electronic equipment. Some authors described more simple methods. An important disadvantage is the diminished precision of these methods.

Cherry (1970) estimated the function of the cilia as a percentage of its initial extension and vigor. This method is simple but very subjective. Schleppy (1975) measured the velocity of an erythrocyte placed on a piece of epithelium of a mouse. This model appproximates reality more than the methods mentioned above, but appeared to be little reproducible in our experiments. Ballenger and Orr (1963) took ciliary tissue from young human subjects under general anaesthesia by gently scraping over the tracheal rings. The shreds of tissue were observed under the microscope. Aggregates of epithelial cells are in a rotating movement caused by the outward directed cilia. The rotation speed can be measured and is dependent on the co-ordination and the force of the beating. The need of sufficient volunteers makes this elegant method not generally applicable. Some characteristics of the different methods are given in table 1. The development of a quick and precise method for direct monitoring the frequency of mucociliary waves would provide a useful tool to investigate medications for the respiratory tract.

We developed a simple and objective technique as a modification of the photoelectric method, that can be employed to study ciliary activity. The method was applied firstly for the determination of the influence of pH and osmotic pressure on ciliary movement.

Table 1. Comparison of several methods to investigate ciliary activity.
Valuation: + positive
0 indifferent
− negative

method	expenses	velocity	simpleness	precision	amount of information	author	species	vitro/vivo
high speed cinem.	0	−	−	+	+	Dalhamn (1955)	rat, guinea pig	vivo
stroboscopy	+	+	+	0	0	Andersen (1971)	rabbit	vitro and vivo
photo-electric	0	+	0	+	+	Mercke (1974)	rabbit	vitro
light scattering	−	+	−	+	+	Lee (1976, 1977)	rabbit (oviduct)	vitro
direct observation	+	+	+	0	−	Galley (1960)	guinea pig	vitro
particle transport	+	+	0	0	+	Schleppy (1975)	mouse	vitro
aggregate rotation	+	−	−	0	+	Ballanger (1963)	human	vitro

MATERIALS AND METHODS

Solutions

Experiments to investigate the effect of difference pH's were performed in solutions, containing:

NaCl	7.72 g
KCl	0.42 g
$CaCl_2 \cdot 2\ H_2O$	0.16 g
Dextrose	1.00 g/1000 ml

The pH was adjusted to 5, 6, 7, 8, 9, 10, 11, with an isotonic HCl solution, or an isotonic NaOH solution. pH was controlled before and after the experiments with a micro-electrode.

Experiments to investigate the effect of osmotic pressure were performed with solutions, containing resp. 0.45%, 0.9% and 1.5% NaCl. A Locke-Ringer (LR) solution was used for a comparison.

LR:	NaCl	7.72 g
	KCl	0.42 g
	$CaCl_2 \cdot 2\ H_2O$	0.16 g
	Dextrose	1.00 g
	$NaHCO_3$	0.15 g/1000 ml (pH adjusted at 7.4).

Tissue preparation

Inseminated chicken eggs (White Leghorns) were incubated for 19 days at 37°C and at an appropriate humidity in a breeding machine. After decapitation of the embryo, the trachea was removed, rinced with Locke-Ringer and sliced in rings, approximately 0.5 mm thick. A second set of experiments was performed with rings of tracheas of Wistar Albino Glaxo rats, sliced as described above.

Procedure

The rings were incubated in Locke-Ringer solution at 25°C for 45 minutes. Between the measurements the rings were stored in petri dishes at 25°C. Before a measurement the ring with a drop of the solution under investigation was transported from the petri dish to a slide with a 0.5 mm deep well. The correct position of the slide under the microscope could be found with the aid of an oscilloscope. Measurements were performed at four rings of one trachea of which one served as a reference. The other three rings were incubated in different test solutions. This procedure was repeated six times with different tracheas.

Microscope

The rings were studied with a Zeiss binocular microscope. The condensor was adjusted to the "Köhler" illimination. The microscope table was connected with a

cryo-thermostate (Colora WK6) to maintain a temperature of 25°C. The microscope was placed on a 350 kg marble slab, which was mounted on shock absorbers. A thermocouple at the place of the tracheal ring demonstrated no variation in temperature during 45 minutes, independent of the power of the illumination. The lamp of the microscope was fed by a stabilized direct current power supply (Eurocart 1035-0680) and was used at full power (6V, 2.5A).

Photo-electric Registration Device.

The device consisted of a phototransistor (BPX25), an amplifier and counter with adjustable trigger level (home made), a dual trace oscilloscope (Jiwatsu SS-5212), a transient recorder (Pauly-DMS-4000) and a T-Y recorder (Goerz RE 511); see fig. 1. On top of the microscope a photocamera was placed. In stead of the film the phototransistor, with a photosensitive surface of 0.64 mm^2, was mounted in the filmcassette. A preamplifier was constructed in the filmcassette to avoid pick up.

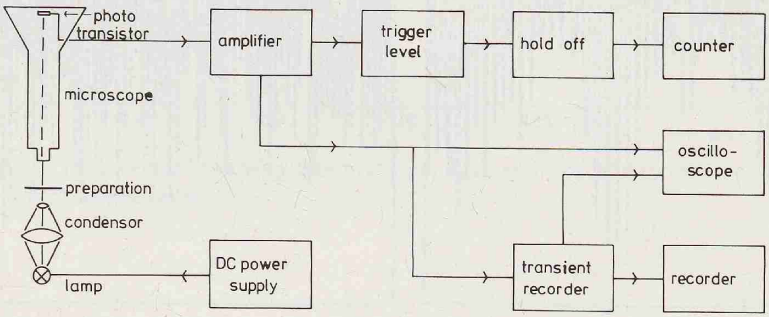

Figure 1. Block diagram of the photo-electric device, including a light source, microscope, phototransistor, amplifier, counter with adjustable trigger level and hold off, oscilloscope, transient recorder and an ink writer.

After amplification the signal was passed to the counter, which counted the times that the trigger level was exceeded. The counter was provided with a hold-off, which blocks the counter, during a time adjustable between 16 and 250 msec. after the trigger level was exceeded. This blocking effect could be visualised on the oscilloscope. The gate of the counter was open during 5 or 10 seconds at choise, the frequency was displayed afterwards for a second before counting starts again. The trigger level was adjusted in a way that peaks, which were three times shorter than the highest peaks, were just counted. When the microscope was focused on an object micrometer, the device was most sensitive to vibrations. In this situation it was checked that no counts were accomplished. The transient recorder made it possible to store a signal. This signal could be transported at a slower rate to the recorder (plotter). The waveform could be displayed flicker free on the oscilloscope, using the roll mode.

RESULTS

Figure 2a, b and c show representative examples of ciliary waves, obtained from the trachea of a chicken embryo. The variation of amplitude appeared to be dependent on the frequency of the ciliary beat. Lower frequencies caused more rapid variation, while very high frequencies caused no variation at all (fig. 2c). The frequencies found for the chicken embryo at pH = 7.4 were 17 to 20 Hz and for the rat 14 to 16 Hz, both at 25°C and during at least 6 hours.

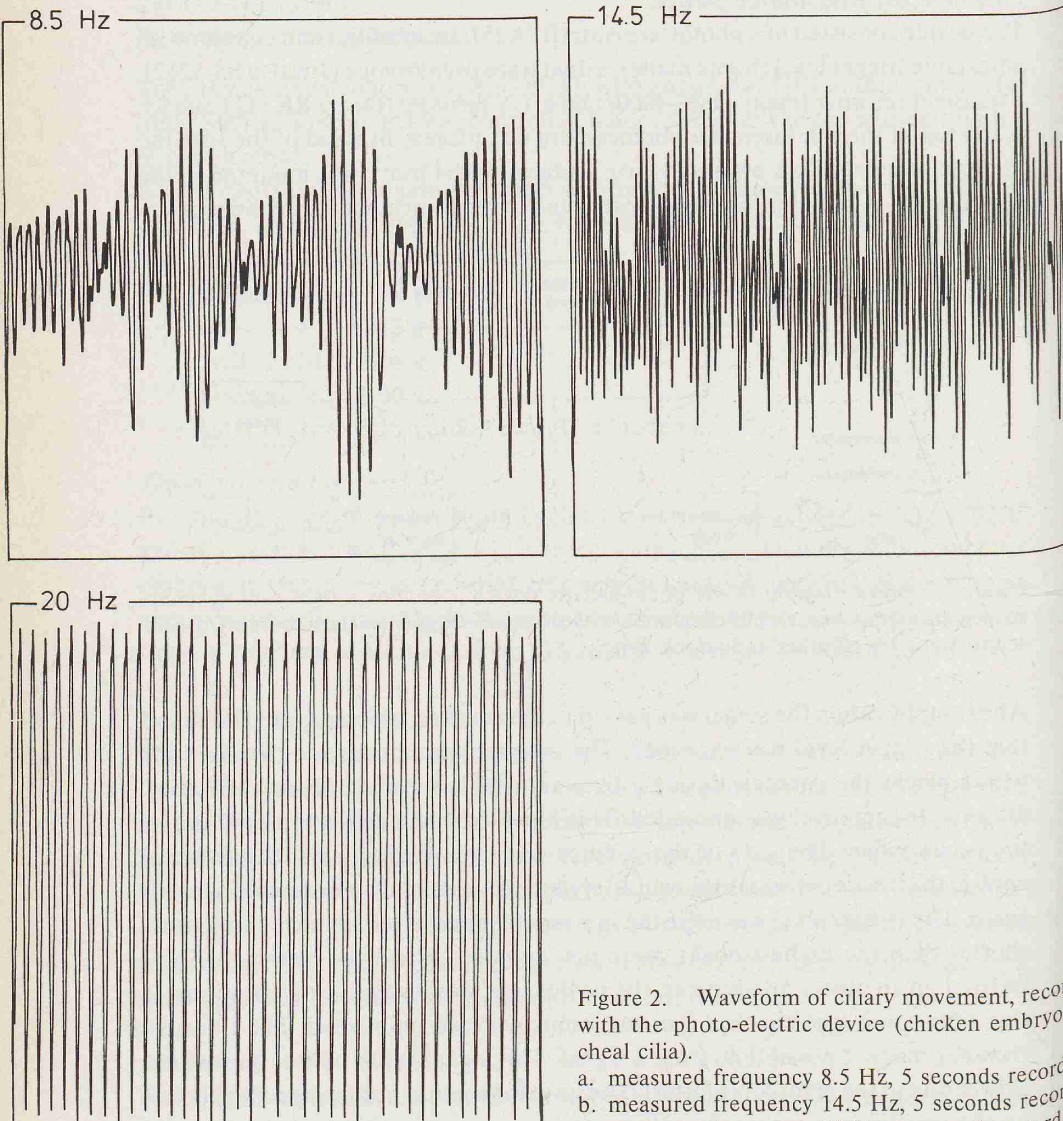

Figure 2. Waveform of ciliary movement, reco
with the photo-electric device (chicken embryo
cheal cilia).
a. measured frequency 8.5 Hz, 5 seconds record
b. measured frequency 14.5 Hz, 5 seconds reco
c. measured frequency 20 Hz, 2 seconds record

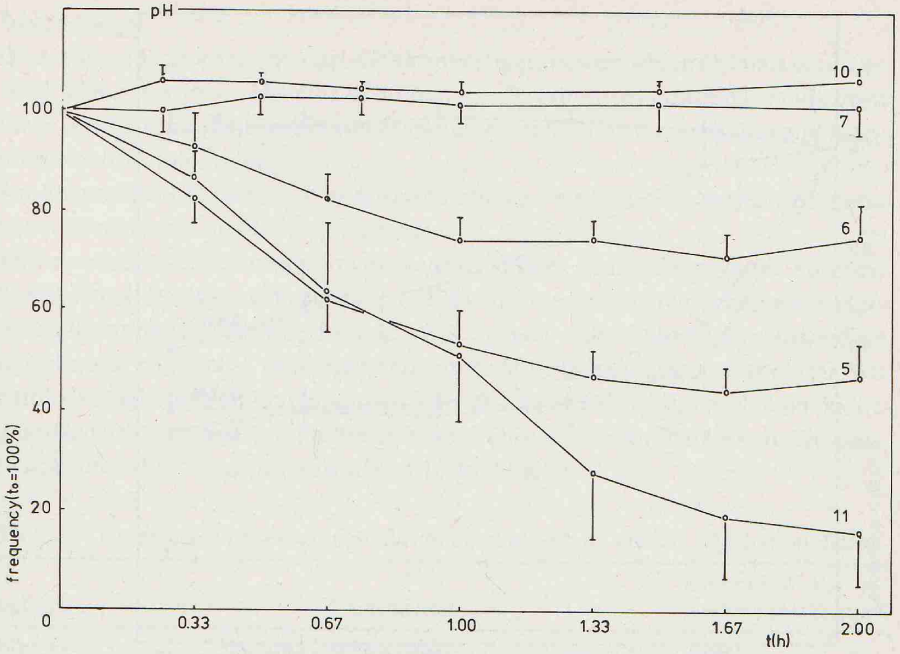

Figure 3. Time versus frequency plot pH = 5, 6, 7, 10 and 11 (chicken embryo tracheal cilia). S.E.M. is indicated.

The influence of the pH is shown in fig. 3. No different effect was found on rat or chicken cilia. The highest ciliary frequency was found from pH = 7 till pH = 10. At pH = 6 the ciliary frequency was decreased about 20%, compared with the frequency at pH = 7, each measured after 0.75 h incubation. Values lower than pH = 6 and higher than or equal to pH = 11 resulted in severe decrease of the ciliary frequency.

Figures 4 and 5 show the results of differences in the NaCl content or osmotic pressure. The isotonic (0.9%) solution shows the best results of the NaCl solutions. Increased and diminished concentrations result in a steeper decay. In the experiments with chicken cilia a concentration of both 0.45% and 1.5% NaCl decreased the frequency about 50% after 1 hour in comparison with the initial frequency. A concentration of 0.9% NaCl decreased the frequency 27% after 1 hour in comparison with the initial frequency. In the experiments with rat cilia a concentration of 0.45% NaCl decreased the frequency 14% and 0.9% NaCl decreased the frequency 5%, both after 1 hour in comparison with the initial frequency. With 1.5% NaCl solution the frequency decreased 38% after 0.75 hour in comparison with the initial frequency.

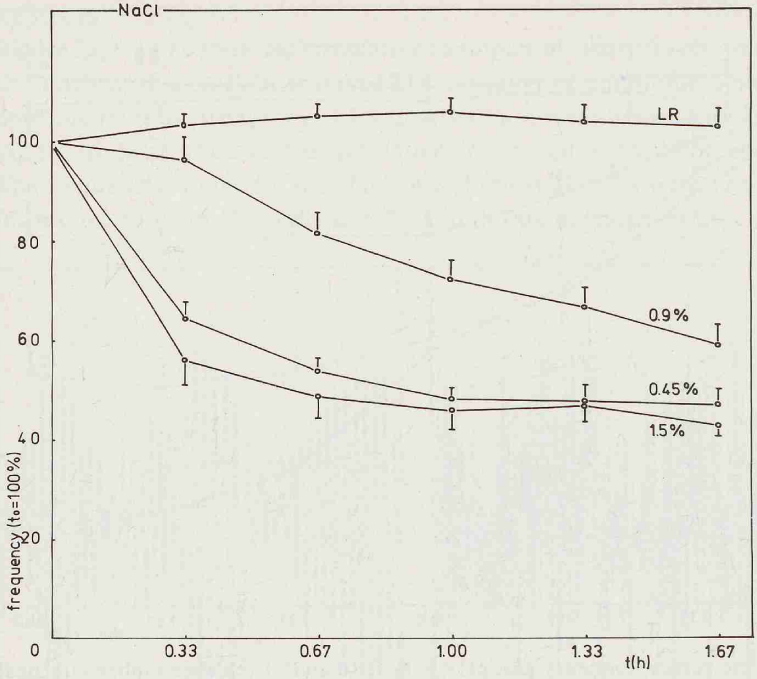

Figure 4. Time versus frequency plot for Locke Ringer, 0.45% NaCl, 0.9% NaCl and 1.5% NaCl solution (chicken embryo tracheal cilia). S.E.M. is indicated.

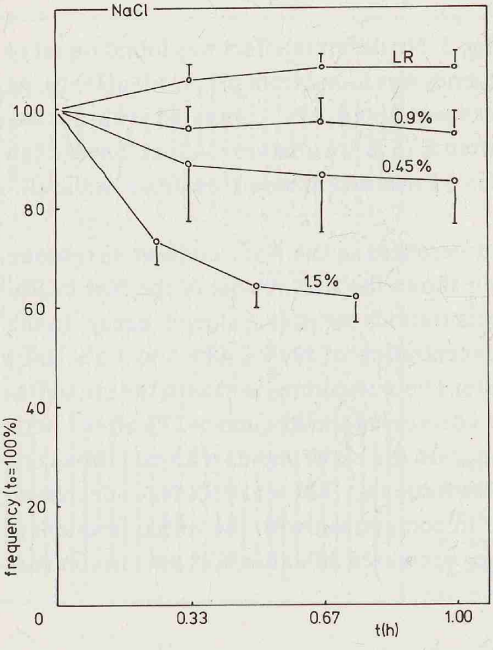

Figure 5. Time versus frequency plot for Locke Ringer, 0.45% NaCl, 0,9% NaCl and 1.5% NaCl solution (rat tracheal cilia). S.E.M. is indicated.

DISCUSSION

One of the questions at the start of this investigation was, which animals could be used. Wanner writes: "The cilia of human (. . .) respiratory epithelia or cilia from lower animals are remarkably similar when compared by transmission and scanning electron microscopy".

So, for practical reasons, we choose the chicken embryo and performed some experiments with rats for comparement.

The number of cilia under investigations differs much from other authors. Mercke et al. (1974) and Yager et al. (1978) observed a larger area and used a different illumination (table 2). Mercke et al. worked with a light beam reflected by the cilia and Yager et al. used light, transmitted through a piece of epithelium. We employed light perpendicular to the cilia. An area of 36 μm^2 was projected on the photosensitive part of the phototransistor. This area contained about 220 cilia (Andersen, 1971) and one row about 15 to 22 cilia.

Table 2. Comparison of several methods to employ a photo-electric registration device.

authors	area	illumination	number of cilia under investigation
Mercke	380 μm^2	reflection	2,400
Yager	314–490 μm^2	transmission through preparation	2,000–3,100
Van de Donk	36 μm^2	transmission through cilia	15–44

The microscope could be focused on one or two rows which corresponded with 15–44 cilia. Our results indicated, that enough cilia were observed to receive a good impression of the co-ordination between several cilia. In Locke Ringer the waveform was regular (fig. 2c), after storage or a contact with drugs which resulted in frequency slowing, the waveform always showed an interference pattern (fig. 2a and b). This interference pattern might be explained by a small difference in the frequencies of neighbouring cilia, due to a lack of co-ordination. The number of ciliary beats, found for the rings in Locke Ringer, was in agreement with the average "normal" frequency for mammals of about 16 Hz (Wanner 1977). The average frequency of chicken cilia was somewhat higher than the average frequency of rat cilia. This might be due to the fact that embryonic tissue is more intact. The pH influence on the frequency is in agreement with the results of Hée and Guillerm (1973) but different from the results of Gallay (1960). Gallay found a very marked decrease of the time, necessary to provoke a cessation of movement for pH smaller than 6.5 or greater than 7.5 (vitro experiments on tracheas of guinea pigs). This might be explained by the fact that Gallay used borate buffers, which are toxic to ciliary epithelium (Grumbach et al., 1965).

The effect of a hypertonic solution on rat cilia is nearly the same as the effect on chicken embryo cilia.

The decrease of the frequency of rat cilia in 0.9% and 0.45% NaCl solutions was less than the decrease of the frequency of chicken cilia in those solutions. An explanation (though not satisfying) of this phenomenon can be, that the tracheal rings of a rat are much bigger than the tracheal rings of a chicken embryo and might give more protection. The frequency of cilia in Locke Ringer had the tendency to increase a little, which was probably due to recovering after the slicing of the trachea.
The effect of the osmotic pressure on chicken cilia is in agreement with the results of Stepper et al. (1965, human mucosa in vitro), but differs from the results of Gallay (1960). Gallay found only a negative effect on the activity with hypotonic NaCl solutions and hardly with hypertonic solutions.
Sumarizing it can be concluded, that our method is adequate for studying the ciliary movement, is easy to handle and very suitable for routine measurements.

RÉSUMÉ

Une méthode de mesure de la fréquence du battement ciliaire in vitro est décrivée. Les variations d'intensité lumineuse, produites par le déplacement des cils, sont détectées à l'aide d'un phototransistor, monté sur un microscope, et la fréquence du battement ciliaire est mesurée immédiatement et le patron d'ondes est montré à l'aide d'un oscilloscope et d'un enregistreur des phénomènes transistoires.
Le degré d'amplification et le mode d'illumination sont choisis de façon qu'environ 30 cils sont projectés sur le phototransistor.
Dans la solution de Locke-Ringer, l'amplitude des signaux enregistrés est très constante. Des interférences de la fréquence ciliaire surviennent sous l'effet de facteurs nocifs et sont dépendantes de la fréquence du battement ciliaire.
L'influence du pH et de la pression osmotique est recherchée au niveau de l'epithélium trachéal du rat et de l'embryon de poulet. La fréquence ciliaire n'est pas influencée par des variations de pH entre 7 et 10; mais elle diminue par des pH inférieurs à 7 ou supérieurs à 10. Des solutions hypertoniques de NaCl aussi bien que des solutions hypotoniques diminuent la fréquence du battement ciliaire de l'embryon de poulet.

ZUSAMMENFASSUNG

Es wird eine Methode zur Messung der trachealen Flimmerfrequenz in vitro beschrieben. Durch die Zilien geführtes Licht wird mit Hilfe eines in einem Mikroskop montierten Fototransistor detektiert. Die Frequenz wird unmittelbar gemessen und zugleich wird die Wellenform in einem Oszilloskop, das an einen Transient Speicher angeschlossen ist, sichtbar. Vergröszerung und Beleuchtungsweise werden so gewählt, dasz etwa 30 Zilien auf dem Fototransistor abge-

bildet werden. In Locke-Ringer Lösung zeigt die Wellenform eine sehr konstante Amplitude. Durch schädliche Einflüsse auf das Zilienepithel treten von der Frequenz der Zilienbewegung bedingte Interferenzen auf.
In dieser Arbeit wurde der Effekt des pH sowie der Tonizität auf die tracheale Flimmerfrequenz des Hühnerembryos und der Ratte untersucht. Die Frequenz wird zwischen pH = 7 und pH = 10 nicht beeinfluszt, während höhere und niedrigere pH-Werte die Frequenz vermindern. Hypertonische und hypotonische Kochsalzlösungen vermindern in gleichem Masze die Frequenz der Hühnerembryonenzilien, Rattenzilien waren dagegen weniger empfindlich für hypotonische Kochsalzlösungen.

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