METHOD OF RECORDING MOTOR ACTIVITY OF THE CILIA OF CILIATED EPITHELIUM

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Key words: cilia; ciliated epithelium; recording system.

Investigations into the structure and function of the cilia of ciliated epitkelium (CE) are being pursued intensively in biology with the aim of studying mechanisms of nonmuscular forms of motility and reception [1, 3, 6, 8]. Medical science is faced with similar problems in connection with the study of the mechanisms of onset and the course of various bronchopulmonary diseases, in whose pathogenesis an important role is played by disturbances of the functions of the bronchial CE [2, 7]. Meanwhile progress in the study of this problem has been delayed because of the lack of adequate methods of recording motor activity of cilia, which would allow the dynamic parameters of this living microobject to be evaluated.

The aim of this investigation was to develop and test a system for recording motor activity of the cilia of CE and to investigate the action of some chemical and physical factors on the frequency of beating of the cilia.

EXPERIMENTAL METHOD

CE from the gills of bivalve mollusks (mussels and scallops), taking account of the high level of development of the ciliary mechanism of animals of this species [6], were used as model to study ciliary motor activity.

Beating of the cilia was recorded by means of a system incorporating the "Biolam L-211" microscope, the "Élektronika 821" television camera, the VL-100 televisor, N-338-1P automatic writer, light guide, and photodiode. By means of the television microscope it is possible to examine a large high-contrast picture on the screen of a video control system (on-screen enlargement 1500), the intensity of illumination of the microobject can be reduced and, consequently, the life span of the preparation can be prolonged. The system operates as follows: against the background of the picture of the test object two lines of a frame are projected on the televisor screen. The arrangement of these lines and the distance between them can be varied, to keep the part of the picture of interest to the observer between them (for example, a single cilium). The end of the light guide is then brought up to the chosen part of the picture and the frequency of changes in its brightness recorded. This frequency in this case is determined by the frequency of oscillation of the cilia (cilium). The synchronization circuit, which we developed for the system, is triggered by the frame synchronizing pulses of the televisor. It controls the sampling – storage circuit and shapes the lighting pulses with a duration of 64 μsec (one line). The electronic circuit of the system contains altogether eight microcircuits of series 155, 544, and 1100. Unlike existing systems with purely electronic processing of the television signal [4, 5] the suggested system is much simpler and cheaper.

EXPERIMENTAL RESULTS

During observation of the specimen of CE and recording motor activity of the cilia, their functional heterogeneity attracts attention. Ciliated rows, the cilia of which beat at a constant frequency (Fig. 1b), and ciliated rows whose cilia sometimes stop spontaneously (the stop effect, Fig. 1a), in contact with one another, can be distinguished. On the addition of particles of activated charcoal measuring 3-8 μm to the medium the frequency of the "stop effects" increases sharply. Small

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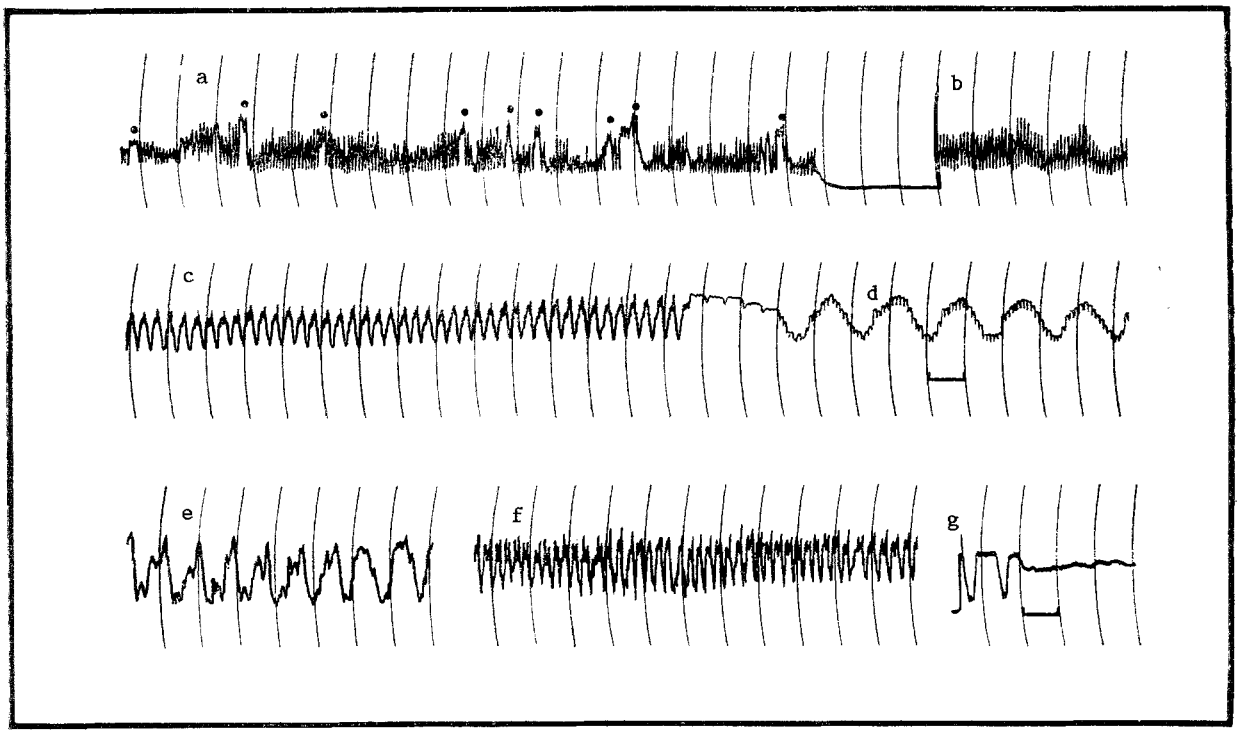

Fig. 1. Types of behavior of cilia of bivalve mollusks. a) "Fast" cilia of a scallop, exhibiting "stop effect." Dots above trace indicate moments of spontaneous cessation of beating; b) continuously working "fast" cilia of a scallop; c) "slow" cilia of a scallop; d) the same cilia, but with a recording speed 5 times greater; e) "slow" cilia of a mussel; g) control. Downward position of trace indicates black level, upward position — white level. Time marker 1 sec, for d: 200 msec, 10°C.

particles of charcoal, moved by the current of water created by the ciliated rows, on reaching one such area, collapse; large particles, however, are thrown off by special cilia which are 2-2.5 times longer than the ordinary kind and are much less frequent. This class of cilia (conventionally called "sorting") on contact with large charcoal particles make several energetic beats against the current of water, as a result of which these particles are thrown off. Incidentally the "sorting" cilia are arranged in rows whose cilia do not demonstrate "stop effects." It will be clear from Fig. 1e that a phase of waving and a phase of effective striking can be distinguished in the trace of ciliary beating. To exclude any graphic recording of an artefact, each recording is followed by a control: the light guide is shifted to a light or dark area of the screen (Fig. 1g).

By means of this system it was possible to record motor activity of the cilia of a single cell of CE. One example of such a trace is given in Fig. 2h. The frequency of beating of the cilia of the single cell at 10°C was 720 min^{-1}. A special series of experiments showed that a steady spontaneous decrease in the frequency of beating of the cilia in a preparation of CE took place at intervals of 3-3.5 h, and this controls the duration of the investigation.

In the next series of experiments we studied the effect of some chemical agents on the motor activity of the cilia. Low concentrations (0.002%) of the surfactant Triton X-305 caused alternate stopping and bursts of beats (Fig. 2a). After rinsing the preparation to remove the surfactant, continuous activity of the cilia was restored, but at a lower frequency than before (Fig. 2b). A 0.05% solution of the surfactant caused virtually instant cessation of ciliary activity (Fig. 2c, d). Addition of 0.1 M KCl solution to the perfusion medium caused a biphasic response: an initial increase in the frequency of beating (Fig. 2e) was followed by a sharp decrease (Fig. 2f). The active phase of the beat remained unchanged but the waving phase was lengthened (Fig. 2f).

An analysis of the literature on the ciliary activity of different organisms [1, 3, 6-8] highlights the wide scatter of data on the frequency of beating of the cilia even of the same organism and tissue. It follows from the data described above that, despite their morphological similarity, cells of ME exist which have a functionally different type of behavioral responses. In this context, the further study of the class of "sorting" cilia and also of the spontaneous "stop effect" is essential, for no description of them could be found in the literature. Meanwhile local cessation of ciliary beating has been described in response to mechanical stimulation, evidence of the mechanoreceptor function of cilia [6]. In the general case the spectrum of

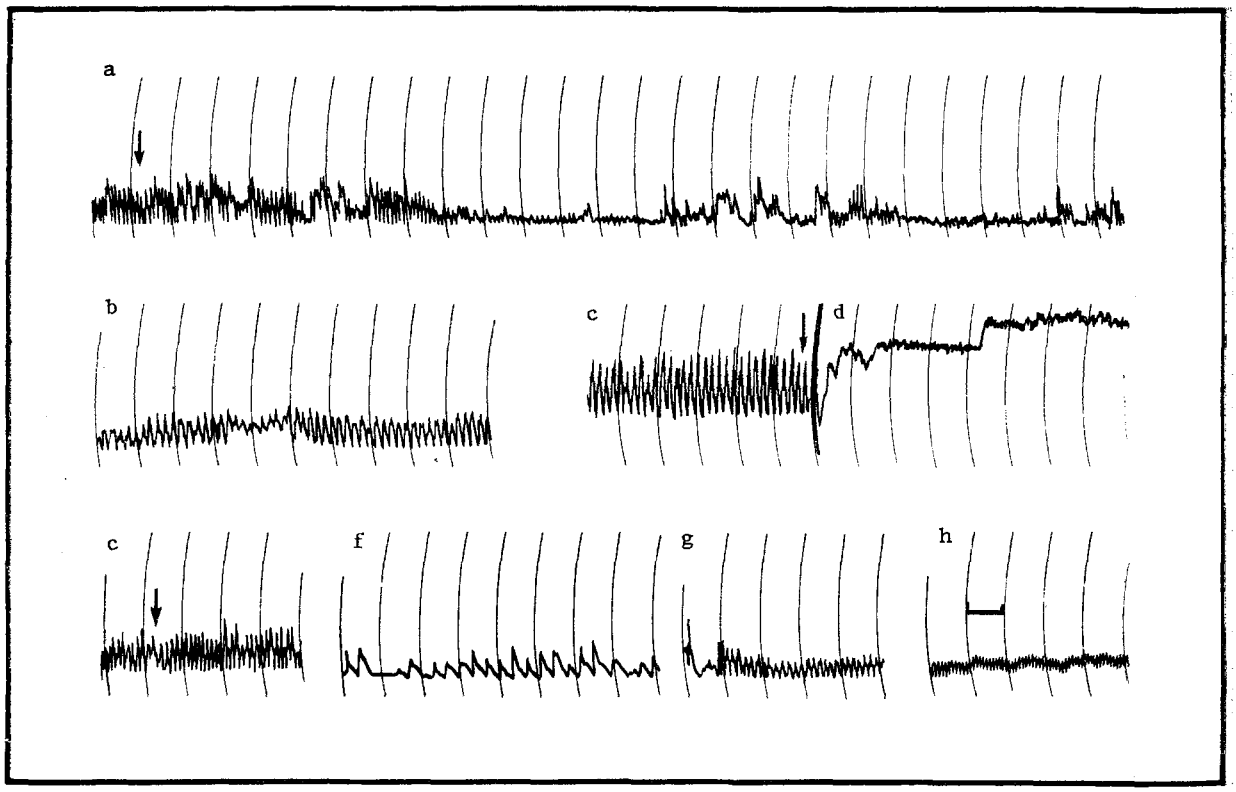

Fig. 2. Action of chemical irritants on ciliary rhythm of CE of scallop gill. a) Action of low concentration of Triton X-305 (0.002%). Arrow indicates time of addition of substance. Periodic decline in beating of cilia can be seen; b) frequency of beating 10 min after rinsing; c) action of high concentration of Triton X-305 (0.05%); d) momentary cessation of beating of cilia. Arrow indicates time of addition of Triton X-305 (0.05%); e) action of 0.1 M KCl. Arrow indicates time of addition of solution of substance; f) marked slowing and desynchronization of beating of cilia. Phases of active striking (leading edges of pulses) and phases of waving (trailing edges of pulses) clearly visible; g) rinsing; h) beating of cilia of a single cell. Frequency 720 min^{-1}. Time marker 1 sec. 10°C.

behavioral responses of the cilia of CE under normal conditions can be represented by the following scheme: beating with different frequencies, mechanically induced "stop effect," spontaneous "stop effect," mechanically induced triggering of beating ("sorting cilia"). The action of factors such as surfactants and KCl on cilia, however, evoked an almost identical set of reactions: temporary cessation of beating, reduction of the frequency of beating, fluctuation of the frequency and complete blockade of the ciliary rhythm. The intensity of these reactions, as we showed, depends on the strength of the stimulus. This is evidently the "standard" set of behavioral responses of the cells of CE, by which they respond to external influences. Meanwhile fine differences do also exist. For example, the reaction to 0.1 M KCl is characterized not only by a decrease in the frequency of beating, but also by a sharp increase in the duration of the waving phase, while the active beating phase remains unchanged compared with the control, possibly due to the depolarizing effect of K^+ on the cell membrane.

The suggested method of recording motor activity of the cilia of CE is thus simpler than existing methods, and it possesses high resolving power, so that it can be used for the intravital study of the functional state of the cells of CE under different conditions of activity.

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EXPERIMENTAL LYMPHOKINE THERAPY OF WOUNDS

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The problem of healing and treatment of wounds was and still remains one of the most urgent in contemporary surgery. Complication of wounds by the pathogenic microflora frequently leads to the development of a secondary immunodeficiency or, on the other hand, wounds which are slow to heal may be the result of a deficiency of the immune system.

Accordingly, the use of methods of immunocorrection during wound healing is of great importance.

The effect of lymphocytes on the regeneration of organs and tissues has been studied experimentally [1]. In recent years the role of lymphokines (interleukins, interferons) in regulation of the function of fibroblasts and epithelial cells has been extensively discussed. Disturbance of the secretion of these mediators is the main cause leading to the development of a severe inflammatory process and of indolent regeneration.

The study of the effect of lymphokines on regeneration in vivo is exceptionally interesting. This was the aim of the present investigation.

EXPERIMENTAL METHOD

A pure suspension of peripheral blood mononuclear cells from the rabbit ear was obtained by Böyum's method [7] and $5 \cdot 10^6$ of the isolated lymphocytes were stimulated with phytohenlagglutinin ("Difco") in a concentration of 10 μg/ml for 3 h. The cells were then washed to remove the mitogen and cultured for 20 h in medium with antibiotics: penicillin 100 μg/ml and streptomycin 100 μg/ml. After the end of culture the cells were removed by centrifugation and the supernatant was sterilized by filtration through membrane filters (pore diameter 0.22 μ, Whatman). Active fractions of lymphokines with mol. wt. of 20-30 kD (M fraction) and 60-70 kD (L fraction) were obtained from the supernatants of the peripheral blood lymphocyte cultures by gel-filtration on Sephadex G-100 [4]. The biological activity of the supernatants and of the isolated fractions was determined in a microversion of the macrophage migration inhibition test [5], and the phagocytic activity of the neutrophils was determined as in [6]. As an experimental model of wound healing, a skin-muscle wound with an area of 400 mm^2 was inflicted on noninbred rabbits in the scapular region (after anesthesia). Treatment of the wounds began on the 2nd day and continued for 7-8 days. The autologous supernatant of the lymphocyte cultures was used in a volume of 0.5 ml (protein concentration 50 μg/ml) and the M and L fractions of lymphokines in a dose of 100 μg/ml, by application to the wound. During the first 3 days treatment was carried out twice, in the morning and evening; subsequent treatments once a day for 4, 5, 6, 7, and 8 days. Lymphocytes (after culture for 20 h) were applied to the wound once at the rate of $7.0 \cdot 10^6$ cells/cm^2. The criteria of wound healing were: a) planimetric parameters: measurement of the area and determination of the rate of wound healing by Popova's method [5]; b) the time of complete epithelization; c) morphological investigation of the wound exudate by the squash preparations method [5]. The results were subjected to statistical analysis by Student's test.

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