treated leaves (figure 1) almost tallies with that of excized leaves treated with kinetin (figure 2). Thus it is tempting to suggest that the normal hormonal balance for chlorophyll biosynthesis is disturbed on excision of etiolated leaves, which causes a prolonged lag phase. A longer period of light exposure probably brings back the proper physiology of leaves for induction of pigment synthesis. In the case of intact leaves, hormones like cytokinins are readily synthesized in roots and transported to leaves causing thereby an earlier induction.

1 Acknowledgment. We thank Prof. M.C. Dash for encouragement and facilities. The assistance of Mr A.N. Misra is acknowledged.
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Stimulation of glycoprotein secretion in dispersed rat submandibular gland acini by cystic fibrosis serum[1]

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Summary. Acini were enzymatically dissociated from rat submandibular gland and their mucous glycoproteins radiolabelled with ^{14}C-glucosamine. Sera from cystic fibrosis patients stimulated the release of labelled TCA/PTA - insoluble material from the cultured acinar cells to a significantly higher degree than did control sera.

Sera from patients with cystic fibrosis provoke ciliary dyskinesia and/or mucus discharge in rabbit tracheal explants[2,3], and cultured gills of oysters[4] and fresh water mussels[5]. These effects have been attributed to a circulating cystic fibrosis (CF) 'factor' believed to be a small polypeptide (mol. wt 1000–11,000) which may be associated with IgG[6, 7]. It has been speculated that the disruptive effect of CF sera on ciliary activity may be secondary to hypersecretion of mucus, since dyskinesia has not been demonstrated in ciliated epithelia which do not contain mucous cells, e.g., ciliated protozoa. In this report, we describe quantitatively the effect of sera from CF patients (homozygotes), the parents of CF patients (obligate heterozygotes), normals, and patients with chronic respiratory diseases, on mucus secretion in a non-ciliated mucous cell model - the isolated acini of rat submandibular gland.

Methods. Rat submandibular glands were dissociated by a technique[8] based on the methods of Mangos et al.[9] and Quissell[10], in a culture medium consisting of calcium- and magnesium-free Hank's Balanced Salt Solution (HBSS), 50 ml; BSA, 0.2%; collagenase, 4200 units (Sigma type III, chromatographically purified) and hyaluronidase 5.0 mg (Sigma). The medium was buffered to pH 7.3 with 10 mM HEPES and maintained under normal atmospheric conditions. The enzymatic digestion of gland connective tissue was coupled with mild mechanical shearing (repeated pipetting) and filtration through a nylon mesh (Nitex bolting cloth, 320 µm mesh). This produced a population containing 75% or more mucous acinar cells, which remained associated in complete or incomplete acini, but were isolated from the duct cells and stroma (figure 1, a). In each experiment, the pooled glands of 6 rats released approximately 8×10^6 cells, which were washed and suspended in complete HBSS containing 0.2% BSA. Assessed by trypan blue exclusion, 85–90% of the cells remained viable during the experimental period of up to 5 h. The cells were established as functionally normal by their capacity to produce typical ultrastructural secretory responses on stimulation with the respective β adrenergic and cholinergic agonists, isoproterenol (IPR) (5×10^{-5} M) and carbachol (5×10^{-5} M). These responses were consistent with those previously described by us[8], the most notable feature being the rapid fusion of the mucous secretory granules with each other and with the luminal plasma membrane, and the subsequent release of their contents (figure 1, b). Degranulation was blocked by the specific antagonists, propranolol and atropine.

Blood samples were collected from 27 patients with CF, aged 2–34 years (homozygotes); 15 parents of CF children (obligate heterozygotes); 14 control children (hospital patients free from chronic infection) and 8 patients with chronic respiratory disorders. The samples were allowed to clot at 4 °C; serum was separated and stored at −20 °C until use (usually within 1 week). In each experiment, the dispersed submandibular cells of 6 male, 150–180 g Wistar rats were washed twice, then incubated for 2 h in culture medium containing 1 µCi/ml D-(1-^{14}C)-glucosamine - HCl (sp. act. 45–60 mCi/mmole). The labelled cells were resuspended in fresh culture medium and divided into 18×5 ml aliquots in Erlenmeyer flasks, each flask containing approximately 4.5×10^5 cells. After 5 min equilibration, serum was added to each cell suspension at a final dilution of 1:25; controls received no treatment. After 40 min incubation the cell pellets and culture media were separated by centrifugation at $200\times$ g. Each pellet was resuspended in 5 mM Na_2EDTA, pH 7.0 and the cells disrupted by sonication. The disrupted cells and media supernatants were precipitated with equal volumes of 20% TCA/2% PTA; the precipitates were washed in 10% TCA/1% PTA, dissolved in 1M NaOH, and their ^{14}C activity measured by scintillation counting. Glycoprotein secretion was estimated quantitatively from the percentage of the total radioactivity released into the media after 40 min[8]. In each experiment the basal glycoprotein release from unstimulated controls was assigned a value of 100, and changes in the secretory index of stimulated cells were expressed as a percentage of this figure.

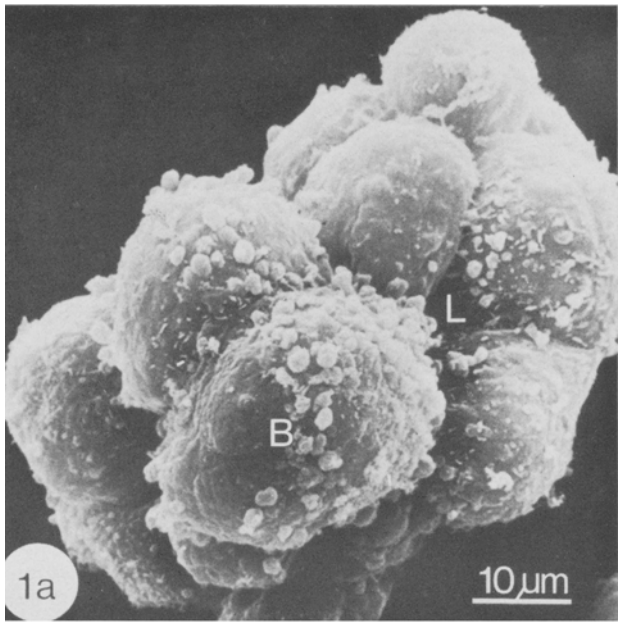
Results and discussion. The effects of sera on glycoprotein secretion from dispersed rat submandibular acini are summarized in figure 2. With normal sera, secretion was a mean of $108\pm$ SD 7.4% of the basal level of release from unstimulated cells; CF heterozygote sera stimulated secre-

tion to 120 ± 9.0% and CF homozygote sera to 125 ± 9.2%. The variation in glycoprotein release produced by individual sera in duplicate or triplicate experiments was less than 6%. Both CF homo- and heterozygote sera were significantly different from control sera ($p < 0.001$; and $0.001 < p < 0.002$ respectively, the Student t-test), but not from each other ($p > 0.05$). The secretion provoked by the sera of CF patients varied from 108–149% of unstimulated controls. To determine whether the value obtained for each patient reflected the severity of the disease in that individual, the glycoprotein release figures were compared with the corresponding clinical scores on the degree of pulmonary and gastrointestinal involvement. The clinical symptoms were assessed at the time of diagnosis on an arbitrary scale of 1 (no symptoms) to 4 (severe symptoms). No correlation was found between the glycoprotein release and the age, sex or the severity of either pulmonary or GI involvement in CF serum donors.

The sera from 8 patients with chronic respiratory diseases (bronchiectasis, immotile cilia syndrome, sinobronchial syndrome) stimulated glycoprotein secretion from 98 to 145% of unstimulated controls (figure 2). These findings suggest that the secretagogue activity in CF sera may not be directly related to the CF biochemical defect, but may be secondary to chronic respiratory infection, or to circulating factors resulting from therapy. This would not, however, explain the high levels of glycoprotein release produced by obligate heterozygote sera. The factor or factors in normal serum which stimulate glycoprotein secretion to a mean of 108 ± SD 7.4% of the basal release are not known. Serum is a complex mixture of bioactive substances including catacholamines, other neurotransmitters and hormones. It may be speculated that one or more of these components can provoke a limited secretory response in cultured submandibular cells.

It has been shown that low molecular weight fractions (mol. wt 1000–10,000) of culture media from cultured CF homozygote- and heterozygote fibroblasts, or amniotic fluid cells produce ciliary dyskinesia in rabbit tracheal explants[11]. We have, therefore, begun experiments to examine the effects of culture media fractions from CF and normal fibroblasts on mucus-secretion in the rat submandibular model, in an attempt to establish whether the factor stimulating glycoprotein release is related to the basic genetic defect in CF.

The serum studies described above show that, although the mean level of glycoprotein release provoked by CF heterozygote sera was significantly different from that of the controls, there was an overlap of values in the 2 groups (figure 2). Since the assay does not adequately discriminate CF carriers, and since it is complicated, time-consuming and expensive, it is not considered appropriate as a screen-



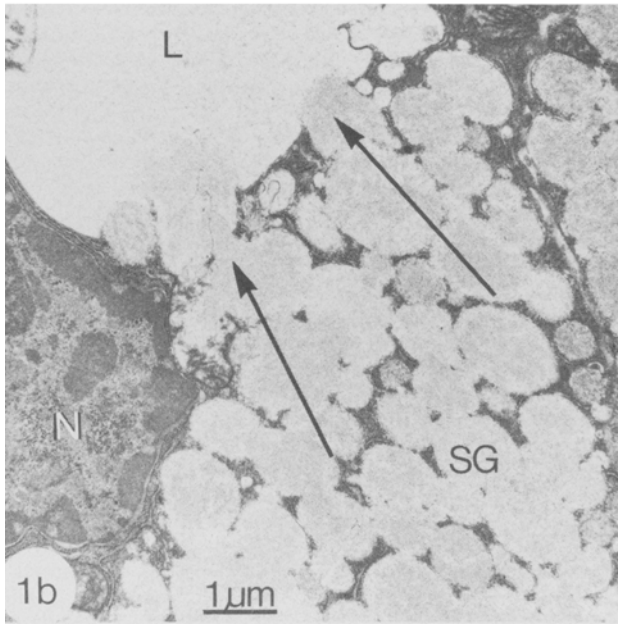


Fig. 1. *a* Scanning electron micrograph of an acinus dissociated from rat submandibular gland. The cells maintain their normal 3-dimensional relationship around a central lumen (L). The surface blebs (B) result from the dispersal procedure. *b* Transmission electron micrograph of an acinar cell exposed to 5×10^{-5} M isoproterenol for 10 min. Mucous secretory granules in the cell apex coalese and fuse with the lumen to form a vacuole like structure representing a luminal extension (L). Granules (SG) in the basal region of the cell release their contents into the enlarged lumen via secretory channels (arrows) produced by granule fusion. N, nucleus.

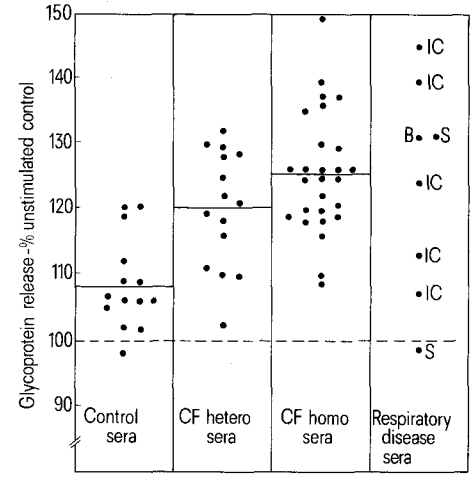


Fig. 2 Comparison of glycoprotein release in rat submandibular acini after 40 min exposure to sera (1:25 dilution) from controls, CF heterozygotes, CF homozygotes and patients with chronic respiratory diseases. Dotted line represents basal release from unstimulated acini. B, bronchiectasis; IC, immotile cilia syndrome; S, sinobronchial syndrome.

ing test. However, the technique is objective, quantitative and sensitive at a serum dilution of 1:25, so that the dissociated rat submandibular model is a valuable research tool for studying the mechanism of the secretory response induced by CF sera and other secretagogues.

1 This work was supported by the Canadian Cystic Fibrosis Foundation and the Sellers Foundation.

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Perception of constant hydrostatic pressure. A physiological basis for the vertical stratification of marine habitats

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Summary. This paper reports an experimental demonstration of the ability of sessile marine animals (*Balanus balanus* L., Crustacea, Cirripedia) to perceive constant hydrostatic pressure. The results suggest the presence in *Balanus* of a previously unknown sensory system with a tonic response. Such a system may also be present in freely moving animals, but be masked by their locomotor activity.

Experiments to test and to record the effects of light-dark cycles on marine animals have frequently been performed. Experiments to study the effects of high-frequency pressure cycles on marine animals have not yet been done, owing to a lack of appropriate methods[2]. In effect, apparatus must be devised whose operation is equivalent to raising an animal quickly to the simulated surface and just as quickly replacing it under water at a changed pressure. The problem has been the requirement that when the animal changes its depth in the water, all the environmental parameters apart from pressure must remain constant; for instance flowing water is necessary for constant conditions to be ensured, because in standing water the oxygen in the vicinity of the animal is rapidly consumed.

Experiments to record the effects of constant hydrostatic pressure are best performed with an animal of sessile habit, because the relatively small pressure changes associated with the ascents and descents of a freely moving animal in the experimental chamber might mask behaviour reflecting detection of the average pressure. The animals used in these experiments were sessile barnacles (*Balanus balanus* L., Crustacea, Cirripedia).

3 methods have been developed to test experimentally the effects of constant hydrostatic pressure over appreciable periods of time.

1. A method for recording the activity of sessile animals at different simulated depths (figure 1). Because the principle of the seesaw, previously used for recording locomotor activity of freely moving animals, is unsuitable for recording the activity of sessile animals, the principle of the swing - not previously used for activity recording - was modified for the requirements of the experiments. 2. A method for ensuring constant experimental conditions at different simulated depth (figure 2). 3. A method for adjusting and maintaining hydrostatic pressure in a physiological experiment (figure 3).

A single apparatus fulfilling the requirements listed has been devised.[1]

The motor activity of the barnacles, the beating of the cirri, is performed with the regularity of clockwork. Each change in stimulus intensity is followed by an interruption of cirrus beating (figure 4, b). Occasional pauses also occur while experimental conditions are at a constant level. It is the frequency and duration of these pauses, rather than a change in the basic beat rate, that determines the average beat rate characteristic of a particular stimulus.

During each experiment the animals were subjected to an alternation, at regular intervals, between surface pressure (p_1) and the pressure at a depth of 20 m underwater (p_2). During such a series of stimuli (p_1p_2 60:60 min, 30:30 min, 15:15 min, 10:10 min, 5:5 min) an equal total time was spent at each pressure (number of measurements see figure 4, a).

In the present experiments the experimental conditions other than pressure are held constant at different simulated depths. When the beat rate is monitored quantitatively over the listed periods of time (p_1p_2 60:60 - 5:5 min) a difference in the beat rate between the simulated surface and 20-m-depth conditions persists. There is a significant ($p < 0.05$) difference in the beat rate even when the pressures are maintained for as long as 60 min (figure 4, a).

Because the difference in the amount of activity per unit time persists, the results can be interpreted as follows. If it is the case that the observed differences in pressure behaviour indicate differences in metabolic adaptation, the metabolic rates during a 60-min period of stimulation must increase while stimulus duration decreases (60 min:5 min $\hat{=}$ 12:1) under otherwise constant conditions. No metabolic system is known which increases metabolic rates only when stimulus duration is decreased. It is unlikely that the differences in beat rate indicate differences in metabolic adaptation (a metabolic model to understand diurnal vertical migration depending on transmitted light has been described previously[5-7]. Light behaviour in sessile animals has been interpreted as a metamorphosis of larval swimming activity[8-10,13-16]). Measurable effects of hydrostatic pres-