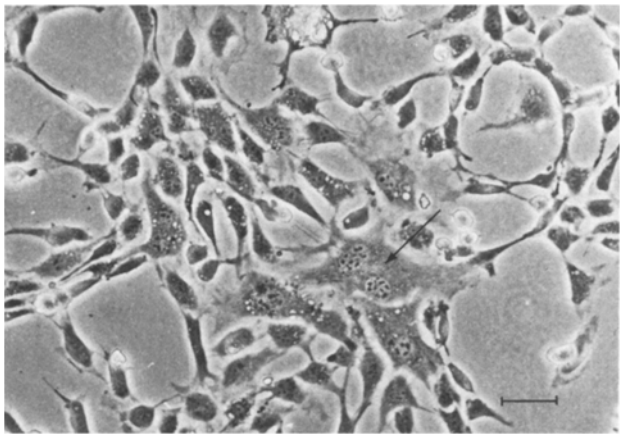

Figure 1. Surface view of an endothelial cell culture. (The arrow marks a multinuclear endothelial cell, surrounded by mononuclear endothelial cells. The scale represents 50 µm.)

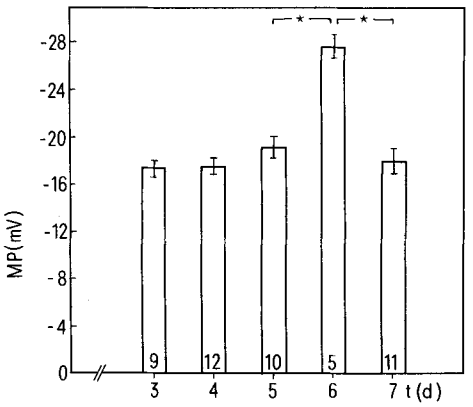

Figure 2. MP of multinuclear endothelial cells in dependence on time after seeding. (The vertical bars indicate mean ± SEM; * $p < 0.01$ by t-test; the numbers in the columns represent the number of measured cells.)

−8.2 mV (SEM = 0.02 mV, n = 4480); average of cells from the 3rd to the 7th day of cultivation. The mean values of MP were similar in all cultures between the 6th and the 8th subculture. Multinuclear cells had a considerably higher mean value, −19.1 mV (SEM = 0.3 mV, n = 47). The difference was found to be statistically significant ($p < 0.001$) by the t-test.

Within a subculture MP of multinuclear endothelial cells depended on the time of cultivation. During exponential growth (3rd day) MP was −17.4 mV. MP had a maximum, −27.6 mV, at the 6th day (see fig. 2). When reaching the stationary growth phase (7th day) MP was −18.1 mV. Hence, MP of multinuclear endothelial cells shows a similar dependence on time of cultivation to that already noted for mononuclear cells[5]. The fraction of multinuclear endothelial cells was about 1% in all subcultures and was roughly independent of the time of cultivation (table).

Discussion. So far, endothelial cells from vessels have only rarely been investigated electrophysiologically. Northover[6] found in vitro an average MP of about −40 mV in endothelial cells of the thoracic aorta from guinea-pigs.

In a previous investigation[5] we found an average MP of −8.1 mV for cultivated mononuclear endothelial cells from the calf aorta between the 2nd and the 7th day after seeding. Higher values were obtained for multinuclear cells only. In the present study we found a similar value for mononuclear and −19.1 mV for multinuclear cells. The difference from Northover's data may result from the differences in subject and experimental conditions.

Since cultivated multinuclear endothelial cells of the calf aorta contain the cell-type specific factor-VIII-antigen they may functionally be completely differentiated. Their higher MP, presumably caused by a changed ion permeability of the membrane, may be a characteristic, which points to a different function whose pathophysiological relevance in vivo is not yet known.

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Modification of membrane cholesterol content affects electrical properties and prolactin release of cultured pituitary cells[1]

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Summary. Treatment of cloned pituitary cells (GH3/B6) with cholesterol-enriched liposomes, which presumably increases membrane cholesterol content, affects the passive and active electrophysiological properties and stimulates the release of prolactin (PRL).

It is now well established that modifications of membrane permeabilities to calcium (Ca^{2+}) and potassium (K^+) ions are associated with hormonal release by pituitary cells[2]. Ca^{2+} in the extracellular space is required for the release process; TRH, which stimulates the release of PRL by GH3 cells, induces Ca^{2+}-dependent action potentials[3-5], and Ca^{2+} influx has recently been demonstrated following TRH administration in GH3 cells[6]. Precocious changes in the permeability to K^+ ions appear to be associated with the subsequent generation of Ca^{2+}-dependent action potentials induced by TRH[7]. Membrane channels to ions are embedded in a complex bilayer of lipids and numerous lines of study suggest that lipids also have a role in the release process of pituitary hormones[8,9]. In agreement with

Effects of liposomes on PRL release by GH3 cells

1h incubation with	Control	TRH (10^{-7}M)	EPC cholesterol-enriched (C/P = 0.5)	EPC (C/P = 0)
PRL (ng/10^6 cells)	22.7	100.8	36.3	24.5
± SEM	1.3	14.1	5.1	2
Stimulation (%)	-	344*	60*	8

Each point is the man ± SEM of 6 experiments. *Significantly different from control ($p < 0.05$).

this hypothesis, we show that experimental manipulation of the membrane cholesterol content of a cloned cell line of pituitary cells (GH3/B6) affects both their electrophysiological properties and the release of PRL.

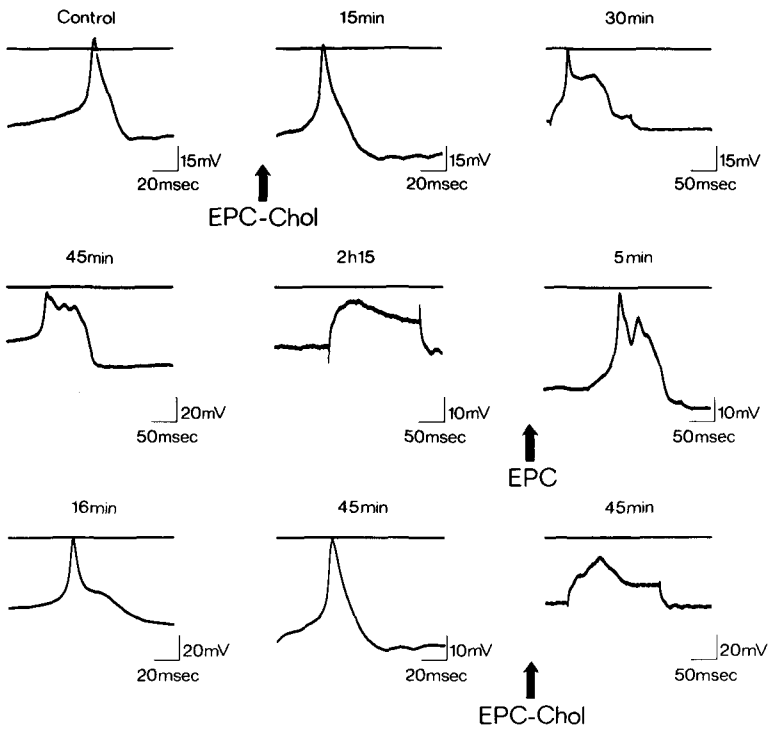
Materials and methods. The GH3/B6 strain, a subclone of the GH3 rat pituitary cell line, was provided by Dr A. Tixier-Vidal (Collège de France, Paris). Cells were routinely grown as a monolayer in Ham's F10 medium enriched with heat-inactivated horse serum (15%) and fetal calf serum (2.5%). The culture medium was changed every 2-3 days. These cells have a diameter ranging from 10 to 15 µm.

Recordings. Recordings were performed between days 5 and 7 following re-plating. 10 min before the beginning of a recording session, the culture medium was replaced by a bathing solution of the following composition (in mM): NaCl 142.6; KCl 5.6; $CaCl_2$ 2.5; Glucose 5; Hepes buffer 5 (pH 7.4). The cells were viewed through an inverted microscope during the experiments, which are conducted in a temperature-controlled Faraday chamber (26 ± 1 °C). Conventional recording techniques were used[4]. Hyperpolarizing pulses for measuring membrane resistance or depolarizing pulses for testing excitability were passed through the recording electrode using a bridge amplifier. Cells were impaled by a KCl (3M) microelectrode having a resistance of 60-90 MΩ.

Prolactin assay. PRL release was measured after a 1-h incubation period. PRL concentrations in the incubation media were determined by radioimmunoassay using reagents provided by the NIAMDD. Separation of bound hormone from free hormone was achieved by anti-rabbit γ-globulin fixed on activated cellulose (double antibody solid phase). Results were expressed in ng of NIAMDD-Rat-Prolactin-RP2 per 10^6 cells. All results are expressed as mean ± SE. Significance was calculated using a 2-tailed t-test.

Preparation of liposomes. Sonicated liposomes having a varying egg phosphatidylcholine (EPC) cholesterol content were used for depletion or enrichment of cell membrane cholesterol. Liposomes were prepared according to techniques described by others[10,11]. Briefly, 80 mg of EPC were sonicated in 5 ml of recording medium and cholesterol was adjusted to obtain the desired molar ratio. Cholesterol to phospholipid ratios in mol/mol were C/P = 0.0; 0.25; 0.5; 1. Freshly prepared liposomes suspensions were diluted in the recording medium (1/5) (V/V) and then applied to GH3 cells during electrical recording.

Results and discussion. In these experiments a total of 98 cells were recorded. Mean resting potential of control cells was VM = −47.6 ± 1.6 mV (mean ± SE; n = 20) and mean membrane resistance was RM = 144.2 ± 13.6 MΩ; 90% of the cells tested were excitable; they displayed action potentials with a positive overshoot (fig.). 1 h following treatment with liposomes having a cholesterol to phospholipid ratio C/P = 0.5, mean resting potential of the treated cells was VM = −33.1 ± 1 mV and mean membrane resistance dropped to RM = 48.57 ± 6.4 MΩ (n = 35). Only 14% of the cells tested were excitable compared with 90% of controls. Moreover the shape of the action potentials was clearly altered; the most obvious changes were a decrease in amplitude and a prolongation of the repolarization phase. Action potentials after treatment with cholesterol-enriched liposomes were markedly prolonged compared to controls (fig.). 2 h after the beginning of treatment the cells were no longer excitable. Similar results were obtained with liposomes having a C/P ratio of 1 whereas a C/P ratio of 0.25 was ineffective.



Effect of treatment with cholesterol-enriched liposomes (EPC-Chol; C/P = 0.5) on the amplitude and shape of action potentials in GH3/B6 pituitary cells. Horizontal lines represent the reference potential (0 mV). The times after EPC-Chol or EPC treatment are indicated above the reference. It can be seen that the action potentials were progressively deformed following EPC-Chol treatment. As it is impossible to record the same GH3 cell for several hours, recordings used for this figure have been obtained in different cells. A subsequent treatment with empty liposomes (EPC; C/P = 0), which presumably reduces membrane cholesterol content, was followed by restoration of control amplitude and shape. Another treatment with EPC-Chol on the same preparation, again resulted in gross modification of action potentials. In this figure, it can be seen that the membrane resting potential was greatly reduced by treatment with cholesterol-enriched liposomes.

In this work, we made no attempt to measure the amount of cholesterol effectively incorporated in the membrane of GH3 cells. However, as the C/P ratio of purified fractions of GH3 plasma membranes is relatively low (approximately 0.3)[19], liposome preparations having a C/P ratio of 0.5 or more would produce a cholesterol enrichment[12].

Basic electrophysiological properties of GH3 cells were partly restored by treating the cholesterol-enriched cells with empty liposomes (fig.). 20 min–1 h after the addition of empty liposomes mean membrane potential rose again to -41.7 mV± 2.1 ($n=22$); mean membrane resistance to 83 ± 10.2 MΩ, and 63% of the cells tested ($n=22$); were excitable. Amplitude and shape of action potentials were also restored by the treatment, which presumably depleted membrane cholesterol from the overloaded cell membranes (fig.). These observations suggest that enrichment and depletion of cholesterol were achieved through specific exchanges rather than through a process of fusion. Moreover they show that the effect of cholesterol on the membrane of GH3 cells is reversible.

It has been shown that altering membrane cholesterol content affects the microviscosity of the membrane[12] and thereby modifies the mobility of membrane enzymes and transport channels. The results reported here suggest that membrane ionic channels associated with the electrophysiological properties of GH3 cells are also affected by changes of membrane cholesterol content. Increasing the cholesterol content of membranes depolarizes GH3 cells and affects the shape of action potentials by prolonging the repolarization phase. These observations suggest that an experimental enrichment of membrane cholesterol of GH3 cells affects potassium channels. Similar actions have been reported for tetraethylammonium. (TEA) and 4-aminopyridine (4 AP)[5,14], 4 AP blocks potassium channels in a variety of excitable cells but enhances the influx of Ca^{2+} during depolarization[15] and thereby stimulates the release of secretory products[14,16]. The release of PRL by GH3 cells has been shown to be stimulated by 4 AP[14]. By prolonging action potentials and depolarizing GH3 cells, cholesterol-enriched liposomes also enhance PRL release by these cells independently of the presence of secretagogues. The table shows that incubation of GH3 cells with cholesterol enriched liposomes ($C/P=0.5$) is associated with a 60% increase of PRL release whereas empty liposomes are ineffective. This increased release of PRL, though less pronounced than the release induced by TRH, is significant.

Various physiological and pathological conditions may affect the microviscosity of biological membranes[17,18]. Such changes, by interfering with the properties of ionic channels of pituitary cells, may induce significant alterations of hormonal release.

1 Supported by grants from CNRS (ERA 493, ATP Endocrinologie). We thank NIAMDD for providing the reagents for PRL assay.
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Relationship between light diffraction intensity and tension development in frog skeletal muscle

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Summary. Laser diffraction intensity decrease in active muscles precedes tension development at sarcomere lengths below 2.76 μm, but not at greater lengths. This suggests that the time lag is caused by random sarcomere shortenings inside each myofibril.

Recently we have shown that activation of skeletal muscle fibers results in a large decrease in the diffraction intensity of first order line and this is due to myofibrillar misalignment[1]. A theoretical approach to this problem has shown that light diffraction studies may be used to investigate the myofibrillar organization of the active fiber[2,3]. On the other hand, many investigators (using the X-ray diffraction method[4–6], measurements of sarcomere shortening and stiffness[7,8], and the light scattering method[9]) have recently found that the crossbridges move out in less time than is necessary for tension to develop in fibers at approximately the slack length of the sarcomere. These findings allow us to suggest that during fiber activation, myofibrillar alignment becomes disordered faster than the process of tension development. The time lag between diffraction intensity decrease and tension development can be considered a consequence of slight and random sarcomere shortenings inside each myofibril.

Materials and methods. Single fibers from the semitendinosus of *Rana nigromaculata* were dissected in Ringer's solution composed of (mM): NaCl(115), KCl(2.5), Na_2HPO_4 (2.15), NaH_2PO_4(0.85), $CaCl_2$(1.8) adjusted to pH 7.0 and maintained at 2 °C. The intact fiber was mounted in a cooled chamber (2–4 °C) fitted with a glass opening through which the laser beam (NEC, Model GLG 5350) was directed. When mechanically skinned fibers were used, the fibers were prepared from *Rana catesbeiana* as described elsewhere[1]. In the present work, the fiber sarcolem-