periment, but subsequently, after 48 h, the following mortality was recorded: with dialysate, 1 out of 6, with whole VSE, 3 out of 6, and with the dialyzed material, 5 out of 6.

About 30 min after injection, there was a body temperature drop of about 2.5 °C in all the experimental groups, but subsequently the lines for the different groups diverged as follows (fig. 2): in those receiving VSE dialysate, the body temperature reverted to normal within 3 h after injection, whereas in the other 2 groups (A and B) the temperature continued to drop, stabilizing only at about 3.5 h after injection.

The lowest temperature recorded in the VSE-injected mice (B) was about 31 °C, while in the group injected with the dialyzed VSE (A), the temperature dropped as low as 27.5 °C. In both these groups the temperature differed from the norm (not shown in the graph), as well as from that in mice injected with VSE dialysate (C). The differences became significant about 1 h after injection ($p < 0.01$) and increased further with time. 2 h after injection, there was already a similar difference between mice injected with whole VSE and those injected with dialyzed VSE only. At this time, there were also marked differences in the body temperature of individual mice within each group, as apparent from the increased standard errors in the graph. In 2 repetitions of the experiment shown in figure 2, the results were similar although not uniformly so and therefore not compatible. In these experiments we noted that the mice injected with VSE dialysate were generally just as active as the control mice, but the mice of the other 2 groups (A and B) displayed decreased activity throughout the experiment.

Discussion. Perusal of the results given in figures 1 and 2 shows that: a) VSE causes a drop in body temperature of the mice (both the naive and immunized ones); b) the drop in body temperature is dose-dependent, increasing in magnitude and duration with increasing size of the venom injection; c) mice receiving repeated injections of VSE and developing immunity to the toxic effect of the venom show a smaller and briefer drop in body temperature. Since the immunizing agents are usually the protein fractions of the venom and inasmuch as the results shown in figure 2 indicate a larger drop in body temperature due to the injection of dialyzed VSE (in which the protein concentration is twice as high as in whole VSE), it is reasonable to presume that the observed drop in body temperature of the mice is due mainly to one or more protein fractions present in the venom (although it is certainly possible that some of the small-molecular-weight fractions of VSE may also exert some hypothermic effect). The hypothermic effects produced by hornet venom sac extract in mice resemble those produced by anticholinesterase in mice and rats[5].

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Membrane potential of vascular mono- and multinuclear endothelial cells cultured in vitro

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Summary. Membrane potential (−19.1 mV) and fraction (about 1%) of multinuclear endothelial cells from calf aorta (in vitro) were determined and compared with mononuclear cells (−8.2 mV).

Under pathological conditions[1] and during aging[2,3], endothelial cells of large vessels often show an enlarged cell body and multiple nuclei. In an endothelial cell line from the calf aorta[4] we observed that the proportion of enlarged multinuclear cells increased throughout the subculturing. Factor-VIII-antigen (as a cell type specific marker for endothelial cells) was shown to be present in mono- as well as multinuclear cells[4].

In a previous electrophysiological investigation[5] we observed a higher membrane potential (MP) in multinuclear endothelial cells. Here we present a more detailed analysis of MP and the fraction of multinuclear cells in different phases of growth using some defined subcultures of endothelial cells.

Material and methods. Cultivation of endothelial cells from the calf aorta has already been described[4] as well as intracellular MP measurement[5]. In brief, we used monolayers from the 6th to the 8th subculture grown on cover glasses and investigated them from the 3rd to the 7th day after seeding. In the MEMPAS cultivation medium used the number of cells doubled within 25 h. The microelectrodes were filled with 3 M KCl (resistance 10–50 MΩ, tip potential ≤ 3 mV). Potential differences were measured by using a high input impedance differential electrometer amplifier ($R_i \geq 10^{12}\ \Omega$).

Results. A phase contrast picture of a monolayer of cultivated endothelial cells is shown in figure 1. Multinuclear cells were larger and seemed to be randomly distributed. They either occurred singularly or were surrounded by mononuclear cells. Mononuclear cells had an average MP of

Fraction and number of multinuclear endothelial cells in the total culture

Time of cultivation (days)	Multinuclear endothelial cells	
	Number	Fraction (%)
3	9	0.96
4	12	1.02
5	10	1.10
6	5	0.61
7	11	1.71
		Mean = 1.08

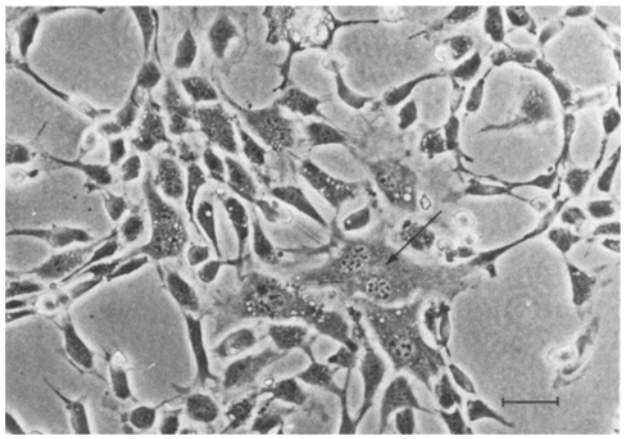

Figure 1. Surface view of an endothelial cell culture. (The arrow marks a multinuclear endothelial cell, surrounded by mononuclear endothelial cells. The scale represents 50 µm.)

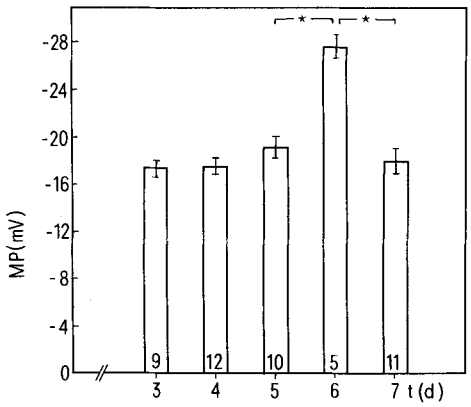

Figure 2. MP of multinuclear endothelial cells in dependence on time after seeding. (The vertical bars indicate mean ± SEM; * $p < 0.01$ by t-test; the numbers in the columns represent the number of measured cells.)

−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