animal is fed whole fish underwater, the fish is sucked into the mouth, while water is expelled at the same time as swallowing occurs. Water expulsion is readily perceived by the hand and can be made visible by staining the water in the mouth (Andersen, personal observation).

These data and the observation on the swallowing behavior indicate that the harbor porpoise does not take in substantial volumes of water by the oral route. We therefore tested the possibility that water molecules could exchange by way of the skin, supported by the findings of Harrison and Thurley[5] that the stratum cornea is very poorly developed in the harbor porpoise and other delphinid species.

In order to demonstrate a free diffusion of water over the skin, molecule by molecule, but no net flux, the following experiment was performed. On the day after administration of tritiated water the experimental animal was taken from its holding basin containing isosmotic water, and placed on a stretcher above an empty plastic tub. The bladder was emptied and catheters left in the bladder and rectum for continuous sampling. For 1 h the animal was sprinkled at a flow-rate of 2 $l \cdot min^{-1}$ with an isosmotic NaCl solution and care was taken to assure that all parts of the skin were continuously wet. Water was collected in the tub below and its total volume determined by weight. Samples for the determination of plasma water radioactivity were taken at -2 h, -1 h, $+1$ h and $+2$ h in relation to the onset of sprinkling. Furthermore, radioactivity was measured in urine and tub water. Plasma and urine water was determined by drying to constant weight at 80 °C. A 1-h experimental period was chosen because the porpoise showed distinct signs of being distressed. The sprinkling procedure was chosen rather than complete submergence in isosmotic solution to avoid oral intake of water during the experiment.

Table 1 shows that the loss of activity from the body water is recovered within 10% and that the cutaneous loss constitutes 90 and 97% of the body water activity loss. The loss of activity by respiration was thus found to be negligible under these circumstances[6].

Table 2. Weight changes during sprinkling experiments

Osmolality of sprinkling water (mosmole $\cdot kg^{-1}$)	Weight change ($g \cdot h^{-1}$)
30	+50, +250
310	+50, 0, 0, 0, −100
1050	−150, −100, −50, 0

The demonstration of a considerable permeability of the skin to water – free diffusion – poses the question of whether this pathway for water molecules can contribute to net water flux in the face of osmotic gradients. The plasma osmolality of our captive animals shows values between 290 and 330 mosmol kg^{-1} plasma water, and harbor porpoises are known to migrate between salinities of 3.5% (1200 mosmol kg^{-1}) and 0.5% (170 mosmol kg^{-1}). Steep osmotic gradients may thus occur between the surroundings and the plasma.

In order to evaluate the magnitude of net water movement through the skin at different osmolalities of the ambient water, we have performed the following experiments: 2 animals were studied in 11 experiments during a period of 5 months. The animals were placed in the set-up described previously, sprinkled with hypo-, iso- or hyperosmotic NaCl solutions for 1 h and weighed before and after. At least 2 days before the experiment the osmolality of the basin water was corrected to the same osmolality as those chosen for the sprinkling water. Urine and faeces were not collected in these experiments since the weight losses by these routes were found to be negligible.

Table 2 shows results from the 11 experiments. The data strongly suggest that net water flux through the skin takes place in the presence of osmotic gradients which occur in the natural environment of the harbor porpoise.

A demonstration of an exchange of water by way of free diffusion and osmosis with the environment in a delphinid species has not been described before. Furthermore, total body water has for the first time been measured in a delphinid.

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Hypothermia induced in mice by injection of venom sac extract of hornets (*Vespa orientalis*, Vespinae: Hymenoptera)

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Summary. Intraperitoneal injection into naive and immunized albino mice of Oriental hornet venom sac aqueous extract induces within 3 h an 8–10 °C and 3–4 °C drop in body temperature, respectively. The fall in temperature is dose-dependent. The responsible fraction(s) in the venom is of high molecular weight.

The oriental hornet *Vespa orientalis* is prevalent in the Mediterranean basin as well as in Southeast Asia[1]. Hornet venom is a mixture of various substances such as biogenic amines, kinins and different sugars (which are not retained during dialysis of the venom) as well as proteins, enzymes and different toxins (which are retained in the dialysis bag)[2]. An interesting recent observation made in our laboratory (as well as in some hospitals) is that shortly after being stung, the victims of hornet attack complain of feeling cold, despite ambient aestival temperatures of about

28 °C. There is no concomitant drastic drop in blood pressure and the drop in body temperature is in the order of 0.5–1 °C. We deemed it worthwhile, therefore, to examine in laboratory animals and under controlled experimental conditions, the extent to which hornet venom interferes with body temperature regulation and also whether this could be abrogated by various means.

Material and methods. Collection and processing of the venom were done as previously described[3]. The experiments were run on male albino mice weighing initially 18–20 g but weighing approximately twice as much by the end of the experiments. Injections of the venom sac extract (VSE) in physiological saline were done i.p. in a maximal dose of 0.3 ml.

Mouse immunization. 100 mice were immunized over a period of 6 months by twice-weekly i.p. injections of whole VSE at a starting dose of 0.5 mg of the fresh (wet) material per animal. This was gradually increased until a terminal dose of 3.6 mg VSE per mouse was reached at the 50th injection. LD_{50} determinations and dialysis were done as described earlier[4]. Temperature was measured via electric thermistor introduced anally. Each measurement was made on individually kept mice in an ambient temperature of 25 °C (±0.5 °C). Body temperature was measured twice before injection – at time 0 and at 22 min – while the injection was given at 37 min. The experiment was repeated 3 times in each animal and the results obtained were generally comparable, but because the VSE had to be prepared anew for each repetition, and the weight of the experimental animals increased with time, the results could not be combined into a single graph. In another experiment the effects of injecting whole VSE and VSE dialysate were compared in mice weighing 35 g.

The statistical significance of the results was determined by transforming all the data to logarithms (natural) and performing the desired analysis (L = line, R = repeat, T = time).

Results. The results of VSE injection (or saline), as shown in figure 1, were as follows: In control group D (saline) there was no significant change in body temperature from start to end, whereas in the groups receiving the venom (A, B and C), there was a significant drop in body temperature (compared to the control group) during the initial 60–70 min ($p < 0.01$). Subsequently, however, the lines for each group diverged as follows: the venom-injected but non-immunized mice (A) continued to show a drop in body temperature to 30.5 °C over a period of 259 min, with the body temperature stabilizing at 220 min but 5 out of 6 mice dying afterwards. The mice receiving twice as much venom as the amount used to immunize them (B) showed lowered body temperatures of about 35 °C throughout the experiment (5 h), and only 1 mouse out of 6 died. Several h after termination of the experiment their body temperatures reverted to normal. The mice of group C, that received the same treatment in the course of the experiment as during the preceding immunization state, had initially increased body temperatures which reverted to normal within 2 h after the start of the experiment, and all remained alive. Comparison between the groups showed that mice of group B had body temperatures significantly different from the control mice throughout the experiment ($p < 0.001$) and also significant was the difference in body temperature between mice of groups A and B.

From several min after the venom injection and until about 3 h later, the injected mice tend to remain immobile in some selected corner of their breeding box, without eating or drinking and with hair erect. Only for a brief period of a few min following the VSE injection is there hyperactivity and even hypersensitivity of the mice (as evident from the cries emitted and the jumps performed in response to slight stimuli such as a sudden noise or abrupt movement of the hand). There was no mortality of mice during the ex-

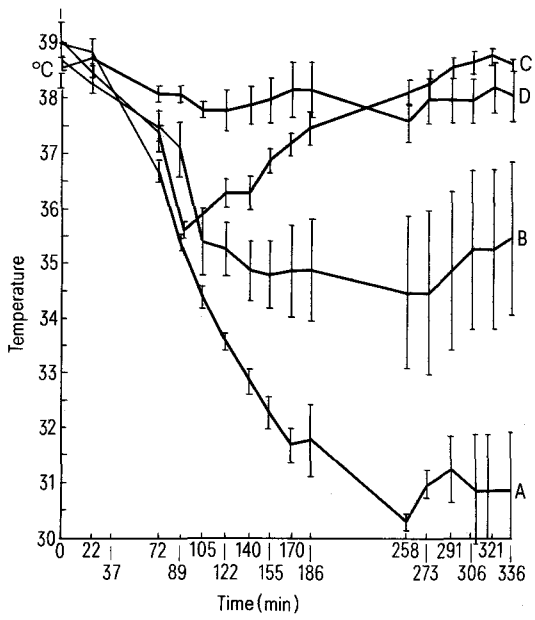

Figure 1. The figure summarizes the results obtained on 4 different groups of mice of identical weight (35 g) which received the following treatments (each line represents the mean temperature of 6 mice receiving the same treatment ±SE): group D, control mice injected with 0.3 ml of an 0.5% saline solution every time the test mice received increasing doses of VSE in saline; group C, mice immunized with VSE and receiving 25 injections of increasing amounts of VSE. From 2 weeks prior to start of the experiment and until day 0, each injection comprised 1.8 mg VSE per mouse (see 'materials and methods' section); group B, mice immunized as in C, but prior to the start of the experiment, they were given twice as much VSE, i.e. 3.6 mg VSE per mouse; group A, naive mice which at the beginning of the experiment were injected with 3.6 mg VSE (i.e., like those in group B).

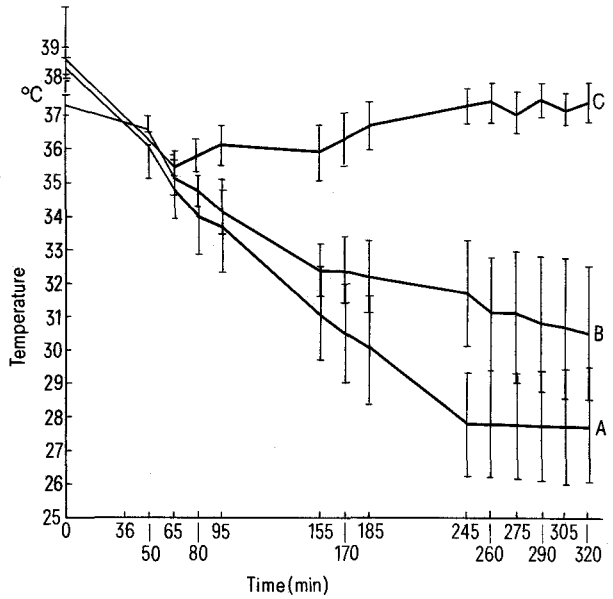

Figure 2. The effects of VSE dialysate as compared to whole VSE. A, The effect of the material retained in the dialysis bag; B, the effect of whole VSE; C, the effect of dialysate. Each line represents the mean temperature of 6 mice receiving the same treatment ±SE.

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