

zymes and their affinity, as judged by apparent K_m values, is higher for NADP(H) than for NAD(H).

GAPDH II gives, on SDS electrophoresis, 2 bands of 43,000 and 37,000 daltons: their mobility is the same as that of the subunits which constitute the 600,000-dalton enzyme, but their relative proportions are different. Although the presence of a minor contaminating band of protein prevents the unambiguous definition of the subunit composition of GAPDH II (mol.wt 300,000 daltons), it is likely that both this, and the 600,000-dalton species, derive from a different assembly of the same subunits: their molar ratio is 1:1 in GAPDH I, while an excess of the lighter subunit is present in GAPDH II (fig. 3,C). Similar results have been also reported by Pawlizki and Latzko¹².

The 150,000-dalton enzyme shows, on SDS electrophoresis, a single band of 37,000 daltons which migrates similarly to one of the corresponding subunits of the two forms of GAPDH reported above (fig. 3,C); therefore this form corresponds to the previously reported A_4 homotetramer⁶⁻⁹, as suggested also by its amino acid composition (table 2) which does not significantly differ from that of the analogous enzyme from mustard seedlings⁹.

The same subunits (43,000 and 37,000, daltons) which constitute, in equimolar amounts, the 600,000-dalton enzyme, can originate, in a different arrangement, 2 minor forms of active GAPDH; a homotetramer, made up of mol.wt 37,000 daltons subunit and the 300,000-dalton enzyme which might arise from an asymmetric assembly of both of them.

From the reported data it seems reasonable to conclude that the most stable arrangement of the 2 subunits occurs in the 600,000-dalton enzyme; consequently, in agreement

with the observation on crude chloroplast extracts¹⁰, this should be the main form of native GAPDH. The other 2 species, rather than true isoenzymes, might originate either from an unbalanced production of the 2 subunits, or from a dissociation and reassociation process of GAPDH I taking place within the chloroplast, or during the purification procedure.

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Exchange of water between the harbor porpoise, *Phocoena phocoena*, and the environment

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Summary. During determination of total body water and net water turnover in the harbor porpoise, *Phocoena phocoena*, it was demonstrated that the porpoise exchanged water with an isosmotic environment by way of free diffusion and with hypo- or hyperosmotic environment by way of osmosis.

We have determined the total water turnover rate in 1 subadult female harbor porpoise (*Phocoena phocoena*) kept in isosmotic salt water (320 mosmol kg⁻¹) by measuring the rate of disappearance from the plasma over a period of 5 days of i.v. injected tritiated water¹. In 2 experiments, the relative rate was quite constant with a turnover rate of 12.0 l and 13.1 l per 24 h. The total body water, obtained from the degree of dilution of the tritiated water, was found to be 14.5 and 14.6 kg, respectively, or 51.8 and 47.7% of the body weight. These low figures for body water are in accordance with a 45% blubber mass as given by Slijper². Thus, the total rate of water turnover was 13.5 and 12.0 kg per 24 h. The high turnover rate of water was inconsistent with the mean daily water intake from the food during the

experiment. This was calculated as 1.5 kg of preformed water to which should be added the water of oxidation, totalling 1.9 kg.

Similar data with respect to water turnover, body water content and water intake were found in 17 experiments with 5 animals including the experimental animal.

The measured turnover rates were also inconsistent with data on oral water consumption measured by 2 other methods in 2 other delphinid species^{3,4}, which report ingestion of saltwater of the order of 5-10 ml/kg b.wt per 24 h corresponding to 150-300 ml per 24 h in our experimental animal.

A high oral intake of water is also in contrast with the way in which the harbor porpoise swallows its prey. When the

Table 1. Recovered activity and losses of radioactivity in 2 experiments in 1 harbor porpoise sprinkled for 1 h with isosmotic saline. Fecal output was negligible

Experiment	Activity recovered in urine (dpm h ⁻¹ · 10 ⁷)	Activity recovered in tub water (dpm h ⁻¹ · 10 ⁷)	Total activity recovered (dpm h ⁻¹ · 10 ⁷)	Loss of activity from body water (dpm h ⁻¹ · 10 ⁷)
1	0.139	4.10	4.23	4.21
2	0.0273	3.67	3.70	4.11

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 Thurley⁵ 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 $2 \text{ l} \cdot \text{min}^{-1}$ 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 , $+1 \text{ h}$ and $+2 \text{ h}$ 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 circumstances⁶.

Table 2. Weight changes during sprinkling experiments

Osmolality of sprinkling water (mosmole $\cdot \text{kg}^{-1}$)	Weight change (g $\cdot \text{h}^{-1}$)
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 $8\text{--}10^\circ\text{C}$ and $3\text{--}4^\circ\text{C}$ 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 Asia¹. 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