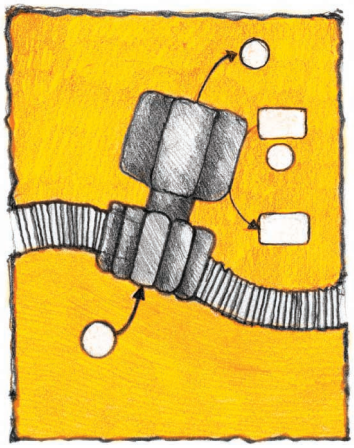
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

GLUCOSE SENSING



SENSOR NOT TRANSPORTER

We all know that glucose occupies a central position in cellular carbon and energy metabolism. However, glucose has another little-known function; it also works as a signalling molecule regulating food intake, body weight, blood glucose levels and gastrointestinal reflexes. Which poses the question, are eukaryotic cells able to sense external glucose concentrations directly?

Apart from a few gustatory chemoreceptors, the only proteins that have so far been suggested as possible glucose sensors are two glucose transporter homologues found in yeast, both of which transduce the external glucose signal to control gene expression in the cell. However, little else was known about these sensor proteins until scientists from Los Angeles and Würzburg recently discovered a novel human glucose sensor hiding in a large family of Na^+/glucose cotransporters called SLC5.

Ernest Wright and Hermann Koepsell had been interested for many years in these types of glucose transporters and knew that the SLC5 cotransporters usually facilitate glucose uptake by coupled transport of sodium into the cell. Therefore, it was not surprising that they would examine a previously undescribed member of the SLC5 family, discovered by the human genome project in 1999, called human sodium coupled glucose transporter 3 (hSGLT3). After cloning the protein, the team began searching for hSGLT3 expression in human tissue, finding it in the plasma membranes of skeletal muscles, at the neuromuscular junction and also in the cholinergic neurons of the small intestine. But what was the protein's physiological role in cholinergic neurons and skeletal muscles?

To answer this question, the team examined the functional properties of the apparent glucose transporter. Using the *Xenopus laevis* oocyte expression system, they first injected hSGLT3 cRNA into the oocytes and then tested for evidence that the RNA was translated to produce protein that was inserted into the plasma membrane. Next, they tested whether the modified oocytes could transport radiolabelled sugars across the cell's membrane with the human glucose transporter. However, they were unable to detect any sugar transport. hSGLT3 seemed to be incapable of transporting sugar.

At first, Wright and his colleagues must have assumed that hSGLT3 was non-functional, either due to misfolding or some other artefact. But when they studied the electrical properties of hSGLT3 in *Xenopus* oocytes they found depolarization of the membrane; the cotransporter was able to transport sodium in response to glucose. hSGLT3 was functional, and the membrane depolarization turned out to be reversible and specific for D-glucose. And when the team looked at the Na^+-dependent membrane potential, it was directly proportional to the glucose concentration. All of these properties make hSGLT3 an ideal glucose sensor. Rather than modulating gene expression as the yeast sensors do, hSGLT3 is a completely novel type of glucose sensor that resembles the behaviour of chemoreceptors and signals glucose levels through membrane depolarisation.

Thus, hSGLT3 may influence both gastrointestinal cholinergic nerve activity and skeletal muscle in response to varying extracellular glucose concentrations by modulating membrane potential. This new and unexpected insight into glucose sensing may also serve as a warning not to rely exclusively on sequence homologies when assigning functions to unknown proteins. Although hSGLT3 looked just like any other transporter, in reality it turned out to have a completely unexpected function.

10.1242/jeb.00796

Diéz-Sampedro, A., Hirayama, B. A., Osswald, C., Gaboulev, V., Baumgarten, K., Volk, C., Wright, E. M. and Koepsell, H. (2003). A glucose sensor hiding in a family of transporters. *Proc. Natl. Acad. Sci. USA* **100**, 11753-11758.

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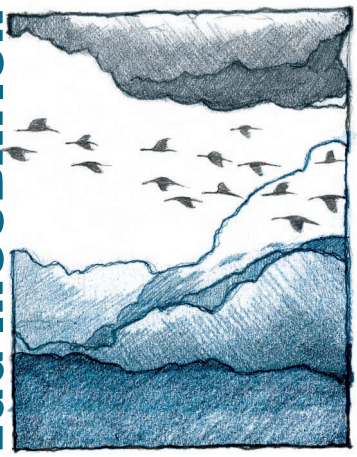
EGG INCUBATION



WARM FEET, COZY EGGS

Birds incubate eggs to provide a constant cozy environment for their developing offspring. The central heater for most eggs is the parent's broodpatch, an area of thick, bare, vascularized skin on the abdomen. When parents sit on their nest, the brood patch touches and warms the eggs. However, some species do not have a brood patch and resort to alternative heating systems to incubate their young. Most famous are the Megapodes that use mounds of decaying material to incubate their eggs. Less well understood is the Pelecaniformes' approach to nurturing their eggs. Pelicans, boobies and gannets partially cover their eggs with their feet and then crouch over them. For decades, this observation has invited speculation about the potential for heat transfer between the feet and eggs, but proper measurements had not been made.

Stephanie Morgan and her colleagues set out to end the speculation. Their main goal was to determine whether the bird's feet directly warm the eggs or if they merely pass on heat from the abdomen. The team studied nazca boobies at the Galápagos Island of Española. Nazca boobies, like all Pelecaniformes, have webbing between all four toes of their feet. During incubation, both parents take turns in wrapping the middle webs around the sides and top of their single egg. To measure heat transfer from the abdomen and the feet separately, the researchers took the booby eggs and temporarily replaced them with larger albatross eggs. The waved albatross' eggs are about twice as long and wide as boobies' eggs. So, the incubating parent was only able to cover the sides of the adopted egg with its feet, leaving the top of the egg uncovered by webbing and in direct contact with the abdomen. The egg was equipped with three temperature sensors, two on the sides where the feet touched the egg and one on the top where the abdomen covered the egg. In addition, Morgan and colleagues measured the soil and egg temperatures before the albatross egg was placed beneath the booby and after the egg was removed.

In all four trials, the incubating booby heated the albatross egg to above ambient and soil temperature. Temperatures at the foot–egg interface exceeded temperatures at the abdomen–egg interface, at least during part of each trial. And the feet of the booby were always warmer than the final temperature of their adopted egg. Temperature-sensitive paint showed that boobies' feet can be warmer than 40°C!

How do incubating boobies get such warm feet? The answer is in the blood. Foot-webbing is always vascularized, but incubating birds have a larger area of blood vessels than birds without eggs. More, or larger, vessels allow for a larger heat flow to the egg. As is often the case for abdominal brood patches in other bird species, female boobies had more vascularized feet than their mates, even though both sexes incubate.

This study ends the speculation about the role of warm feet in providing a cozy environment for developing embryos. Just like regular brood patches, the foot-surrogates are well vascularized and transfer heat from the parent to the egg. And, as usual, good science not only answers but poses new questions too. The next piece in the puzzle is an efficiency test: how do the feet compare with the abdomen for keeping their precious eggs warm?

10.1242/jeb.00797

Morgan, S. M., Ashley-Ross, M. A. and Anderson, D. J. (2003). Foot-mediated incubation: nazca booby (*Sula granti*) feet as surrogate brood patches. *Physiol. Biochem. Zool.* **76**, 360-366.

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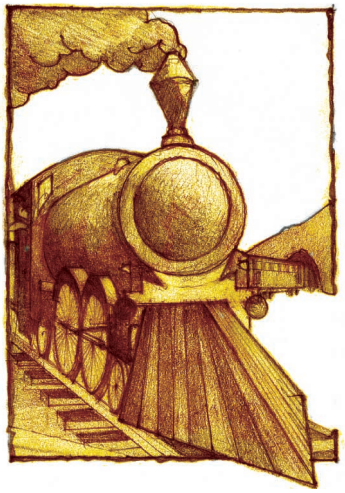
VORTEX INTERACTIONS



NEW TWISTS IN THE LEADING-EDGE VORTEX

Insects aren't airplanes. If they flew like airplanes, they would fall right out of the sky because their wings are much too small. But since they flap their wings, they can generate a 'leading-edge vortex', a rotating element of fluid along the front of the wing that dramatically increases lift briefly and helps keep them aloft. A recent debate has focused on how and when the leading-edge vortex forms and what it does once formed. Gregory Lewin and Hossein Haj-Hariri have added more complications to the argument in their computational study of small flapping wings, published in *J. Fluid Mech.* They describe how the leading-edge vortex can interact with other wing vortices to produce forces that differ on the up- and downstroke, even when the wing movements are symmetrical.

Normally, each time a wing changes direction when it is flapping, it sheds a vortex off the back edge, the 'trailing-edge vortex', which then drifts backwards to form the wake. Sometimes, it also produces a leading-edge vortex at the front of the wing, which can remain attached at the front edge or drift back to interact with the trailing-edge vortex.

Using a 2-D computational model of a wing moving up and down in steady viscous flow, Lewin and Haj-Hariri found that the leading-edge vortex can interact with the trailing-edge vortex in either a completely symmetrical way, a completely asymmetrical way or can produce a type of interference pattern with the trailing-edge vortex.

In the symmetrical mode, which happens at low frequencies and amplitudes, the wake consists of alternating vortices with a

backward-pointing jet, like in ordinary flapping propulsion. By contrast, in the asymmetrical mode, which can happen at high frequencies and amplitudes, the wake deflects to one side for many beats but occasionally flips sides. The same effect has been seen with flapping airfoils in wind tunnels, but this is the first numerical study to reproduce it. For insects, it would probably be detrimental, since the lift force would increase or decrease unpredictably. At intermediate frequencies, Lewin and Haj-Hariri observed something like an interference pattern between the leading-edge and trailing-edge vortices, with dramatic changes in the output power over four or five beats. They showed that the leading-edge vortex usually moves backwards, interacting with the trailing-edge vortex, but sometimes, because of subtly different background conditions, the leading-edge vortex from one stroke wraps around the front of the wing to interact with the leading-edge vortex that forms on the next stroke, producing a dramatic drop in the power output.

What do these results mean for our understanding of insect flight? First, it isn't clear whether these strange effects even happen during insect flight. Insects might avoid these effects by tuning how they flap their wings. Nonetheless, Lewin and Haj-Hariri show that small changes in the background flow, perhaps caused by gusts of wind or turbulence, can produce large qualitative changes in the wake and ultimately in efficiency and power output. Specifically, the wing's propulsive efficiency depends critically on when the leading-edge vortex separates. Efficiency is highest when the leading-edge vortex stays attached to the wing for a whole stroke and merges with the next trailing-edge vortex. But Lewin and Haj-Hariri have shown that when the leading-edge vortex remains attached for longer, or even wraps around the front edge of the wing, flight efficiency decreases. So, although the leading-edge vortex can increase efficiency in some circumstances, it isn't beneficial in all. With these results, the picture of the leading-edge vortex is growing even more convoluted.

10.1242/jeb.00798

Lewin, G. C. and Haj-Hariri, H. (2003). Modelling thrust generation of a two-dimensional heaving airfoil in a viscous flow. *J. Fluid Mech.* **492**, 339-362.

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DIVE RESPONSE

GO AHEAD, VENT YOUR SPLEEN!

For diving and exercise physiologists, 'venting your spleen' has a whole different meaning than it does to the layman. In diving mammals and heavy exercisers, the spleen serves as an oxygen reservoir, storing highly viscous 'thick blood', rich in red blood cells, during periods of rest, and injecting these stored red blood cells into the general circulation when oxygen levels are stressed and increased transport is required. Such injections of oxygenated red blood cells occur during periods of heavy exercise in some mammals, such as horses, and are particularly well developed in diving mammals. The spleen of the Arctic Weddell seal contains so much extra oxygenated blood that it often is referred to as a SCUBA tank; in fact, the release of red blood cells into the circulation is so tightly regulated that arterial oxygen content doesn't decrease for the first 15–18 min underwater. Splenic emptying under these circumstances is due to an active contraction of the spleen triggered by adreneric innervation; spleen volume in some diving seals may reduce by as much as 85%! However, in general, humans have a less well-developed dive response and a relatively small splenic reservoir (about 8% of total body red blood cells *versus* more than 20 litres of sequestered RBCs in the Weddell seal), and the human spleen is poorly innervated by adrenergic fibers. It has thus been suggested that the observed decrease in human spleen volume following sympathetic activity is due to passive collapse as blood supply to the spleen decreases rather than to an adaptive, active contraction. Recent work by D. Bakovic and coworkers set out to investigate whether splenic contraction is part of the human dive response too.

The team began examining spleen volume and blood flow during simulated human dives in which volunteers submerged their faces while holding their breath. They reasoned that a decrease in spleen volume, caused by a decreased arterial inflow and increased or unaltered venous outflow, would indicate passive collapse. Conversely, an active contraction would result in increased venous outflow without changing arterial inflow. Their results showed that over five successive periods of breath-holding, the diameter of the splenic artery was not altered, whereas the diameter of the splenic vein increased after the first apnea and then gradually returned to baseline. Spleen volume decreased by 14–18% after the first apnea and did not change significantly during subsequent breath-holds.

While the decrease in spleen volume during human 'diving' thus appears to be an active process, rather than a passive collapse, the question of its functional significance remains. Does an injection of red blood cells from the human spleen during apnea increase how long you can hold your breath? For this, the authors looked at dive length in trained apneic divers (members of the Croatian national apneic diving team, no less!), in untrained individuals and in persons who had undergone a complete splenectomy at least 2 years previously. Apneic periods were longer during subsequent dives than for the first dive in both trained and untrained individuals but not in splenectomized persons. As the spleen did not relax in the 2-min recovery periods between apneas, the splenic emptying of the first dive thus increased circulating red blood cells for subsequent dives, without a further reduction in spleen volume. The authors suggest that subsequent apneas were longer because there were more red blood cells and thus a higher oxygen carrying capacity from the outset, although they were unable to definitively measure blood gases.

While numerous other factors, including experience, can increase apneic 'dive time' over sequential trials, these other factors apparently did not extend repeat dive times in splenectomized individuals, leaving the intriguing possibility that, as with other facets of the dive response, humans share similar, if less well developed, adaptations exemplified by such champion mammalian divers as Weddell and elephant seals.

10.1242/jeb.00794

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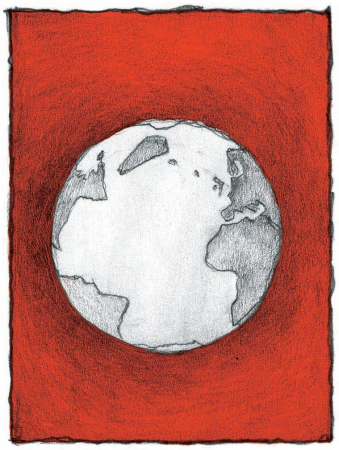
FREEZE TOLERANCE



FROZEN FROGS FALL BEHIND

The terrestrial wood frog, *Rana sylvatica*, can tolerate sub-freezing environmental temperatures because of biochemical adaptations that protect cells from freezing. As frogs freeze, extracellular water is lost to ice crystals, and intracellular glycogen is converted into glucose. Both these events raise the intracellular solute concentration, thereby lowering the freezing point of the cell and preventing intracellular ice crystal formation. The physiological costs of these responses are not well known, and Irwin and colleagues have investigated the impact of the protective responses on locomotor endurance.

In the spring, thawed frogs can travel more than 400 m from their over-wintering sites to breeding ponds. As their breeding success depends on how quickly the frogs get to the pond, there's a lot at stake if the amphibians arrive too late or too exhausted to mate. To investigate the impact of freezing on locomotor endurance, frogs frozen for 36 h, were thawed and allowed to recover for 24, 48 or 96 h at 4°C. Then, the frogs were warmed to 15°C and placed on a treadmill where they walked until exhausted.

Despite the fact that thawed frogs displayed normal behavioural patterns, their endurance was significantly reduced relative to frogs that had been cooled to 0°C but not frozen. After 1 day of recovery, frozen frogs had about half the endurance of cooled frogs. After 4 days of recovery, both the thawed and cooled frogs' endurance increased, although frozen frogs only had the stamina that cooled frogs had recovered after 1 or 2 days. Increased endurance in the cooled frogs could be related to deleterious effects of cold exposure on both groups of frogs.

Because locomotor endurance may be limited by oxygen transport, metabolic efficiency and energy reserves, the team sampled blood and tissue from frozen, thawed and cooled frogs to see whether freezing had affected any of the processes. These samples were used to assess levels of erythrocyte damage during freezing (plasma hemoglobin), to assess the energetic poise of frogs during each stage of the thaw process (lactate accumulation) and to examine energy reserves (glycogen and glucose available for ATP generation).

Frozen frogs had elevated plasma hemoglobin, suggesting that extracellular ice crystals had lysed erythrocytes. However, within 24 h of thawing, hemoglobin was no longer detectable in plasma samples, and hematocrit was at pre-freeze levels, indicating that blood water content had returned to normal.

Metabolite levels changed dramatically as the amphibians froze, and the responses were most pronounced in liver tissue. As the frogs froze, hepatic glycogen levels plummeted 95% and rose to just 17% of control levels after two days post-thaw. Hepatic glucose levels rose more than 5-fold during the freezing process and remained elevated after thawing. These observations demonstrate that the frozen frogs had dramatically lower energy reserves than control frogs, as glycogen degraded to glucose during the freezing process was only partially replenished by 48 h post-thaw. Hepatic lactate levels rose as the frogs froze but returned to control levels within a day of recovery, suggesting that in resting frogs oxygen delivery to tissues was adequate to maintain aerobic poise.

There was no clear indication that levels of tissue glycogen or glucose were correlated with locomotor endurance. Lactate levels increased in both cooled and frozen frogs after exercise to exhaustion, suggesting exhausted frogs were experiencing physiological hypoxia. Thus, endurance may be better correlated with oxygen delivery to metabolically active tissues than to tissue energy reserves.

Future studies with frogs acclimatized to different seasons and acclimated to different nutritional states may help resolve the roles of oxygen transport and delivery and tissue metabolites in locomotor endurance in thawed frogs.

10.1242/jeb.00795

Irwin, J., Costanzo, J. and Lee, R., Jr (2003). Postfreeze reduction of locomotor endurance in the freeze-tolerant wood frog, *Rana sylvatica*. *Physiol. Biochem. Zoo.* **76**, 331-338.

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