If we make similar measurements on the photographs of a cast of the counterslab of the Berlin specimen provided by Rietschel[2], which were used by Norberg, for regions of the fourth primary which are not overlapped, we obtain almost the same result as that we obtained previously[1] from measurements of a cast of the main slab (1.51 instead of 1.46). These measurements are also consistent across the only two specimens of *Archaeopteryx* where feathers are sufficiently preserved to make measurements. Although we previously measured several feathers, we included only measurements from feather 4 in the statistical comparison[1]. We also showed that vane asymmetry at the 25% point does not vary between the first and fourth primaries of extant birds. Our measurements and statistical treatment are correct. However, if we accept Norberg's estimates of vane asymmetry, these clearly fall at the very lower end of the range for extant flying birds and well within the range of current flightless birds. Any inference that the bird was capable of powered flight from these data is extremely weak.

(2) The coincidence that Norberg's estimates of vane asymmetry fall within a hypothetical 'optimal' region is irrelevant to the discussion of flight performance. Many currently flightless birds have feathers with asymmetries which fall within this region: most extant birds using powered flight do not (Fig. 2 of ref. 1).

(3) Norberg provides no quantitative data concerning comparisons of vane curvature in current flying and flightless birds. However, the powered flight capabilities he infers from his vane asymmetry estimates and unsupported vane curvature are inconsistent with many other aspects of these fossils: in particular the wrist structure which is incompatible with execution of a flapping motion[7], the pectoral muscles which were too small for powered flight[8] and the long bony tail which may have been aerodynamically inefficient[9]. Rather than claim from our analysis that *Archaeopteryx* was not capable of sustained flapping flight, we stated that the poorly developed asymmetry was consistent with these other features.

J. R. Speakman
S. C. Thomson
Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN, UK

1. Speakman, J. R. & Thomson, S. C. *Nature* **370**, 514 (1994).
2. Rietschel, S. in *The Beginnings of Birds* (eds Hecht, H. K., Ostrom, J. H., Viohl, G. & Wellnhofer, P.) 251–260 (Freunde des Jura-Museums, Eichstätt, 1985).
3. Norberg, R. Å. in *The Beginnings of Birds* (eds Hecht, H. K. *et al.*) 303–318 (Freunde des Jura-Museums, Eichstätt, 1985)
4. Norberg, R. Å. *Biol. Rev.* **48**, 561–596 (1973).
5. Lowe, P.R. *Ibis* **86**, 517–543 (1944)
6. Fedducia, A. & Tordoff, M. *Science* **203**, 1021–1022 (1979).
7. Vasquez, R.J. *J. Morph.* **211**, 259–268 (1992).
8. Speakman, J.R. *Evolution* **47**, 336–340 (1993).
9. Balmford, A. *et al. Nature* **361**, 628–631 (1993).

Proton movement on membranes

SIR — Lateral proton diffusion along membrane surfaces represents certainly the most efficient system for proton movement between source and sink on a membrane. For the ATP-synthase activity in alkalophilic bacteria, this kind of diffusion may be a prerequisite because of the extremely low proton concentration in the medium. However, the proton diffusion along membrane surfaces has been a subject of continuous debate. Heberle *et al.*[1] used surface-bound pH indicators to measure light-induced pH changes on the extracellular and cytoplasmic surface of the purple membrane. The pH changes detected on the cytoplasmic side were slower than on the extracellular surface, the proton release side, but were clearly faster than those measured in the aqueous bulk phase.

These data were interpreted by the authors as the result of a fast proton migration along the purple membrane surface to the opposite side, with retarded surface-to-bulk transfer. Although this interpretation is certainly plausible, it is ambiguous and similar data could be obtained by a transient deprotonation on the cytoplasmic surface. Therefore, it is necessary to find direct experimental evidence for this preferential lateral proton migration. If the protons do move along the membrane surface, this lateral diffusion will most probably be mediated by the surface-bound buffer groups (acidic and basic surface residues, for example, lipid head groups). The dwell time of protons on lipid head groups (the inverse of the dissociation rate constant) was shown to increase rapidly with their pK_a (refs 2, 3). Therefore, the lateral proton movement along membranes would be expected to depend on the pK_a of the lipid head groups.

An experiment that would conclusively demonstrate lateral diffusion of protons along the membrane surface would show that this diffusion can be modulated by varying the chemical character of the lipid head groups. Detection of a proton, released on the extracellular side, should be affected exclusively on the cytoplasmic surface when lipids with different pK_as are introduced. Unfortunately, such an experiment cannot be done easily with purple membrane without causing alterations in the photocycle and proton release kinetics. We characterized the proton movement in a bacteriorhodopsin–lipid–detergent micelle system[4–6]. The pH indicator fluorescein was covalently bound to cysteine residues, introduced into selected positions on the extracellular and cytoplasmic surface of bacteriorhodopsin by site-directed mutagenesis through reaction with the iodoacetamido derivative of fluorescein[4,5]. In this system, the light-induced proton concentration changes detected on the extracellular and cytoplasmic surfaces were also clearly faster than in the aqueous bulk medium. Moreover, this micellar system allowed us to exchange lipids without affecting the photocycle kinetics.

The proton mobility along the micellar surface could be varied, as predicted, by adding phospholipids with head groups of different pK_as (refs 4, 6). Head groups with a low pK_a enhanced the lateral movement of protons from the extracellular release side to the cytoplasmic surface, resulting in a larger amplitude for the proton signal measured on the latter side. When lipids with higher pK_a head groups were introduced, the amplitude of the proton signal was considerably reduced. No changes were observed on the extracellular side. These results clearly demonstrate that the proton released on the extracellular side does move along the micellar surface to the cytoplasmic side and the role of the pK_a of lipid and detergent head groups. The protons move significantly faster from the extracellular to the cytoplasmic side than from the micellar surface to the aqueous bulk phase. Furthermore, we could demonstrate a rate-limiting step for the lateral proton movement from the protein to the lipid/detergent surface[7].

We have also investigated the proton movement in the purple membrane, applying the technique described above for the micellar system using mutant purple membrane with cysteine in selected positions[8]. In principle, our results do agree with the interpretation presented by Heberle *et al.*[1], and the proton is also detected faster on either surface of the purple membrane than in the aqueous bulk phase. In our case[8], however, the time difference for detection of the released proton between the two surfaces is smaller, with 71 ± 4 μs on the extracellular and 76 ± 5 μs on the cytoplasmic side compared with 76 ± 18 and 228 ± 39 μs, respectively, reported by Heberle *et al.* Based on our data, the surface diffusion constant is much larger than estimated by Heberle *et al.* ($D > 3 \times 10^{-5}$ cm^2 s^{-1} compared with $D = 9.6 \times 10^{-7}$ cm^2 s^{-1}).

Peter Scherrer
Department of Biochemistry, The University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

1. Heberle, J., Riesle, J., Thiedemann, G., Oesterhelt, D. & Dencher, N.A. *Nature* **370**, 379–382 (1994).
2. Nachliel , E. & Gutman, M. *J. Am. chem. Soc.* **110**, 2629–2635 (1988).
3. Gutman, M. & Nachliel, E. *Biochemistry* **24**, 2941–2946 (1985).
4. Scherrer, P. *et al. Struct. Funct. retinal Prot.* **221**, 205–211 (1992).
5. Alexiev, U., Marti, T., Heyn, M.P., Khorana, H.G. & Scherrer, P. *Biochemistry* **33**, 298–306 (1994).
6. Scherrer, P., Alexiev, U., Marti, T., Khorana, H.G. & Heyn, M.P. *Biochemistry* **33**, 13684-13692 (1994).
7. Alexiev, U., Marti, T., Heyn, M.P., Khorana, H.G. & Scherrer, P. *Biochemistry* **33**, 13693–13699 (1994).
8. Alexiev, U., Scherrer, P., Mollaaghababa, R., Khorana, H.G. & Heyn, M.P. *Proc. natn. Acad. Sci. U.S.A.* **92**, 372–376 (1995).