



Fig. 3 Difference spectrum of 5-dm bathorhodopsin at -191°C . Conditions are given in Fig. 2a and b. The curve drawn results from subtracting spectral curve (4), from curve (3) in Fig. 2b.

shown in Fig. 2a, curves 2–7. The appearance of an isosbestic point at 503 nm argues that Fig. 2a illustrates the single-step transformation of 5-dm isorhodopsin into one, or a mixture of, long-wavelength absorbing intermediate(s).

Photoreversibility of this pigment system is shown in Fig. 2b, first by the transformation of the long wavelength absorbing intermediate(s) into shorter-wavelength-absorbing species, presumably 5-dm rhodopsin and 5-dm isorhodopsin. The first process (curves 1 and 2) is followed by the reformation of the long wavelength absorbing intermediate(s). Thus spectrum 3 of Fig. 2b contains thermostable pigments, presumably 5-dm rhodopsin and 5-dm isorhodopsin, as well as a thermally unstable component which decomposes to 5-dm retinal oxime and opsin when the solution is warmed to 20°C , as shown in spectrum 4. The difference spectrum of the thermally unstable compound, drawn in Fig. 3, has a positive peak with λ_{max} at 525 nm and a band width at half maximum of 95 nm. This spectrum characterises an intermediate which, because of its spectral and thermal analogies to cattle bathorhodopsin⁹, I call 5-dm bathorhodopsin.

When the violet irradiated mixture of 5-dm pigments (Fig. 2b, curve 3), containing 5-dm bathorhodopsin, and presumably 5-dm rhodopsin and 5-dm isorhodopsin, is warmed to only about -165°C , a decrease in absorbance at wavelengths greater than 500 nm and an increase in absorbance in the 400–500 nm region occurs. This change is consistent with the transformation of 5-dm bathorhodopsin into a pigment with λ_{max} at 497 nm and band width at half maximum of 80 nm. I call this pigment 5-dm lumirhodopsin. The spectrum of 5-dm lumirhodopsin and its formation from a longer wavelength precursor at around -165°C suggest it is analogous to cattle lumirhodopsin¹⁰.

These results show that a batho-intermediate can be formed from a pigment lacking an allylic methyl group in the ionone ring of the chromophore. Without such a methyl group it is impossible to transfer a hydrogen atom or ion from a group on the ionone ring to opsin and thereby form the structure proposed^{1,2} for bathorhodopsin. Since 9-dm pigments and 13-dm pigments also form batho-pigments at -190°C (my unpublished results), it seems reasonable to conclude that photochemical proton transfer from an allylic methyl-group on the chromophore to a site on opsin is not responsible for the rhodopsin (or isorhodopsin) to bathorhodopsin transition. *Cis* to *trans* isomerisation

still seems to be the process which can best account¹¹ for the experimental observations bearing on the initial photochemical event in the photochemistry of visual pigments, both natural and artificial.

This work was supported by a grant from the National Eye Institute. The low temperature experimentation was performed while I was a visiting professor in the Department of Biophysics of Kyoto University and I thank Professor T. Yoshizawa and his colleagues for hospitality and assistance. I also thank Professor B. Honig, Hebrew University of Jerusalem, for encouragement and R. B. Kropf for helpful emendations in the manuscript.

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Received July 13; accepted September 9, 1976.

- ¹ Fransen, M. R., *et al.*, *Nature*, **260**, 726–727 (1976).
- ² Kropf, A., in *Processing of Optical Data by Organisms and Machines*, Proc. int. School Physics Enrico Fermi (edit. by Reichardt W.), **XLIII** 28–43, Academic, New York, 1969.
- ³ Oseroff, A. R., and Callender, R. H., *Biochemistry*, **13**, 4243–4248 (1974).
- ⁴ Cookingham, R. E., Lewis, A., Collins, D. W., and Marcus, M. A., *J. Am. chem. Soc.*, **98**, 2759–2763 (1976).
- ⁵ Yoshizawa, T., and Wald, G., *Nature*, **214**, 566–571 (1967).
- ⁶ Kropf, A., Whittenberger, B. P., Goff, S. P., and Waggoner, A. S., *Expl Eye Res.*, **17**, 591–606 (1973).
- ⁷ Rotmans, J. P., and Kropf, A., *Vision Res.*, **15**, 1301–1302 (1975).
- ⁸ Hubbard, R., Brown, P. K., and Bownds, D., in *Methods in Enzymology* (edit. by McCormick, D. B., and Wright, L. D.), **XVIII**, 615–653 (Academic, New York, 1971).
- ⁹ Yoshizawa, T., in *Handbook of Sensory Physiology* (edit. by Dartnall, H. J. A.) **7I**, 146–179 (Springer, Berlin, 1972).
- ¹⁰ Yoshizawa, T., and Wald, G., *Nature*, **197**, 1279–1286 (1963).
- ¹¹ Rosenfeld, T., Honig, B., Ottolenghi, M., Hurley, J., and Ebrey, T. G., *Pure app. Chem.* (in the press).

Corrigendum

In the article “Perception of melodies” by H. C. Longuet-Higgins (*Nature*, **263**, 646; 1976) there are some errors in the figures. In Fig. 3 the top left-hand number should be -9 ; in Fig. 4 the second x in the left-hand column should be a z ; in Fig. 7 the trilled minim should be prefixed by a #.

Errata

In the article “Male emigration and female transfer in wild mountain gorilla” by A. H. Harcourt *et al.* (*Nature*, **263**, 227; 1976) the second author should be K. J. Stewart and not as printed.

In the article “Axial, magnetron, cyclotron and spin-cyclotron-beat frequencies measured on single electron almost at rest in free space (geonium)” by R. Van Dyck, Jr, P. Ekstrom and H. Dehmelt (*Nature*, **262**, 776; 1976) $(n + \frac{1}{2})\nu_c$ in the first equation should read $(n + \frac{1}{2})(\nu_c - \nu_m)$. In the acknowledgment, for Schwingberg read Schwinberg.

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