The existence of isosbestic points, at 420 and 437 nm, in the *p*H-dependent difference spectra, is in agreement with the presence of only two spectral species in *p*H-dependent equilibrium. The experimental data in *bis*-Tris (0.05 M) can be fitted with a simple titration curve, with *p*K 6.5 (Fig. 3), suggesting that only one type of ionisable group is involved in the Root effect. In the presence of IHP (10^{-3} M) a similar effect is observed

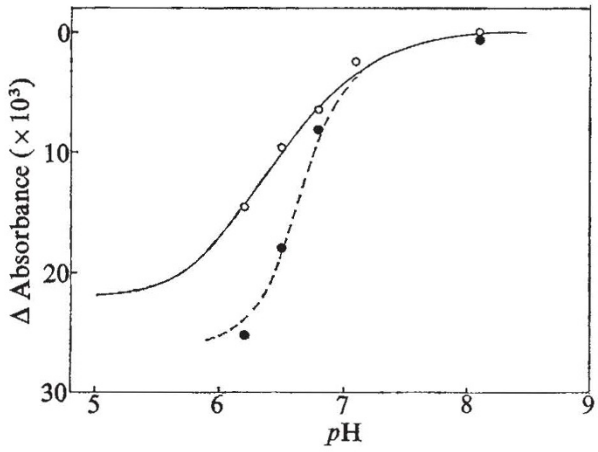

Fig. 3 *p*H-induced transition in Hb trout IV–CO in the absence (○) and in the presence (●) of 10^{-3} M IHP (solvent 0.05 M *bis-Tris* buffer) at 20 °C; Δ Absorbance as in Fig. 2.

(Fig. 3); the higher *p*K of the transition is consistent with the cumulative effect that both protons and the organic phosphate exert on the stabilisation of the "low affinity" form. It cannot be excluded, however, that in the presence and absence of IHP the proton induced transition may occur among different states. The greater steepness of the transition observed in IHP is probably related to the fact that Hb trout IV is not fully saturated with the phosphate at all *p*H values.

It is difficult to interpret the redshift of the maximum of absorption of the CO derivative of Hb trout IV, induced by lowering *p*H. For instance, it is not possible to correlate unequivocally the position of the peak to the strength of the bond between the iron and the ligand, although obviously the observed changes must reflect a perturbation in the distribution of electrons in the porphyrin–metal–ligand complex. The observed redshift may suggest a weakening of the π-bonding and an increase in the iron–ligand bond length correlated to a movement of the proximal imidazole relative to the metal (R. J. P. Williams, personal communication). Apart from detailed interpretations of the observed effect, it is of interest to correlate the spectral change with other properties of Hb trout IV, which are also *p*H dependent and occur over the same *p*H region. First, the dramatic change in functional properties and in particular the well known drop in ligand affinity which is experienced between *p*H 8 and 5. It seems especially relevant to remark that such a drop in affinity, common to fish haemoglobins, can be largely attributed to a great increase in the dissociation velocity constant of the ligand[8], which in itself is an indication of a destabilisation of the ligand–protein complex. Second, the fact that *p*H-dependent ultraviolet difference spectra of Hb trout IV saturated with CO have been observed (M. F. Perutz, personal communication and unpublished data from this laboratory). These difference spectra show a characteristic "fine structure", centred between 280 and 290 nm, which in the case of human haemoglobin has been directly correlated with the ligand-induced change in the quaternary structure of haemoglobin, and thus should reflect the allosteric conformational transition[9].

By correlating this information, it seems reasonable to propose that the spectral changes we have reported are themselves an indication of the *p*H-induced structural transition which, according to the simple model outlined above, is expected to occur in liganded Hb trout IV. This being the case, we may be provided with a method to test possible correlations between the spin state of the molecule and its quaternary structure, and to follow the dynamics of the allosteric transition in Hb trout IV. Experiments along these lines are in progress.

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1 Brunori, M. in *Current topics in cellular regulation*, 9 (edit. by Horecker, B. L., and Stadman, E. R.), 1–39 (Academic, New York and London, 1975).
2 Binotti, I., *et al.*, *Archs Biochem. Biophys.*, **142**, 274–280 (1971).
3 Brunori, M., *et al.*, *Eur. J. Biochem.*, **39**, 563–570 (1973).
4 Brunori, M., Falcioni, G., and Rotilio, G., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2470–2472 (1974).
5 Brunori, M., Falcioni, G., Fortuna, G., and Giardina, B., *Archs Biochem. Biophys.*, **168**, 512–519 (1975).
6 Gray, R. D., and Gibson, Q. H., *J. biol. Chem.* **246**, 7168–7174 (1971).
7 Barra, D., Bossa, F. Bonaventura, J., and Brunori, M., *FEBS Lett.*, **35**, 151–154 (1973).
8 Giardina, B., Brunori, M., Binotti, I., Giovenco, S., and Antonini, E., *Eur. J. Biochem.*, **39**, 571–579 (1973).
9 Perutz, M. F., Ladner, J. E., Simon, S. R., and Ho, C., *Biochemistry*, **13**, 2163–2173 (1974).

Corrigendum

In the article "Domoic and quisqualic acids as potent amino acid excitants of frog and rat spinal neurones" by T. J. Biscoe, R. H. Evans, P. M. Headley, M. Martin and J. C. Watkins (*Nature*, **255**, 166–167; 1975) the structural formula given for quisqualic acid in Fig. 1*d* and Table 2 is incorrect. The structure proposed by Takemoto *et al.* (Takemoto, T., Nakajima, T., Arihara, S., and Koike, K., *Yakagaku Zasshi*, **95**, 326–322 (1975) and **95**, 448–452 (1975) is reproduced below.

In paragraph 2, line 11, the word isoxazolidinedione should read dioxo-oxadiazolidine.