Clearly, pH (50 per cent) cannot equal pK_L except in very special circumstances. If other interactions of, for example, Van der Waals or electrostatic nature are involved in the binding, the relationship of pH (50 per cent) to pK_L becomes even more complicated. Thus measurements of this kind cannot lead to values of pK_L unless the other factors are known.

To interpret potentiometric studies of horse carboxyhæmoglobin[2], seven different pK values were necessary, representing acid groups, histidine groups and lysine, tyrosine or arginine groups, but the authors did not suggest which of these groups were linked to hæm. Wyman[3] has presented evidence that the bonding groups in horse hæmoglobin are imidazole portions of histidine as suggested originally by Küster[4] and more explicitly by Conant[5]. Carboxyl and sulphydryl groups also have been suggested as the bonding group from globin, and some evidence for and against these, and histidine, has been summarized by Haurowitz[6]. For reasons discussed above, O'Hagan's results throw no light on the nature of the bonding group.

If there are changes in the degree of aggregation of any of the reacting species, it is even less justifiable to identify pH (50 per cent) with pK_L. It is true that pH (50 per cent) will equal pK_L if the experimentally unrealistic assumption is made that complex formation between hæm and globin is so slight that the hæm-globin complex has a stability constant approaching zero ; in other words, that the hæm is not bound to the protein.

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THE equation deduced by Drs. Falk, Phillips and Perrin would seem applicable only when interaction between the 'metal' and ligand would be high, for example, in the hæm compounds which have reacted with oxygen or carbon monoxide. If the interaction were low, as it seems to be in the hæmatin compounds on which my experiments were performed, where dissociations appear to be simple (for example, in hydroxide formation) and the linkages 'essentially ionic', then the second and third terms on the right-hand side of the equation could be discarded. My interpretation assumed this, and that removal of the insoluble hæmatin, with polymerization and loss of absorbance, 'indicated' the dissociation of the bonding group of the protein. It was also assumed that over the range of hydrogen ion concentration studied, contributions to the absorbance from linkages to the hæmatin propionate side-chains would be low. The latter opinion now appears confirmed, since the dissociation of ferriætiomyoglobin (prepared from ætiohæmin, with no carboxyl groups) apparently follows the theoretical curve for an acid group with $pK = 4 \cdot 95$ (at 21°, $\mu = 0 \cdot 05$)[1].

In discussing the potentiometric studies on horse carboxyhæmoglobin, Cohn, Green and Blanchard[2] listed four carboxyl groups under a Kp'_3 value of 4·8. They stated : 'The total curve fits better if a small number of groups—possibly representing four of the groups of hæmatin—is assumed to dissociate in the neighbourhood of pH 4·9'. This statement is perhaps ambiguous, but the 'small number of groups' apparently refers to the groups of the protein attached to the four hæmatins, rather than to four of the eight carboxyl groups of the hæmatins, since these would be expected to dissociate with $pK \sim 5 \cdot 7$ (ref. 3). Haurowitz[4], on completely different evidence, does not consider the hæm iron to be bound to imidazole residues, but that hydroxyl or carboxyl group binding is more likely. A group of more negative character than imidazole has previously been considered in the case of myoglobin[5], and it has perhaps been forgotten that German and Wyman[6] considered the groups might be 'either imidazole groups of histidine, or the second carboxyl groups of dicarboxylic acids'.

It has been shown elsewhere[1] that rather than those imidazole side-chains of horse oxyhæmoglobin ionizing at neutrality being co-ordinated with the iron atom, their electrostatic linkage to the propionate side-chains of the oxygenated hæms is more likely. Consideration of the curve for the apparent heat of dissociation of horse oxyhæmoglobin[7] shows that in the region about pH 5, where more than half the ligand groups of the protein are still linked to the hæm iron, the groups dissociating have $\Delta H_{app.} \sim 0$, which is very suggestive of carboxyle groups. The hæmoglobin shows little loss of capacity to combine reversibly with oxygen down to pH 4·5 (ref. 8), so that linkage of the hæm iron to carboxyl rather than imidazole groups is indicated.

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Multiple Hæmoglobins in Fish

THOUGH there have been reports by different workers[1,2,3] about the concentration of hæmoglobin in different species of fish, no information seems to be available in the literature about hæmoglobin patterns of fish. This communication deals with the findings on the blood samples of 10 different species of fresh-water fish : *Catla catla*, *Labeo rohito*, *Cirrhina mrigala*, *Labeo calbasu*, and *Labeo bata* (family Cyprinidæ), *Ophicephalus punctatus* Bloch and *Ophicephalus striatus* Bloch (family Ophicephalidæ), *Heteropneustes fossilis* (Bloch) (family Heteropneustidæ), *Clarias batrachus* (Linn) (family Claridæ) and *Tilapia mosambica* (family Cichlidæ). After this note had been prepared, a report[4] on the occurrence of multiple hæmoglobins in some species of fish has appeared.

Blood samples were obtained direct from the heart