A Factor Which Regulates Bohr Effect in Poikiloterms Animals and Man

The aim of the present research is to show that there is in various groups of vertebrates a factor which specifically regulates the Bohr effect, different from the well-known system based on organic phosphates[1], and that it has an adaptive value. Several studies[2–6] show the existence of a new effector(s) of the haemoglobin (Hb) molecule; in research carried out by us on the Peruvian Indians[2] and on the Nepalese Sherpas[6], and in the work of LENFANT[4], there is a strong suggestion that the effector(s) has an adaptive value.

Material and methods. In the present research Goldfishes (*Carassius auratus*) and Newts (*Triton cristatus*) were used as experimental animals. In these 2 species we studied the oxygen dissociation curves of the haemolysates of animals raised at different temperatures for a minimum of 1 month. Oxygen dissociation curves were determined at different pH's and temperatures in 0.1 *M* phosphate buffer with a spectrophotometric method as previously described[7,8].

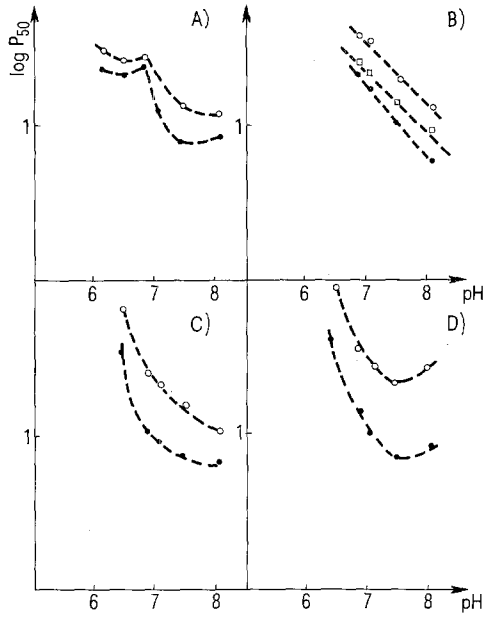

Fig. 1. Oxygen Bohr effect for Goldfishes haemolysates: a) after acclimatization to 4 °C; b) after acclimatization to 21 °C; c) after acclimatization to 37 °C. In all cases acclimatization lasted 1 month. d) after filtration through Sephadex G25 Phosphate buffer 0.1 *M*. ○, determined at 37 °C; □, At 21 °C; ●, At 4 °C.

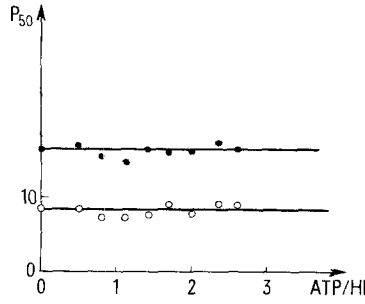

Fig. 2. Effect of ATP on the P_{50} (partial pressure of oxygen required for half-saturation of haemoglobin) on haemolysates of Goldfishes filtered through Sephadex G 25. Phosphate buffer 0.1 *M*, Temperature 4 °C. ○ pH 7.4 ●, pH 6.8.

The haemolysates of the fishes and newts were examined by electrophoresis in starch gel at pH 8.5 according to GOLDBERG[9]; 24 Goldfishes, in 3 groups, raised respectively at 4°, 21° and 37 °C showed a single Hb band. 5 haemolysates of fishes raised at 21 °C were also examined by electrophoresis in polyacrilamide gel with a discontinuous buffer system according to ORNSTEIN[10] and DAVIS[11] with the same result. In the Newts a genetic polimorphism of the Hb[12] is present; only the Newts with a single 'slow' band were used. All the haemolysates were tested at the beginning and at the end of the experiment for the presence of methaemoglobin: the data refer only to haemolysates in which no appreciable amount of methaemoglobin was detected. ATP concentration has been measured with the enzimatic test of the Biochemia.

Results and discussion. Figure 1 (a, b, c) shows the variation of the oxygen affinity of the haemolysates of Goldfishes depending on the temperature of acclimatisation of the fishes. The data are expressed as variation of $log P_{50}$ (partial pressure of oxygen required for half saturation of Hb) at different pH's and temperatures. The Figure 1(d) shows the same parameters on haemolysates filtered through a column of Sephadex G 25 (stripped haemoglobin). The oxygen affinity of the stripped haemoglobin is independent of the temperature of acclimatisation. The changes produced by acclimatisation in the Newts were already reported[7] and lead to the same conclusion, i.e. that following acclimatisation a changement of the oxygen affinity curves occurs without a changement of the Hb molecule. Because all the oxygen affinity curves were determined in 0.1 *M* phosphate buffer, it is a priori unlikely that the effector of the Hb molecule is an organic phosphate. Actually organic phosphates at least on human Hb, are practically ineffective in Hb solutions with high ionic strength[13]. We added however increasing concentrations, of ATP to stripped haemoglobin of Goldfishes. The results are shown in Figure 2. It is evident that ATP in physiological range is completely ineffective. In Goldfishes the ATP/Hb ratio is between 1 and 2.3 (Table). The situation is slightly different in the Newts, because in the haemolysates of these animals ATP at high concentration is still effective in 0.1 *M* buffer. The ATP/Hb ratio, which is necessary to have a nul Bohr effect, is around 3. However,

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No. of animals	ATP/Hb	Temperature of acclimatisation (°C)	Acclimatisation time
Goldfishes			
4	1.536 ± 0.153[a]	21	—
3	1.663 ± 0.016	4	96 h
2	1.547 ± 0.039	4	168 h
2	2.035 ± 0.275	4	216 h
3	1.346 ± 0.043	4	2 months
2	1.630 ± 0.007	34	20 h
2	1.326 ± 0.076	34	96 h
3	1.230 ± 0.180	37	2 months
Newts			
4	0.53 ± 0.09	21	1 month
6	0.53 ± 0.08	4	1 month
5 pooled	0.38	21	2 months
12 pooled	0.16	4	2 months

[a] standard error of the mean

also in this case, ATP is not responsible for the phenomena of adaptation, because the ATP/Hb ratio does not exceed 0.5 (Table) Both in fishes and in the urodeles, ATP is the main organic phosphate in the blood[14]. The hypothesis that the adaptation phenomena observed are caused by organic phosphates is therefore ruled out. This does not means that organic phosphates do not play any role in the adaptation of these animals. In 0.01 *M* phosphate buffer at pH 6.8 stripped haemoglobin of Goldfishes is sensitive to ATP.

The experiments reported unequivocally lead to the conclusion that both in the Goldfishes and in the Newts a new factor(s) exists which is capable of modifying the oxygen affinity of the Hb. We have therefore tested whether this factor is species specific or not. Haemolysates of Goldfishes and of Newts were made free of Hb through Amicon XM 50 filters or precitating the proteins with percloric acid. The Hb free haemolysates were then added to the stripped Hb of Newts in 0.1 *M* phosphate buffer and the addition of the filtrate produced the inversion of the Bohr effect at 4 °C. Bohr effect on stripped Hb is negative in Newts[7]. The same addition to stripped Hb of Goldfishes produces the effect shown in Figure 3, shifting towards the right the curve at the acidic pH. In both cases the change in the oxygen affinity simulate that produced by the acclimatisation. The same type of experiment was repeated utilizing Human stripped Hb and Hb-free haemolysates of fishes. ATP was previously added to the human haemolysates to have an ATP/Hb ratio 10/1 to be sure that the effects cannot be due to organic phosphates possibly added with the precipitate. Results are reported in Figure 4. The filtrate of fish shifts toward the right the curve at the

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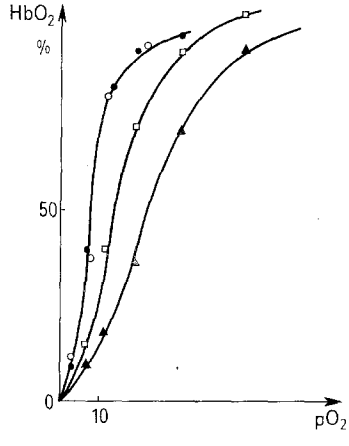

Fig. 3. The effect of Newts haemolysates filtered through Amicon XM 50 filters on the oxygen dissociation curves of Goldfishes haemolysates filtered through Sephadex G25. Phosphate buffer 0.1 M, Temperature 4 °C. ○ □ Goldfishes haemolysates filtered through Sephadex G25, pH 7.4 and pH 6.8; ● ▲ Same conditions plus Hb-free heamolysates of Newts, pH 7.4 and pH 6.8.

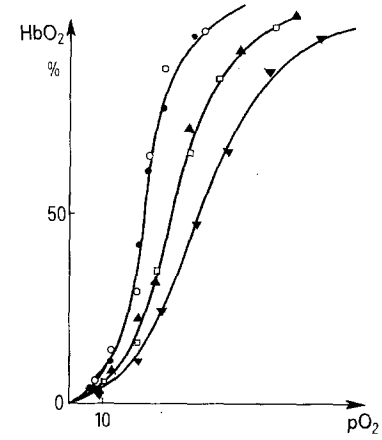

Fig. 4. The effect of Hb-free haemolysates of Goldfishes filtered through Amicon XM 50 filters on the oxygen dissociation curves of human haemolysates filtered through Sephadex G25. Phosphate buffer 0.1 *M*, Temperature 25 °C. ● ▲ Human haemolysates filtered through Sephadex G25, pH 7.4 and pH 6.8; ○ □ Same conditions with added ATP (ratio ATP/Hb 10:1), pH 7.4 and pH 6.8; ▲ Same conditions plus Hb-free haemolysates of Goldfishes, pH 6.8.

acidic pH, while the curve at the alkaline pH remains unaltered. This is exactly what happens in the haemolysates of Peruvian Indians[2] and in diabetic patients[3]. This fact strongly supports the idea that the factor(s) present in the haemolysates of the Peruvian Indians and in diabetic patients is the same or similar to that effective in producing the acclimatisation in cold-blooded animals.

The enhancement in the Bohr effect should increase the release of the oxygen to the tissues; at least in poikiloterms animals this factor should be of primary adaptive value.

Riassunto. In emolizzati di Tritone (*Triton cristatus*) e di pesce rosso (*Carassius auratus*) si è dimostrata la presenza di un fattore differente dai fosfati organici che influenza l'effetto Bohr modificando l'affinità dell'Hb per l'ossigeno a pH acido. Il fattore è specie aspecifico e influenza anche l'effetto Bohr della Hb umana.

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Studies on the Quaternary Structure of the First Enzyme for Histidine Biosynthesis

The results of several studies suggest that the feedback sensitive first enzyme of a biosynthetic operon is involved in repression[1–5]. In *Salmonella typhimurium* the involvement of the first enzyme for histidine biosynthesis (G enzyme)[6], in regulation of the operon[2,4] is well documented. Genetic studies in the *his* system of *E. coli* K12 by Garrick-Silversmith and Hartman[7] and Goldschmidt et al.[8] show the great similarity between the 2 operons of these 2 organisms.

Studies with *E. coli* have been carried out in the present work with the aim of dealing with a slightly different system to which the findings of Ames, Hartman and Goldberger[9] in *Salmonella* could be applicable. We have purified the G enzyme from *E. coli* K12 and report here the results of Sephadex G-200 gel filtration experiments of association-dissociation with substrates and ligands, correlating the quaternary structure of the enzyme with its regulatory role in the *his* operon.

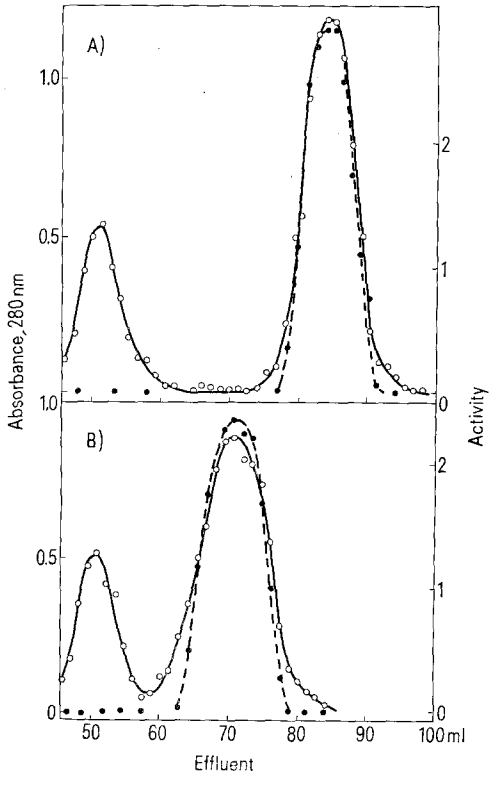

Fig. 1. Elution patterns in the absence of histidine (1A) and in the presence of 0.4 m*M* histidine (1B). (---○---, A_{280}; ---●---, specific activity in arbitrary units). The standard proteins were: Cytochrome C, mol. wt. 13500; Chymotrypsinogen A, 25000; Ovalbumin, 45000; Bovine serum albumin, 67000; Aldolase, 147000; Catalase, 240000; and Ferritin, 540000.

Materials and methods. The source of the enzyme was a regulatory mutant, OA111, 30-fold derepressed, obtained by ethyl methanesulfonate mutagenesis[10] in strain JC5459 (F^- trp$^-$ thi$^-$ lac 74 str^r, from Clark's collection[11]), and selected by its resistance to triazolalanine[12]. The mutant was grown with aeration in a New Brunswick gyrotory shaker at 37 °C, and the pelleted cells were kept frozen.

The purification procedure, following the method of Whitfield[13], is based on the solubility changes in $(NH_4)_2SO_4$ of the enzyme in presence or absence of histidine[14]. Using acid precipitations (S. M. Parsons, personal communication), about 90% of the enzyme precipitated with 1 m*M* histidine at pH 5.1. The enzyme was purified

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