The effect of phosphate and folic acid on cream xanthine oxidase. 0·1 μM xanthine, 0·005 μM folic acid (FA), xanthine oxidase (Diluted 1:100 in Tris), as indicated. For dye reduction, 0·1 ml of 2,6-Dichlorophenol indophenol (30 mg/100 ml). Completed to 3·0 ml with 0·05 M Tris, pH 7·8, or with 0·1 M phosphate, pH 7·8. Values correspond to the change in optical density ($\times 1000$) at 298 mμ—in assays # 6 and 7 change in optical density at 600 mμ—for the first 10 min after the addition of substrate. All reactants, except xanthine, were preincubated for 5 min at 24°C. Assay temperature: 24°C. All values are averages of three determinations.

Assay #	Enzyme	Tris		Phosphate	
		−FA	+FA	−FA	+FA
1	0·5 ml fresh	258	154	258	191
2	0·5 ml aged	203	86	244	139
3	0·5 ml aged	168	44	222	128
4	0·3 ml fresh	207	106	237	126
5	0·3 ml fresh	160	79	190	99
6	0·5 ml fresh	201	175	140	126
7	0·3 ml fresh	150	129	103	94

Several other instances of the effect of orthophosphate on metalloflavo-proteins have been reported. MACKLER *et al.*[3] stated phosphate to be required for reduction of one-electron acceptors by cream xanthine oxidase. DPNH-peroxidase is inhibited by phosphate[4]; this is also true for DPNH-cytochrome c reductase[5]. MAHLER *et al.*[5] attribute this inhibition to a 'coordination of phosphate with the iron of the enzyme during the catalytical process'.

The present data indicate that the processes of loss of enzymic activity due to aging or mild heating, the inhibition by folic acid, and the effect of orthophosphate, are all concerned with the same site of cream xanthine oxidase; it is likely, that the site affected is the molydenum or iron, or both metals. The data indicate further, that the level of orthophosphate determines, which pathway of electron transport is favored for xanthine oxidase activity in a given system. Phosphate shunts enzymic activity away from the 'dye pathway' in fresh enzyme. The aging process can be interpreted as a gradual removal of endogenous phosphate from an active site, an effect which can be partially reversed by the addition of orthophosphate.

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R. FRIED

Research Division, Veterans Administration Hospital, Hines (Illinois), and Department of Biochemistry, Northwestern University Medical School, Chicago (Illinois), January 9, 1958.

Zusammenfassung

Die Einwirkung von Orthophosphat auf die Milch-Xanthinoxydase wurde unter verschiedenen experimentellen Bedingungen untersucht. Phosphat hemmt einerseits die Übertragung des mobilisierten Wasserstoffes auf 2,6-Dichlorophenol-indophenol mit frischen Enzym-Präparaten und aktiviert andrerseits die Akzeptorfunktion des Sauerstoffes bei gealterten, oder anderweitig inaktivierten Präparaten.

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Heme-Heme Interaction in the Oxygen Equilibrium of Reconstituted Hemoglobins[1]

Object of this note is to demonstrate effective heme-heme interaction in the O_2 equilibrium of reconstituted hemoglobin (Hb) and to investigate the role played in the interaction by the vinyl side chains of the porphyrin.

Apohemoglobin was prepared by acid acetone splitting of human Hb, as reported elsewhere[2]. Protohemin IX was furnished by BDH; crystalline deuterohemin IX and mesohemin IX were prepared according to FISCHER[3]. The recombination of hemes with the globin and other physicochemical properties of reconstituted Hbs will be described elsewhere. The O_2 dissociation curves were determined by the spectrophotometric method developed by us[4]. The O_2 capacity of reconstituted ferro-Hbs is the same as that of native Hb.

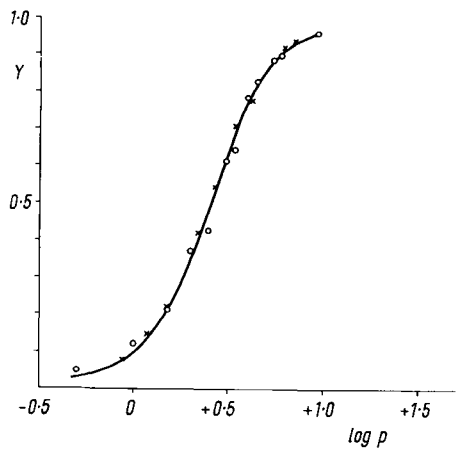

Fig. 1.—Oxygen equilibrium of reconstituted = o and native = × protoHb (2×10^{-4} M as Fe). 20°C; Tris (trishydroxymethyl-aminomethane) buffer 0·1 M, pH 7·4. Y = fractional saturation with oxygen. log p = log oxygen pressure (at 20°) in mm Hg.

Reconstituted protoHb. It can be seen from Figure 1 that the O_2 dissociation curves of native and reconstituted Hb are almost identical to each other with n = 2·6–2·7 (in the Hill empirical equation).

In the plot of Y versus log p the curve obtained for the two proteins appears to be symmetrical. In reconstituted Hb (as in native Hb) there is a slight decrease of interaction in the absence of salts (in phosphate buffer 0·016 M, pH 7·6 n = 2·2). For both proteins we found with urea and para-chloromercurybenzoate respectively n = 1·7 and 1·5.

Reconstituted deuteroHb. The O_2 dissociation curve of deuteroHb (Fig. 2) is still sigmoid in the shape but it is much less inflected than that of protoHb, with n = 1·7 (instead of 2·7 as in reconstituted protoHb). In different conditions of temperature, pH and concentration of the pigment, the value of n was found ranging between 1·5–1·9 (most frequent value 1·7). The salt effect on the shape of the curve is analogue to that observed for protoHb (in phosphate buffer 0·016 M pH 7·6 n=1·4). The

[1] Aided by a grant of the Rockefeller Foundation.

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addition of reduced glutathione does not modify the shape of the curve in deuteroHb.

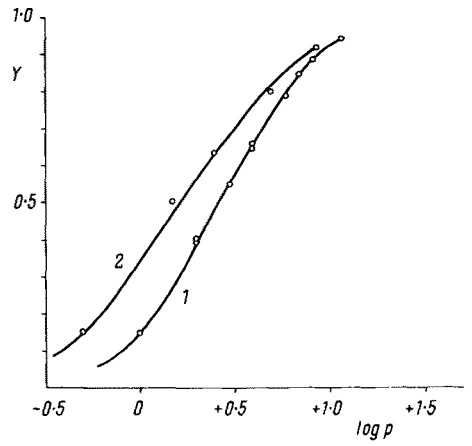

Fig. 2.–Oxygen equilibrium of reconstituted deutero Hb (2×10^{-4} *M* as Fe) curve 1 and meso Hb (1×10^{-4} *M* as Fe curve 2). 30°C; Tris buffer 0·1 *M*, pH 7·7.

Reconstituted mesoHb. The results obtained with meso-Hb (Fig. 2) are similar to those obtained with deuteroHb. For the curve reported in the Figure, the value of n in the Hill equation is 1·5.

The results reported above demonstrate that reconstituted protoHb shows the same heme-heme interaction as the native pigment. The behaviour of deutero- and meso-Hb points out the role of the vinyl groups of the porphyrin in heme-heme interaction.

The oxygen equilibrium of the reconstituted Hbs in different conditions of temperature, pH, etc. and the discussion of these results will be reported *in extenso* in another publication.

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Institute of Biological Chemistry, University of Rome (Italy), January 21, 1958.

Riassunto

È stato dimostrato che nella protoemoglobina ricostituita l'interazione fra gli emi è identica a quella della emoglobina nativa mentre è fortemente diminuita nella deutero e mesoemoglobina.

Electrophoresis of the Venom and Haemolymph of the Scorpion *Buthus Judaicus* E. S.

During work on the toxicity of the black scorpion which occurs commonly in the Mediterranean regions of Israel, a study of the protein components of its venom was undertaken. Specimens of the venom were obtained by electric stimulation of laboratory-kept scorpions. The first clear drop was collected apart from the remainder of the secreted venom. The venom parts, denoted *A* and *B* respectively, were delivered into small quantities of distilled water and stored at 4°C until freeze drying. The total protein of the fresh venom as estimated by biuret reaction was 8·7 g/100 ml and amounted to about 50% of the dried venom. No marked difference in the protein content was observed between parts *A* and *B* of the venom. Electrophoretic separation at pH 8·6 revealed that the venom is composed of at least 6 protein fractions (Fig. 1 and 2), three of them of cathodic mobility. The relative concentrations of the protein fractions varied somewhat with different venom pools, the anodic fractions A_1 and A_2, as well as the cathodic fraction K_1, being affected in particular. These variations seemed to be caused by differences in composition of parts *A* and *B* of the venom. On the other hand, the protein pattern obtained after direct application of the venom from scorpion's telson to the filter paper was essentially the same as with freeze dried venom. No lipoproteins were detected on venom electropherograms stained with Oil Red 0, while a feeble spot reflecting protein-bound carbohydrate appeared at the site of the K_2 fraction after periodic acid-Schiff staining[1].

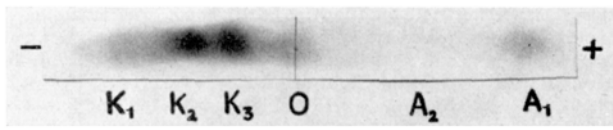

Fig. 1.–Paper electrophoretic separation of pooled *Buthus judaicus* venom. The venom was separated on Whatman 3 MM filter paper in the apparatus of Köiw, Wallenius, and Grönwall[2], in barbital buffer pH 8·6 and ionic strength 0·05. The strips were stained with Amido Black and scanned in an automatic photoelectric recorder (Spinco Analytrol). The relative concentrations of the fractions were: A_1: 11·0%, A_2: 9·9%, *O*: 21·1%, K_3: 19·8%, K_2: 23·1%, and K_1: 15·1%.

The venom was also separated preparatively by electrophoresis on filter paper. Preliminary assays of the eluted fractions on mice indicated that most of the toxicity resided in the cathodic fractions of the venom.

Fig. 2.–Descending boundary pattern of pooled *Buthus judaicus* venom analysed in Perkin-Elmer electrophoresis apparatus at total protein concentration of about 1%, barbital buffer pH 8·6, ionic strength 0·1 and 1°C. The venom was dialyzed for 24 h prior to analysis. The mobilities of the five fractions from left to right, are in $cm^2 \cdot V^{-1}s^{-1} \times 10^{-5}$: A_1: –3·36, A_2: –1·57, K_3: +0·58, K_2: +1·9 and K_1: +2·2. The arrow indicates the position of the boundary at start. The non-moving 0 fraction encountered in paper electropherogram was not discernible in the moving boundary pattern. The distended peaks of the cathodic fractions indicate their heterogeneity.

The protein pattern of *Buthus judaicus* haemolymph as shown in Figure 3 does not reveal any similarity with that of the corresponding venom. It is interesting that the albumin-like main protein fraction of the haemolymph comprised at least 3 components. On paper electropherogram, this fraction contained also 46% of haemolymph glycoprotein (total concentration 170 mg/100 ml[3]). Only traces of lipoprotein staining material were detected at the origin of haemolymph electropherogram.

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