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A full account of the work which also comprises a study of the minute innervation of the hair cells and other cytological characteristics will be published elsewhere.

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## HÆMATOLOGY

### Decrease in Acid Strength of Propionate Side-chains of Hæmatin on Reduction to Hæm

THAT the hæm carboxyl groups might participate in reactions of hæmoproteins has received little attention, apparently because they were considered<sup>1,2</sup> to be too far away to be affected by addition of ligands to the iron atom. It was suggested<sup>3</sup> that the acid strength of hæm propionate side-chains might change, on the basis that substitution of *R* in  $RCH_2CH_2COOH$  has a significant effect on the *pK* value<sup>4</sup> and that any alterations in the high state of resonance of the porphyrin ring system was likely to have the same effect as substitution of *R*. Further, since side-chain variation has a marked influence on the oxidation-reduction potential of the iron of hæmochromes<sup>5</sup>, it is, conversely, not unreasonable to expect that a change in electronic structure of the iron atom could effect the acid strength of the propionates.

Experiments indicating that a change in acid strength of at least one of the propionates does occur on reduction have now been carried out. Hæmatin and hæm combine with native human serum albumin through their propionate groups, not through their iron atoms<sup>5</sup>. The extent of combination may be studied by measuring the absorbance increment (at the Soret peaks of the compounds formed) after equilibrating the metalloporphyrin and protein in buffer solutions, separately and together, at 21° for 3-4 hr. (Fig. 1). The increment represents  $A_{ha} - A_h - A_a$ , where  $A$ =absorbance,  $h$ =hæmatin or hæm,  $a$ =albumin.

The decreased absorbance increment of the ferrohæmalbumin occurs whether the reducing agent (dithionite) is added initially or after the ferrihæmalbumin has formed. No appreciable amount of verdohæm-type compounds was detected, except, as expected, when attempts were made to reoxidize the ferrohæmalbumin solutions.

That the effect resulted from reduction of the hæmatin and was not due to either interference by the reducing agent itself or to its effect on the albumin

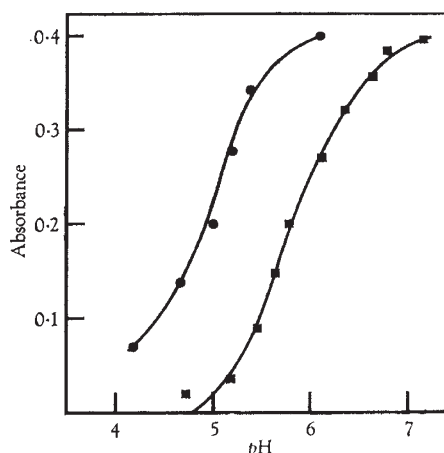


Fig. 1. Absorption increment curves indicating extent of attachment of hæmatin and hæm to human serum albumin. ●, hæmatin; +, albumin, measured at 404  $m\mu$ ; ■, hæm+albumin measured at 414  $m\mu$ . (Hæmatin and hæm,  $2.86 \times 10^{-4} M$ ; albumin 2.5 per cent; 10 mm. cuvette. Buffers, pH 4.2-6.2 phthalate, 6.2-7.2 phosphate;  $I=0.05$ ;  $T=21^\circ$ ; time for equilibrium, 3 hr.)

was demonstrated by using nickel mesoporphyrin (unaffected by dithionite under these experimental conditions) with the albumin. On adding reducing agent, no significant change in attachment occurred.

Hæmatin couples to caffeine in the neutral pH range<sup>6</sup> and the extent of this attachment is also markedly decreased (by about 75 per cent at pH 7.0) on reduction to hæm. Linkages of this type are most likely to involve the propionate groups. Theophylline, having an unsubstituted imino group, is comparable with the imidazole side-chain as it would be expected to occur in proteins. In a preliminary single series of experiments, theophylline produced increments in the hæmatin Soret peak, but on reduction no absorbance increment was detected. These experiments indicate that the hæm propionate groups may play a very important part in the reactions of hæmatin compounds.

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## PATHOLOGY

### Loss of Plasticity of Erythrocytes coated with Incomplete Antibodies

TETTEL and Rădulescu were the first to describe in 1952 a method for evaluating the plasticity of red blood cells from their ability to pass through filter paper. Here we have used a modification of this method.

2 ml. of washed erythrocytes are placed in a funnel lined with filter paper (Schleicher Schül Nr. 589 'Schwartzband'), previously wetted with saline. The red cells although much greater in diameter than the pores in the paper do pass through<sup>1</sup>, due to their