Formation of Haematins and Haemochromogens

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In the course of studies on methods of estimating haemoglobin, observations were made which seemed to throw some new light on current theories of the formation of haematins and haemochromogens from haemoglobin and haemin.

METHODS

Preparation of purified haemin and haemoglobin (see Delory, 1943). Determination of the optical density of the various haematins and haemochromogens. An amount of haemin, or of haemoglobin (or blood), was dissolved in 0.1N-NaOH so as to give a solution containing about 0.45 mg. Fe/100 ml. 5 ml. of the solution were pipetted into each of two test-tubes. To the first were added 5 ml. of 40% pyridine (v/v) and to the other 5 ml. of 0.1N-NaOH. The colours were read either in the King (1942) photoelectric colorimeter with a Chance green filter or in a Leitz colorimeter with a Hg-green filter. A small knife-point of sodium hydrosulphite was added to each tube, and after careful mixing the resulting colours were re-read in the colorimeter. The optical densities of the pigments were divided by the mg. Fe/100 ml. solution to give the specific extinction coefficients for a cell of 1 cm. thickness. The iron figure used was either the known iron content of the haemin or haemoglobin, or in the case of blood was calculated from the determined extinction coefficient relative to alkaline haematin (Clegg & King, 1942).

For experiments with diethylamine, haemin or haemoglobin was dissolved in a 20% solution (v/v) of the organic base. As before it was arranged for the solution to contain about 0.45 mg. Fe/100 ml.

Spectroscopic examination. A Beck-Hartridge reversion spectroscope was used. The calibrations of the instrument were checked with a neon lamp. For each experiment twelve readings were taken and the average recorded.

RESULTS

Table 1 shows the colour intensities of haemoglobin and haemin in 0.1N-NaOH. It will be seen that, iron for iron, haemoglobin gives over 30% more colour than haemin (cf. Clegg & King, 1942). The absorption curves (Fig. 1), for which we are indebted to Mr S. Y. Thompson of Reading, show that there is also a qualitative difference in colour.

The findings with diethylamine are given in Table 2. Again there is a difference between the corresponding haemin and haemoglobin compounds. Table 3 gives the colour intensities of globin haemochromogen, pyridine haemochromogen and 'blood

Table 1. Comparison of the colours produced by haemoglobin and by haemin dissolved in NaOH, relative to their iron contents

(Extinction coefficients, E (Leitz colorimeter, Hg 5467), for 1 mg. haemoglobin-Fe, and 1 mg. haemin-Fe/100 ml. Mean values are given for 13 specimens of haemoglobin and 13 specimens of haemin.)

E for	
Haemoglobin in 0.1N-NaOH (i.e. globin haematin)	Crystalline haemin in 0.1N-NaOH ⁻ (i.e. haematin)
0.996 $\pm$ 0.025	0.769 $\pm$ 0.009

Fig. 1. Spectrophotometric comparison of alkaline haematins from haemoglobin, A, and haemin, B. Photoelectric spectrophotometer; spectral impurity 10 m μ .

in pyridine' haemochromogen. Whilst the extinction coefficients for globin and pyridine haemochromogens are almost equal, that of 'blood in pyridine' haemochromogen is perceptibly higher. Table 4 shows similar results obtained in other experiments and with a different light filter (Chance yellow-green). It includes the specific extinction coefficients of globin and pyridine haematins and haemochromogens and the positions of the absorption bands of the haemochromogens. The colour intensities of the globin haemochromogens and the pyridine haemochromogen from pyridine and haemin are almost equal, but the pyridine haemochromogens from blood and haemoglobin show higher extinction coefficients. Reference to the spectroscopic readings

Table 2. *Colour intensities of haemoglobin and haemin in diethylamine*

(Extinction coefficients, E (photoelectric colorimeter, Chance green filter) for 1 mg. haemoglobin-Fe or 1 mg. haemin-Fe/100 ml. Mean values are given for 12 specimens of haemoglobin and 16 specimens of haemin.)

E for	
Haemoglobin in 20 % diethylamine (i.e. diethylamine + globin haematin)	Crystalline haemin in 20 % diethylamine (i.e. diethylamine haematin)
0.910 $\pm$ 0.018	0.864 $\pm$ 0.016

Table 3. *Colour intensities of globin haemochromogen, pyridine haemochromogen, and 'blood in pyridine' haemochromogen*

(Extinction coefficients, E (photoelectric colorimeter, Chance green) for 1 mg. of haemochromogen-Fe/100 ml.)

E for		
Globin haemochromogen (blood in 0.1N-NaOH + Na ₂ S ₂ O ₄) (mean of readings for 28 specimens)	Pyridine haemochromogen (haemin in pyridine + Na ₂ S ₂ O ₄) (mean of readings for 10 specimens)	'Blood in pyridine' haemochromogen (blood in pyridine + Na ₂ S ₂ O ₄) (mean of readings for 23 specimens)
1.48 $\pm$ 0.09	1.47 $\pm$ 0.03	1.59 $\pm$ 0.09

Table 4. *Colour intensities of haematins and haemochromogens, and spectral bands of the haemochromogens*

	E^* for				λ for			
	Alkaline haematins	Globin haemochromogens	Pyridine haematins	Pyridine haemochromogens	α Band of globin haemochromogens	β Band of globin haemochromogens	α Band of pyridine haemochromogens	β Band of pyridine haemochromogens
Haemin	0.77	—	0.84	1.04	—	—	5548	5230
Capillary blood	0.97	1.04	1.09	1.22	5576	5266	5557	5243
Purified haemoglobin	0.97	1.02	1.07	1.20	5564	5250	5550	5231
Washed cells	0.97	1.05	1.07	1.24	5575	5266	5557	5243
Whole oxalated blood	0.97	1.04	1.08	1.23	5576	5266	5557	5243

(Each figure is the average of 12 estimations.)

* E stands for the specific extinction coefficient for 1 mg. pigment-Fe/100 ml. in a cell depth of 1 cm.; photoelectric colorimeter, Chance yellow-green filter.

in this table shows that the bands of the blood pyridine haemochromogen are between those of the globin haemochromogen and the pyridine haemochromogen from haemin. An interesting finding is that the wave-lengths of the α and β bands of the haemochromogens from purified haemoglobins are lower than those of the corresponding blood compounds.

DISCUSSION

Current theories of the formation of haematins and haemochromogens may be represented by those of Keilin (1926) and of Anson & Mirsky (1930). According to Keilin, when haemoglobin is dissolved in

sodium hydroxide solution, free haematin is formed together with free denatured globin. On reduction the conjugated protein haemochromogen is formed, which on reoxidation gives once more haematin and free globin. On Anson & Mirsky's view, however, haemoglobin yields a conjugated protein in sodium hydroxide solution. Drabkin also favours this view (Drabkin & Austin, 1935). He believes that the conjugated protein can exist in either an oxidized (globin haematin or globin ferriprophyrin) or in a reduced (haemochromogen or globin ferroporphyrin) form. The two theories may be represented schematically as follows.

Schemes for haematin and haemochromogen formation

* A conjugated protein existing in either oxidized or reduced form.

If Keilin's view is correct, it would be expected that haemoglobin in sodium hydroxide solution

would give a colour equal both quantitatively and qualitatively to that obtained with haemin of the same iron content. Our results indicate that this is not the case. We therefore conclude that a conjugated protein is formed when haemoglobin is dissolved in NaOH.

With diethylamine we also find a discrepancy between the corresponding haemin and haemoglobin compounds, and it is suggested that the substance obtained from treating haemoglobin with the base is either a mixture of diethylamine haematin and globin haematin, or, more likely, a 'double' haematin in which both diethylamine and globin are conjugated with the haemin. Likewise with pyri-

dine. In both cases, the colour density is greater when haemoglobin is treated with the organic base than when an equivalent amount of haemin is treated with the organic base. The extinction coefficient of the pyridine haemochromogen from haemin is almost equal to that of globin haemochromogen whilst those of the pyridine haemochromogens from haemoglobin and blood are considerably higher. This suggests again that we are dealing with a substance which is a mixed or 'double' globin+pyridine haemochromogen. This is further suggested by the difference in the position of the absorption bands, those of the blood pyridine substance being between those of the other two. Drabkin & Austin (1935) have also noted a difference in the positions of the bands of haemin and haemoglobin pyridine haemochromogens. The difference between the pyridine haematins from haemin and haemoglobin respectively is consistent with the idea of a mixed haematin being obtained with haemoglobin.

Two explanations may account for the fact that the absorption bands of the purified haemoglobin derivative are lower than those of the corresponding blood derivative: (1) the presence of substances other than haemoglobin in blood; (2) degradation of the globin part of the haemoglobin molecule produced during the purification. The first

explanation seems less likely, since the positions of the absorption bands in the case of the blood are independent of its source, identical figures being obtained whether the blood is from normal individuals or anaemic patients. The position of the absorption bands is also identical for whole blood and washed cells.

SUMMARY

1. The colours given by solutions of several haematins and haemochromogens prepared from haemoglobin and from haemin are compared.

2. The results favour the theory that a conjugated protein is formed when blood is dissolved in 0.1N-NaOH, pyridine or diethylamine, with the haematin in the oxidized state.

3. The haematins formed by haemoglobin when treated with diethylamine and with pyridine, and the haemochromogen it forms with pyridine, appear to be different from those formed by haemin, and may be mixed compounds containing globin as well as the organic base.

4. The pyridine haemochromogen from purified haemoglobin differs slightly in the position of its spectral bands from that formed from blood.

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The Relationship between the Weight of Young Vitamin A-deficient Rats and the Increase in Weight after β -Carotene Administration

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Different workers hold different opinions on the correlation between a rat's body weight and its need for vitamin A. Greaves & Schmidt (1936) claim to have shown, on the basis of changes in the vaginal contents, that within the limits of age and body weights of the rats used by them, the vitamin A requirements are independent of these factors. They used cod-liver oil as their source of vitamin A. On the other hand, using the same criterion, but a slightly different dosing technique, Goss & Guilbert (1939) found that the rat's requirement of vitamin A was dependent on its body weight. The tests were carried out with β -carotene as well as cod-liver oil. Irving & Richards (1939) seemed to confirm this by using pathological changes in teeth and other organs

as their criteria. These last workers treated their rats prophylactically, from weaning, with solutions of vitamin A.

It was thought that some further light on this problem might be obtained by examining the weight response of rats deficient in vitamin A to the same daily dose of that factor. If, in fact, the rat's requirement of vitamin A is proportional to its body weight, then the increase in weight in response to a given dose of that vitamin, after the rat's stores have been depleted, should be in inverse proportion to its body weight at that time. For investigating this possibility, the results of vitamin A determinations carried out in this laboratory over many years were available.