Letter to the Editors

LIGHT ABSORPTION SPECTRA OF HEMOGLOBIN OF p-CHLORONITROBENZENE POISONED RABBITS

Previously^{1,2)}, it has been reported that the oxygen affinity of hemoglobin obtained from p-chloronitrobenzene or nitroglycol poisoned rabbits increased markedly and the value of n in Hill's equation, $Y=Kp^n/(1+Kp^n)$: Y ; fractional oxygenation of hemoglobin, p ; oxygen partial pressure, K ; constant, n ; the order of sigmoid curve, decreased from 2.8 of the normal to 1. $n=1$ shows that the heme-heme interaction in one hemoglobin molecule, which is observed in the combining reaction between heme and oxygen, disappears completely and each heme combines with oxygen independently to one another.

Formaline³⁾, p-chloromercuribenzoate³⁾ and urea⁴⁾ have been known as an effective

Fig. 1. Difference spectra of attacked hemoglobin.

Difference spectra were obtained by measuring the difference of optical density, (O. D. of 1 mM attacked oxyhemoglobin) - (O. D. of 1 mM normal oxyhemoglobin).

- 1: 1 mM hemoglobin + 8 mM formaline,
- 2: 1 mM hemoglobin + 1 mM p-chloromercuribenzoate,
- 3: 1 mM hemoglobin + 4.6 M urea.

All experiments were carried out at pH 7.2 and 27~28°C. The concentration of hemoglobin was expressed in terms of that of heme.

weapon for cutting off the heme-heme interaction. By the action of these substances upon the hemoglobin molecule the value of n was decreased. At present, any difference of physicochemical properties between these two species of hemoglobin, that is, normal and the attacked by formaline *et al.*, has not been found, and as a matter of course, the absorption spectra of attacked hemoglobin in the visible region were the same with that of the normal.

But by the following experiments, it was found out that the absorption spectra of attacked hemoglobin differed from the normal. The difference spectrum between 1 mM normal oxyhemoglobin and 1 mM attacked oxyhemoglobin was measured by using a Cary spectrophotometer model 14 and the results were shown in Fig. 1. As seen from the figure, the maximum of the difference spectrum was at 590 m μ for all of the attacked hemoglobin.

As reported previously¹⁾, hemoglobin solution obtained from p-chloronitrobenzene poisoned rabbits contained methemoglobin. When a mM methemoglobin and (1-a) mM oxyhemoglobin were contained in 1 mM total hemoglobin, the difference spectrum represented by the following equation,

$$\begin{aligned} & [(1-a) \text{ mM oxyhemoglobin} + a \text{ mM methemoglobin}] \text{ of poisoned rabbit} \\ & - \{ [(1-a) \text{ mM oxyhemoglobin}] + [a \text{ mM methemoglobin}] \} \text{ of normal rabbit,} \end{aligned}$$

was measured. The results accorded with those in Fig. 1, and the absorption maximum was at 590 m μ .

It may be certain that when hemoglobin molecule is modified by some reagents and the heme-heme interaction is attacked, the light absorption spectrum differs from that of the normal at 590 m μ . Accordingly, it may be possible to presume whether the hemoglobin molecule is attacked or not by measuring the difference spectrum.

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