MECHANISM OF HAEMOLYSIS CAUSED BY VARIOUS SUBSTANCES

I. COMPARISON OF HAEMOLYTIC CURVES BY VARIOUS SUBSTANCES

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For the purpose of understanding the mechanism of haemolysis caused by various substances, the comparison of haemolytic action of various substances was carried out from the relation between the concentrations of substances and the incubation time with red blood cell.

The phenomenon of haemolysis caused by various substances could be divided into two groups. The first group is haemolysis produced by the substances such as surface detergent, alcohols, urea and tauroglycocholate, and the difference between the concentration producing complete haemolysis and that causing scarce haemolysis is very narrow. Haemolysis of the second group shows a very wide range of concentration, and is produced by metals and organometallic compounds. The difference of mechanism of haemolysis caused by those two groups was discussed.

The comparison of haemolytic action by chemical compounds having similar structure was also studied.

The studies of interaction between cell membrane and toxic substance are important in industrial poisoning. The author used the red blood cell as a model of cell membrane and the interaction between the red blood cell and various toxic substances was observed from the haemolytic phenomenon.

In recent years, many toxic substances which have a haemolytic action have been reported. The haemolysis caused by chemical substances occurs ultimately by chemical reaction with biochemical substances of red blood cells. Accordingly, the mechanism of haemolysis may be different with the physicochemical property of each substance, though the mechanism of haemolysis is still obscure.

In the present series of experiments, the author attempted to investigate the haemolytic action of various substances and elucidate the mechanism of haemolysis. This paper reports the comparison of haemolysis by various substances from the relationship between the concentrations of haemolytic substances and the incubation times.
Red blood cells from heparinized rabbit blood by heart puncture were washed thrice in 0.9% NaCl solution.

The substances were made up to a preparative concentration in 0.9% NaCl solution. The red blood cell suspension of 2% (v/v) was prepared with these sample solutions. This red blood cell suspension was incubated at 37°C, and a part of the suspension was centrifuged at 3,000 r.p.m. for 15 minutes every 30 minutes for 4 hours and the color intensity of supernatant solution was measured at 530 mµ. In each experiment, one sample which contained 2% red blood cell in water was prepared and the absorbance of this sample was taken as 100% haemolysis reading. The per cent haemolysis of samples was obtained by dividing the absorbance of the sample by the 100% haemolysis reading, and the haemolytic curves were prepared from these per cent haemolysis.

Haemolytic substances used in this study were urea, sodium tauroglycocholate, alcohols, surface detergents, metals, organomercury compounds, and alkyltin compounds.

RESULTS

Comparison of Haemolysis Caused by Various Substances

The haemolytic curves with different concentrations of butyltin compounds and dodecylbenzensulfonate were described already in the previous reports. The haemolytic curves by several substances used in the present experiment are shown in Fig. 1.

From the observations of the extent of concentrations of substances for a complete haemolysis and for a scarce haemolysis, the type of haemolysis may be divided into two groups, that is, one group showed such a wide extent as from ten to one hundred times and the other group showed a very narrow extent as only several times. The substances which belong to the former are metals and organometallic compounds, and those to the latter are surface detergents, alcohols, urea, and sodium tauroglycocholate. In order to compare the haemolytic action of various substances, the time required for 50% haemolysis in each concentration of the various agents was calculated from the haemolytic curves shown in Fig. 1, and these times were plotted against the above mentioned concentration on log-logistic grid as shown in Fig. 2.

From Fig. 2, it is interesting to know that the lines obtained with various substances were divided into two groups of nearly vertical line and of inclined line. The substances included in the former are the surface detergents (dodecylbenzenesulfonate, trimethyllaurylammonium chloride, trimethylbenzylationmum
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Fig. 1. Haemolytic curves obtained from various compounds.
chloride, and NaN-lauryl sarcosinate, etc.), alcohols (methyl-, ethyl-, butyl-, and octyl-alcohol), urea, and sodium tauroglycocholate, etc. The substances included in the latter are metals (cadmium and arsenic) and organo-metallic compounds (butyltin compounds and ethylmercury phosphate). It seems reasonable to assume that the haemolysis of these two forms is based on the different mechanism.

Comparison of Haemolytic Curves by Chemical Compounds Having Similar Structure

Although the author and Ishii reported previously the haemolytic action of butyltin compounds, it is very interesting to note that haemolytic action of compounds having similar structure is different according to their chemical constitution, especially to the difference of the kind or the number of radical of these compounds. These results are shown in Fig. 2 partially and in Fig. 3.

That is, the intensity of haemolytic action of compounds having similar structure in 6 series decreased in following order:

\[(\text{C}_2\text{H}_5\text{Hg})_3\text{PO}_4 > (\text{C}_2\text{H}_5\text{Hg})_2\text{HPO}_4 > \text{C}_2\text{H}_5\text{HgH}_2\text{PO}_4\]
\[\text{C}_2\text{H}_5\text{HgOCOCH} > \text{C}_2\text{H}_5\text{HgOCOCH} \]
\[(\text{C}_4\text{H}_9)_3\text{SnCl} > (\text{C}_4\text{H}_9)_2\text{SnCl}_2 > (\text{C}_4\text{H}_9)_4\text{Sn}\]
\[(\text{C}_2\text{H}_5)_2\text{SnCl}_2 \simeq (\text{C}_4\text{H}_9)_2\text{SnCl}_2 > (\text{C}_8\text{H}_{17})_2\text{SnCl}_2\]
\[\text{Na stearate} > \text{Cd stearate} > \text{Pb stearate}\]
\[\text{CH}_3\text{OH} > \text{C}_2\text{H}_5\text{OH} > \text{C}_4\text{H}_9\text{OH} > \text{C}_8\text{H}_{17} \text{OH}\]

**DISCUSSION**
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In the studies of patho-physiology of circulatory red blood cells in industrial poisoning the following two problems seem to be important; the one is the interaction between the red blood cell and the toxic substance which may be related to the haemolytic anaemia, and the other is the change of haemoglobin by toxic substance. The author attempted to investigate the interaction between the red blood cell membrane and various chemical substances by means of haemolysis.

The haemolysis caused by chemical substances will be produced by the interaction between physico-chemical property of substances which cause haemolysis and that of biochemical substances of the red blood cell. Although the mechanism of haemolysis by each substance may be different, the process of red blood cell destruction by various substances may be divided into several groups. The haemolysis by various haemolytic substances was studied from the relation between the concentration of substances and the incubation time with red blood cell.

From the observations of the extent of the concentrations of substances causing a complete haemolysis and a scarce haemolysis, these substances could be divided into two groups; that is, one group showed the narrow extent (A group) and the other group showed the wide extent (B group). And the former showed the vertical line and the latter the inclined line when plotted on log-logistic grid (Fig. 2).

If we assume that \( n \) or more molecules of a substance are necessitated for the destruction of one red blood cell and \((n-1)\) or less molecules of a substance could not destroy one red blood cell, the relation between the concentration of this substance and the time for 50% haemolysis should show the vertical line in log-logistic...
grid (Fig. 2). Haemolytic action of the substances which belong to A group may be explained by the above assumption. Concerning haemolysis of the substances in A group, it has been reported that the haemolysis by alcohols\textsuperscript{4) is produced by dissolving the lipids of red blood cell membrane, and the haemolysis by taurocholate\textsuperscript{5) is produced by dissolving the lecithin of red blood cell membrane. And also, it is said that the haemolysis by dodecylbenzene sulfonate\textsuperscript{6) is caused by the interaction between cholesterol of red blood cell membrane and dodecylbenzene sulfonate. And these compounds are easily permeable to the red blood cell membrane.

On the contrary, the extent of concentration causing haemolysis is very wide in B group's substances, and the relation between the concentration of substances and the time for haemolysis showed the decline line as shown in Fig. 6. The mechanism
of haemolysis caused by these substances may be assumed as follows; 1) as the reaction between the red blood cells and haemolytic substances progresses slowly, it may require a long time to cause haemolysis in case of low concentration, 2) the haemolysis take places on more fragile red blood cells primarily, and haemolytic substances which are released by destruction of fragile red blood cells attack on the other red blood cells together with remaining substances, and the haemolysis take place in turn. However, the mechanism of haemolysis caused by the substances in A group is still obscure. Organo-metallic compounds have a more powerful haemolytic action than inorganic metals. It may be necessary to consider the action of alkyl group conjugated with metals.

Furthermore, the author investigated the difference of haemolytic action caused by chemical compounds having similar structure. Haemolytic action of alcohols became more powerful with the increase of the number of carbon atoms as far as studied. This fact may be explained by the distribution coefficient between water and fat. However, in the haemolytic action by dialkyltin dichloride, the haemolysis became more weak with the decrease of number of carbon atoms of alkyl groups. In the comparison of haemolytic action of di-, tri-, and tetra-butyl groups of butyltin chloride, the haemolysis by tributyltin chloride was most remarkable, that by dibutyltin dichloride was moderate and that by tetrabutyltin was scarce. Ethylmercury phosphate groups showed more powerful haemolytic action with the increase of number of ethyl radical. Therefore the strength of haemolytic action of compounds having similar structure could not be assumed only from the difference of alkyl radical. It is still difficult to explain the systemic difference of haemolytic action of chemical compounds having similar structure.

REFERENCES