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SHORT COMMUNICATIONS

Effect of some phenothiazine derivatives on the hemolysis of red blood cells *in vitro*

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THE ability of phenothiazine derivatives to preserve stored blood was recognized in 1950 by Halpern *et al.*[1] These workers observed that the spontaneous hemolysis of stored blood containing a phenothiazine derivative was greatly delayed when compared with controls. This antihemolytic effect on stored blood was confirmed by Chaplin *et al.* in 1952,[2] and again by Greig and Gibbons in 1955.[3] Recently, Spirtes and Guth[4] showed that very low concentrations of chlorpromazine prevented the swelling of rat liver and brain mitochondria *in vitro*. They suggested the possibility that the drug might have a primary action on the permeability characteristics of the subcellular membranes tested.

The present experiments were designed to study the effects of phenothiazine drugs varying in clinical potency on the permeability of a *cell* membrane. Towards this end, the hemolysis time for human and dog red blood cells in distilled water was employed. Furthermore, the effect of chlorpromazine on changes in the erythrocyte membrane brought about by urea and glycerol was studied.

The method used consisted of drawing the blood, heparinizing it in a small Erlenmeyer flask and placing the flask in an ice-bath. Control hemolysis times were then determined by adding, in a test-tube, a drop of whole blood to 10 ml of an NaCl solution equal in osmolarity to the concentration of the drug being studied. Control values of this nature were based on an ionization of 100% for hydrochloride salts of phenothiazine derivatives. The drugs to be studied were contained in 10 ml of distilled water at the proper dilutions before addition of the drop of blood. On contact of the drop of blood with the solution, the tube was shaken vigorously and a stopwatch started. A tungsten filament lamp was switched on and the time for the appearance of the clear tungsten filament image viewed through the tube was taken as the end point. Some of the results are assembled in Table 1.

TABLE 1. EFFECT OF SOME PHENOTHIAZINE DERIVATIVES ON HEMOLYSIS TIME OF HUMAN RED BLOOD CELLS

Drug	Molar concentration of drug giving maximal effect	Control* hemolysis time in seconds	Hemolysis time in seconds with drug	% increase over control time
Chlorpromazine sulfoxide	1×10^{-2}	12	42	250
Promazine	5×10^{-3}	4	22	450
Chlorpromazine	1×10^{-3}	3	20	560
Prochlorperazine	5×10^{-4}	3	6	100

* Control hemolysis times are obtained when an eqiosmolar concentration of NaCl is used in place of the drug being tested. Each figure represents the average of three determinations in one sample of human blood. Order of clinical potency: Prochlorperazine > chlorpromazine > promazine > chlorpromazine sulfoxide.

It is important to note that all the drugs listed were tested, in the system described, at concentrations of 1×10^{-2}, 5×10^{-3}, 1×10^{-3}, 5×10^{-4} and 1×10^{-4} molar; however, only that concentration of each drug, which gave the the maximum increase in hemolysis time, as compared with the control, has been noted in the table. A comparison of such values indicates the relative affinity of the drugs for the red blood cells, the highest concentrations corresponding to the lowest affinities.

From Table 1 it is evident that the phenothiazine derivatives studied do change the hemolysis times in the system used, and that the concentrations resulting in a maximal effect for the individual drugs are inversely related to the clinical potency of the compounds. It can also be seen that the actual percentage increase in hemolysis time does not correlate with clinical activity. Thus, chlorpromazine causes the greatest percentage change, whereas prochlorperazine is effective at the lowest concentration. These data indicate that the affinity of the drug for human red blood cells varies directly with the order of clinical potency, whereas the quantitative percentage effects do not.

Since this preliminary experiment was performed on one sample of human blood and clearly indicated an effect of the drug on the system employed, a similar study was undertaken using dog blood which could be more conveniently obtained.

The experiments performed were identical to those carried out with human cells except that promethazine was subsituted for promazine.

TABLE 2. EFFECT OF SOME PHENOTHIAZINE DERIVATIVES ON HEMOLYSIS TIME OF DOG RED BLOOD CELLS

Drug present	Hemolysis time in seconds				
	Molar concentration of drug				
	1×10^{-2}	5×10^{-3}	1×10^{-3}	5×10^{-4}	1×10^{-4}
Saline control	4·3 ± 1·0	2·2 ± 0·8	1·8 ± 0·8	1·8 ± 0·5	1·8 ± 0·4
Chlorpromazine sulfoxide	7·8 ± 1·7 $P < 0.01$	3·0 ± 0·6 $P > 0.05$	2·0 ± 0 $P > 0.5$	1·7 ± 0·5 $P > 0.7$	1·7 ± 0·5 $P > 0.7$
Promethazine	22·5 ± 6·4 $P < 0.001$	8·0 ± 0·9 $P < 0.001$	2·8 ± 0·4 $P < 0.05$	1·7 ± 0·5 $P > 0.7$	1·8 ± 0·4
Chlorpromazine	5·8 ± 1·2 $P > 0.05$	14·3 ± 0·8 $P < 0.001$	9·7 ± 0·8 $P < 0.001$	3·3 ± 0·5 $P < 0.01$	1·8 ± 0·4
Prochlorperazine	1·5 ± 0·6 $P < 0.01$	2·5 ± 0·6 $P > 0.4$	3·3 ± 0·6 $P < 0.01$	3·0 ± 0·2 $P < 0.02$	1·7 ± 0·5 $P > 0.7$

The saline values represent the osmotic controls performed as noted under Table 1. Each recorded value represents the mean and standard deviation obtained from six animals. *P*-values are calculated by employment of the *t* test for small samples[5], comparing values at each drug concentration with the saline control at the same concentration.

Table 2 clearly shows that the phenothiazine derivatives studied affect the permeability of dog red blood cells in a similar manner to that noted for human cells.

A pH fall of 0·5 to 1 unit was observed upon the addition of 5×10^{-3}M or higher concentrations of all the drugs employed. The activity of the drugs on the red blood cell system cannot, however, be attributed to such a change. On the contrary, control experiments simulating such pH changes by the addition of HCl resulted in *increased* hemolysis rather than in protection against it. The latter results were in agreement with those of Hampson and Maizels[6], who amply demonstrated the increased hemolytic effect of hydrogen ions on erythrocytes.

Table 2 reveals that chlorpromazine decreases hemolysis times at 5×10^{-4}M, whereas the presence of twenty times as much chlorpromazine sulfoxide is necessary to obtain a similar effect. This relative inactivity of the sulfoxide derivative is strongly indicative of the specificity of chlorpromazine action, since osmotic and pH considerations are inherently controlled with its usage.

The effect of chlorpromazine on the hemolysis times of human red blood cells in water, urea, and glycerol was also examined. The drug increased these figures in all three cases; the effectiveness, as measured by the per cent increase over control time, was nearly the same in all. A hemolytic effect of chlorpromazine itself at higher concentrations in 0·9% saline can also be demonstrated. Table 2 shows that prochlorperazine similarly causes hemolysis at 10^{-2}M. The latter effect is consistent with the results of Chaplin *et al.*,[2] who demonstrated both a hemolytic *and* a protective effect of a phenothiazine derivative on stored blood.

It is suggested that, under the conditions of the experiments reported, the phenothiazine derivatives decrease the permeability of erythrocytes to water by altering cell membrane structure. However, the possibility must also be considered that chlorpromazine may affect the exit of intracellular ions, or act on the membrane in such a manner as to increase the critical hemolytic volume of the cells.

Work is presently under way testing these possibilities. Preliminary experiments employing techniques other than the one used in this study have already indicated that 10^{-5} chlorpromazine, the addition of which produces no pH change, can cause red blood cell membrane permeability changes. This low concentration affecting a cell membrane compares favorably with that needed to influence a subcellular (mitochondrial) membrane.[4]

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A mechanism for the induction by carbon tetrachloride of fatty liver in the rat

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THE occurrence of a fatty liver following poisoning with chlorinated hydrocarbons, such as chloroform or carbon tetrachloride, has long been established.[1] The mechanism by which the fatty infiltration of the liver is produced, however, has not been understood. It has been demonstrated recently that, following the administration of CCl_4 to intact animals, there is a decrease in the concentration of plasma triglycerides and a simultaneous increase in liver triglycerides.[2] This observation is suggestive in part either of an inhibition of release of triglyceride by the liver or increased uptake of triglyceride by the liver, or both. We have investigated these possibilities using the isolated perfused rat liver. It is clear from our observations that the release of triglyceride by the liver into the perfusate is completely inhibited, whereas uptake proceeds at normal or moderately increased rates when the livers are obtained from CCl_4-poisoned animals.

EXPERIMENTAL

Male rats weighing 250–350 g, maintained on a balanced ration *ad libitum*, were given by stomach tube, per 100 g of body weight, 0·5 ml of a 1 : 1 (v/v) mixture of CCl_4 and mineral oil.* After 3·5 hr, the liver was isolated and perfused through the portal vein *in vitro*. The perfusion procedure, substrates and analytical methods have been reported previously.[3, 4]

Triglycerides were estimated by the method of Van Handel and Zilversmit.[5] The results presented in Table 1 demonstrate the inhibition of release of triglyceride by the liver induced by administration of CCl_4. All animals treated with CCl_4 exhibited a significant decrease in the concentration of serum triglycerides. The release experiments measured net changes in total perfusate triglyceride in the absence of an exogenous source of triglyceride. The negative number observed after treatment with

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