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Effect of Quinine Hydrochloride on Resistance of Rabbit Red Cells.*

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Clinical observations on blackwater fever have suggested either that red cells of an individual treated with large doses of quinine salts are unusually susceptible to intra-vascular lysis, or that quinine salts act as lysins for red cells already weakened by intra-vascular lysins which appear in the disease. The purpose of this paper is to show that red cells of rabbits receiving quinine hydrochloride in proper amount are less resistant to lysins such as saponin and sodium taurocholate used *in vitro*. The experiments fall into 2 groups: (a) *in vitro* experiments, which show that quinine hydrochloride is a simple hemolysin, and (b) *in vivo* experiments, which show that the resistance of the animal's red cells is lessened by the administration of the quinine salt.

(a) *In vitro experiments.* The time-dilution curve for quinine hydrochloride and rabbit red cells can be found in the usual way,[1] and, at 25°C., is:—

Dilution, 1 in	25	30	40	50	60	100
t, minutes	3.0	4.0	9.5	17	25	85

This is a typical time-dilution curve for a simple hemolysin, and the endpoints are sharp and regular. As might be expected, the addition of quinine hydrochloride to systems containing saponin or sodium taurocholate produces a marked acceleration of hemolysis, and this can be measured by the usual methods.[1] If the cells are left in contact with 1 in 100 quinine hydrochloride for 10 minutes (after which time there is no lysis), and then washed and re-suspended, the resulting suspension has a smaller resistance to saponin and taurocholate than has an untreated suspension, and the R-values which measure the acceleration are about 0.70 to 0.75. The simplest explanation of what happens is that the quinine salt combines with some component of the cell membrane,[2] transforming it sufficiently to bring about hemolysis if the concentration of the

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[1] Ponder, E., "The mammalian erythrocyte and the properties of hemolytic systems," Protoplasma Monographien, No. 6, Borntraeger, Berlin, 1934.

[2] Gibbs, O. S., *J. Pharm. and Exp. Ther.*, 1928, **33**, 210.

quinine is great, and transforming it partially, but sufficiently to decrease its resistance to a subsequently added lysin, if the concentration of quinine is less. The phenomena seem to be like those observed in the case of the "additive" effect of 2 lysins.[3] In all cases the pH of the quinine solution was between 6.0 and 7.0, and so the effects cannot be attributed to H ion.

(*b*) *In vivo experiments.* In these experiments quinine hydrochloride was given to rabbits in varying dosage and over various periods, and the resistance of the animal's red cells to saponin and to taurocholate was measured at intervals. The quinine salt was given by daily subcutaneous injection in doses of from 20 mg. to 250 mg. per kilo, and samples of blood were taken by heart puncture, 2 cc. being withdrawn daily every 5 days in routine experiments, but more often when the dose was expected to be lethal. So that all suspensions to be used for determining red cell resistance should be comparable, red cell counts were made on each sample of blood, and the suspensions were prepared so as to contain 1.25×10^8 cells/cc. Reticulocyte counts were carried out in wet mounts.

The cell resistances were determined by a simplified method.† A dilution of saponin in 1% NaCl (1 in 20,000), and one of taurocholate in 1% NaCl (1 in 1,200, pH = 7.3) were selected as the lysins with respect to which resistance was to be measured, for these dilutions produce complete lysis of an average rabbit red cell suspension containing 1.25×10^8 cells/cc. in about 10 min. at 25°C. The hemolytic systems always contained 0.8 cc. of the lysin in the dilution just stated, 0.8 cc. of isotonic NaCl, and 0.4 cc. of the cell suspension. As a check on the stability of the lysins, a determination of the resistance of the cells of our own blood (J.C.A.) was made on each occasion. The resistance of the cells of the various samples of rabbit blood is expressed as the time required for complete lysis in the systems described.

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[3] Ponder, E., *Biochem. J.*, 1934, **28**, 384.

†The proper method of comparing resistances is to compare in their entirety 2 time-dilution curves, one for the lysin acting on the cells alone, and the other for the lysin acting on the cells in the presence of the accelerator or inhibitor. This method is very laborious, and is quite unnecessary in connection with this investigation. We have accordingly compared the times for complete lysis, under more or less standard conditions, for the cells of the animal at different stages of the quinine administration. It ought to be pointed out, however, that such a comparison gives an exaggerated idea of the change in cell resistance; in Table II, for instance, we have reductions in times for lysis from 10 min. to 3 min., but this corresponds to a resistance change of only some 30%, and not to one of some 300%.

The results can be divided into 3 groups, depending on the amount of quinine hydrochloride administered.

Group 1. Dose 20-50 mg./kilo/day. With small doses (20 mg./kilo/day), there is no significant effect either on the red cell count or on the red cell resistance over a period of at least 15 days. If at the end of this time, however, the dose is increased to 50 mg./kilo/day, there is a sharp rise in the number of reticulocytes, a slight (10%) fall in the red cell count, and a decrease in the red cell resistance (decrease in the time for lysis from about 12 min. to about 8 min.). These changes are transient, and with a continuation of the 50 mg./kilo/day dose the count and number of reticulocytes tend to return to the original values. The cell resistance, however, remains low until the quinine is discontinued, after which the original resistance is attained in from 15-20 days.

Group 2. Dose 75-125 mg./kilo/day. Within 5 days there is a sharp rise in the reticulocyte count, a 10% fall in the red cell count, and a decrease in the time for lysis from one of about 12 min. to one of about 8 min. With continued administration, the red cell count falls another 20% within a further 10-day period, and the resistance decreases to a time for lysis of about 4 min. The reticulocyte count falls to its usual value, the sharp rise which occurs

TABLE I.

Day	Dose, mg./kilo	Red cell count, $\times 10^{-3}$	Reticulocytes, %	Resistance to saponin, min.	Resistance to taurocholate, min.
0	0	5,756	0.3	12	10
5	75	5,240	1.2	8	7
10	75	4,444	0.6	5	4
15	75	4,020	0.2	4	4
20	0	4,982	0.4	6	7
25	0	5,578	0.3	10	10
30	0	5,802	0.3	12	11

TABLE II.

Animal	Max. fall in r.b.c.'s, %	Change in resistance to saponin, min. From	To	Change in resistance to taurocholate, min. From	To
1	26	9.5	4.5	—	—
2	31	10.0	2.5	10.0	4.2
3	22	10.0	3.0	10.0	4.0
4	30	10.3	3.2	11.5	4.5
5	31	10.5	3.2	11.0	4.0
6	30	12.0	4.0	10.0	4.1
7	23	10.0	3.0	10.5	3.5
8	32	11.5	2.2	10.5	2.0
9	31	12.5	2.0	10.0	2.5
10	26	10.3	3.1	10.8	2.9

initially apparently representing a transitory marrow activity which later ceases. As an illustration, the results of a typical experiment are given in Table 1, and a summary of the principal results for 10 rabbits in Table II.

Group 3. Dose greater than 150 mg./kilo/day. Doses of about 170 mg./kilo/day produce diarrhoea, and are usually fatal within a few days, while doses as great as 250 mg./kilo/day often produce death within 24 hours. The fall in the red cell count is not as great as it is in the case of animals of Group 2, principally because the animals do not live long enough; there is always marked decrease in red cell resistance, however, the time for lysis being reduced from about 12 min. to about half that time.

These results can be simply accounted for on the basis of quinine hydrochloride being a simple hemolysin with properties similar to those of simple hemolysins in general. Thus *in vitro* it is lytic in sufficient concentration, and in smaller concentration it is an accelerator of saponin and taurocholate hemolysis (the "additive" effect of 2 lysins). *In vivo,* successive administrations bring about a decreased resistance of the red cells to saponin and taurocholate used *in vitro,* and the fall in the red cell count which occurs with suitable doses of the drug is probably due to an intra-vascular lysis, brought about by intra-vascular lysins present in the blood stream (e.g., the bile salts and the unidentified lysins of urine), the action of which is accelerated by the quinine. It is unlikely that the quinine salt itself produces the intra-vascular lysis, for the greatest concentration in which it could exist, under conditions in which the effect occurs, is about 1 in 1000, and this concentration would not be lytic even for cells in saline.