THE COMPARATIVE RESISTANCES OF THE RED CELLS OF VARIOUS SPECIES TO HAEMOLYSIS BY STREPTOLYSIN O AND BY SAPONIN.

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ALTHOUGH the comparative resistances of the red cells of various species to lysis by saponin and other haemolytic agents have been investigated (Rywosch, 1907 ; Ponder, Saslow and Yeager, 1930), no comparable observations for streptolysin O have yet been made. In view of the probable importance of this lysin in the lethal intoxications resulting from inoculation with streptococcal filtrates, the present study was made to find out how far the red cells of some common laboratory animals differ in resistance to its lytic action. Since the kinetics of haemolysis for nucleated and non-nucleated red cells are essentially the same (Shattuck, 1928), the range of species examined was extended to include red cells of the former as well as the latter types. Further, because of certain similarities between the modes of action of streptolysin O and saponin, notably their common neutralizability by cholesterol, comparative resistances were determined for both lytic agents.

MATERIALS AND METHODS.

Streptolysin O.—This lysin and its thioglycollate reducing solution were made in the manner described in the preceding paper (Howard, Wallace and Payling Wright, 1953).

Red cell suspensions.—The rabbit was used throughout as the reference species against which the others were compared. Fresh blood, collected in 3 per cent sodium citrate solution, was washed thrice in Herbert's buffer-saline solution and finally made up to a 2·5 per cent suspension in the same fluid. For studying the action of lysins on red cells of such differing sizes, suspensions based on constant percentages of packed cell volumes are preferable to those based on fixed cell counts.

Estimations of haemolysis.—(a) *Streptolysin :* Serial dilutions of this lysin were made in buffer-saline solution : to 1·5 ml. of each were added a further 1 ml. of buffer saline and 0·5 ml. of sodium thioglycollate solution. The mixture was placed in a water bath at 37° for 10 min. to reduce the lysin ; 3 ml. of the appropriate red cell suspension was then added to each tube. After a further 30 min. incubation the tubes were centrifuged and the percentage haemolysis determined with a " Spekker " photoelectric absorptiometer. (b) *Saponin :* Serial doubling dilutions of saponin in normal saline were made from an initial solution containing 200 μg./ml. ; fresh preparations were made for each haemolytic series since the activity of saponin deteriorates in solution. To 3 ml. of each dilution of saponin was added 3 ml. of the appropriate red cell suspension, and after the tubes had been immersed for 30 min. in a water bath at 37° the percentage haemolysis was determined as for the streptolysin.

Rabbit's cells were used as the reference for those of other species, which were examined two or three at a time. Resistances to streptolysin and to saponin were tested on the same day to improve comparability of results. The same two rabbits were used throughout the experiments.

Comparison of results.—The dilutions of streptolysin and of saponin that produced 50 per cent haemolysis of rabbit's cells under these conditions were regarded as containing one

haemolytic unit (H.U.)/ml. From this defined standard the number of units needed to produce the same degree of haemolysis with the other species was derived. With the preparation of saponin used, 1 H.U. was equivalent to 15 μg./ml.

RESULTS.

From the comparative resistances to haemolysis shown in Table I, it can be seen that streptolysin O is active against the cells of all the species examined.

TABLE I.—*The Comparative Resistances of the Red Cells of Various Species to Lysis by Streptolysin O and by Saponin.*

Species.	Mean red cell diameter (μ).*	Number of H.U. for 50 per cent lysis.	
		Streptolysin O.	Saponin.
Rabbit	7·0	1·0	1·0
Human	7·4	0·25	1·25
Guinea-pig	7·2	1·0	1·5
Dog	7·1	0·75	4·0
Rat	6·8	2·0	0·75
Mouse	6·7	> 32	0·75
Hamster	6·6	1·5	1·0
Ox	6·0	0·75	4·0
Pig	6·0	0·75	1·0
Cat	5·8	1·0	2·0
Horse	5·5	0·75	2·0
Sheep	4·8	0·75	4·0
Goat	4·0	1·5	4·0
Duck	13·1×7·4	1·0	0·75
Pigeon	12·9×7·0	1·5	1·0
Chicken	12·1×7·3	5·0	0·75
Frog	23·0×14·0	2·0	1·0

* Data from Gulliver (1846) and Wintrobe (1951).

Those of mice, however, possess a resistance of a wholly different order from the relatively narrow range found with the other 16 species. At a concentration of 32 H.U./ml. only 10 per cent haemolysis had occurred; 50 per cent haemolysis was never attained with this species because sufficiently potent preparations of streptolysin were not available. That this high resistance was not peculiar to any particular group of mice (to obtain enough cells for each test, blood from several mice was pooled) is shown by finding consistent results from batches of animals drawn from each of two laboratory strains. Of the other species, the cells of the hamster, rat and goat were rather more, and those of the horse, ox, sheep, dog, pig and human rather less resistant than those of the rabbit. Cat and guinea-pig cells had about the same resistance as rabbit cells. Of the nucleated cells examined, those of the fowl were much more, and those of the pigeon rather more, resistant than rabbit's cells.

The red cells of none of the species presented the same striking resistance to lysis by saponin as had been found for mouse cells by streptolysin. Several species required higher concentrations of saponin to effect an equivalent degree of lysis, but with most, the resistance did not differ much from that found for the rabbit.

It is of interest that when the species are listed in two series according to resistances to the two lysins, some degree of negative correlation emerges.

DISCUSSION.

We have no explanation for the exceptional resistance of mouse red cells towards streptolysin O. Although their resistance to this toxin has not been described before, there are records of their high resistance to two other oxygen-labile, cholesterol-inhibitable, haemolysins, *viz.*, pneumolysin and *Cl. welchii* θ-toxin. Cohen, Halbert and Perkins (1942) found that mouse red cells withstood ten times the concentration of pneumolysin that lysed those of the rabbit, sheep or man, and Oakley (1943) noted that *Cl. welchii* θ-toxin "is vigorously haemolytic for the red cells of most laboratory animals except those of the mouse, which are only slightly attacked." Since it seemed possible that the relative resistance of mouse cells might extend to all four known haemolysins of this type, we compared the lysabilities of mouse and rabbit cells to two preparations of tetanolysin. With this toxin the resistance of mouse cells was less distinctive; they were only four times more so than rabbit cells. It would nevertheless seem that mouse erythrocytes possess some constitutional feature rendering them less vulnerable than those of other common laboratory species to lysins of the oxygen-labile, cholesterol-inhibitable, kind.

The exceptional resistance of mouse cells does not seem to depend on any distinctively different cholesterol content, for we have determined the concentrations of this sterol in packed mouse, guinea-pig and rabbit red cells and found them to be 190, 180 and 155 mg./100 ml. respectively. Moreover, were a high cholesterol content of the cell envelope responsible for the differences in resistance to streptolysin O, mouse cells might be expected to be similarly resistant to saponin, which they are not. Without discarding the possibility, however, that lysis by streptolysin O ultimately depends on a complex formation with cholesterol in the envelope, two possible explanations might account for this lack of correlation. The cholesterol in the cells of some species may be more accessible to this lysin than is that in mouse cells, or in these latter cells a higher proportion of the cholesterol may be in esterified form.

We have applied three tests in the hope of seeing whether mouse red cells are distinctively protected against the streptolysin molecules by some surrounding protein film. The lysin was allowed to act on cells previously treated with trypsin (Morton and Pickles, 1951); no change in resistance followed. A second test was based on the observation of Mudd and Mudd (1926) that after the attachment of a film of agglutinating protein rabbit red cells lose their ability to pass through an oil-water interface; the comparative behaviours of normal mouse and rabbit cells at such a boundary was examined, and both appeared to have the same hydrophobic properties. Thirdly, the possibility that the source of the high resistance of the mouse cells might be found in the participation of some protein constituent of their serum seems to be lessened by the finding that erythrocytes of mouse and rabbit after transference to, and incubation for one hour in, the serum of the other species, undergo no sensible change in their relative resistances to streptolysin O.

Finally, before any further attempt is made to analyse this species idiosyncracy, it may be pointed out that the enhanced resistance of mouse erythrocytes to streptolysin O seems not to be peculiar to this type of cell, for, as will be shown in the following paper, mice are also much more resistant to the lethal action of this toxin than either rabbits or guinea-pigs.

SUMMARY.

The comparative resistances of the red cells of 17 common laboratory animals to haemolysis by streptolysin O and saponin have been determined. Although the cells of all these species can be lysed by both agents, mouse cells were found to be exceptionally resistant to streptolysin O.

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