XLVIII. THE INFLUENCE OF BLOOD SERUM AND OF SUGARS ON HAEMOLYSIS.

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OF late considerable interest has been shown in the phenomenon of inhibition of haemolysis, a matter of no little importance. The experiments of Ransom [1901] have been widely quoted, and passed from book to book, as though worthy of complete reliance. His conclusion that the inhibition of saponin haemolysis is due to the cholesterol content of the serum is negatived, however, by the fact that the quantity of cholesterol in serum is not sufficient to bring about the effects. Stocks [1919] more recently investigated the question, and concurs with Ransom, but his results are directly at variance with those of Ponder [1923], who has shown that the protein content of the serum is the chief factor in the production of inhibition. Stocks also believes that the inhibition is the result of a simple chemical reaction, but it can easily be shown that the inhibitory process is a much more complicated one than he would make out. It has therefore been thought well to confirm the findings of Ponder, who has investigated only the action of serum and of simple proteins, and to find out whether other inhibitory substances conform to the particular relation described by him.

Method.

The method adopted is the neat and simple one devised by Ponder, whose papers may be consulted [1921, 1922].

The materials used require some note. The haemolysin is pure white saponin dissolved in 0.85 % NaCl. The cell suspension is one of human cells, prepared by drawing off 1 cc. of blood into citrated saline, washing the cells three times, and then suspending them in 20 cc. of 0.85 % NaCl. A fresh suspension is made up every day. The sera employed were obtained from the clotted blood of the animal concerned, and were used fresh. The sugar solutions were made from pure sugars, dissolved in distilled water, and were isotonic with the cells.

Technique.

The technique of Ponder was closely followed, the result being the production of two curves for each sugar, one showing the relation between the time for the production of complete haemolysis and the dilution of haemolytic

agent in the absence of any inhibitory substance, and the other the relation in the presence of the sugar or serum. The logarithm of the difference between the δ coordinates of the two curves at any time t , is then plotted against the logarithm of the δ coordinate of the lower of the two curves at time t , the dilutions being first converted into concentrations. The result of such a plotting is a straight line as shown in Fig. 2. A typical experiment is set out in the following table; δ_1 is the dilution of lysin employed, c_1 its corresponding concentration, T the time for haemolysis, and δ_2 the dilution of lysin which is

Fig. 1

considered as acting on the cells and not inhibited; c_2 is its corresponding concentration. From these figures the curves shown in Figs. 1 and 2 are derived. In this case glucose is the inhibitory substance.

δ_1 1 in	c_1 mg.	T min.	δ_2 1 in	c_2 mg.	x mg.	$\log c_2$	$\log x$
10,000	0.2	1.0	18,000	0.11	0.09	1.04	2.95
20,000	0.1	2.4	30,000	0.06	0.03	2.82	2.52
25,000	0.08	3.5	36,000	0.055	0.027	2.74	2.43
30,000	0.06	5.1	43,000	0.046	0.02	2.66	2.30
35,000	0.057	7.2	50,000	0.04	0.017	2.60	2.23
40,000	0.05	10.7	57,000	0.035	0.015	2.54	2.17

Results.

(1) *Serum.* For these experiments rabbit serum was used in a dilution such that 0.01 cc. was present in each tube with the saponin. The following results were obtained:

Animal	Value of n	Value of A
1	0.93	1.2
2	0.93	1.09
3	1.0	1.0
4	1.3	0.4
5	0.9	1.12

Ponder gives only one case of the rabbit, in which the constants are $n = 1.125$, $A = 1.02$; it will be seen that all the above results give considerably lower values of n than the figures for the sera of the cat and of man, so that, for

identical dilution, rabbit serum appears to be less inhibitory. The errors of these values are exceedingly slight, and no difficulty was found in any case in reducing them to the linear form.

(2) *Glucose and Fructose*. Very pure samples were used, and the solutions made up fresh every time they were required. No effect on the cells was observed as a result of the sugars *per se*. It was found that glucose and fructose acted identically when in the same concentration, the curves being so nearly coincident that the one given by fructose has been omitted from

Fig. 2

Note. The uppermost point lies somewhat off the line, but this divergence is really insignificant, and is occasioned by its greater liability to experimental error, and to the sensitiveness of logarithms in this region.

the graph in Fig. 1. It will be seen that the effect of these sugars on saponin haemolysis is substantially the same as that of serum, the linear form of graph being obtained by plotting $\log x$ against $\log c_2$. The values of the constants are $n = 0.77$, $A = 1.0$. This value of n is much less than is usually found in serum experiments.

(3) *Galactose*. A very different effect was here observed. The action is to accelerate in a marked manner the haemolytic rate, as can be seen from the uppermost curve in Fig. 1, although the sugar has no effect on the cells *per se*. So far no method of analysing the acceleration curve has been devised.

Careful experiments were conducted to discover whether the acceleration effect was in any way comparable to the accelerations met with in systems of bile salts and serum, or bile salts and histamine. In such systems the histamine or serum acts as an inhibitor if added to the cell suspension before the glycocholate, and as an accelerator if added after the cells are mixed with the lysin [Ponder, 1922]. This was not found to be so in the galactose-saponin system, nor indeed, was it expected to occur, since no such inhibition-acceleration effects have been observed with saponin, which shows either a clear inhibition as with serum, or a clear acceleration as with aspartic acid and allied substances.

(4) *Starch*. Further experiments were carried out with a highly purified form of rice starch, in many dilutions, but no effect on the saponin haemolysis was observed.

It is not proposed to discuss these results, which are in part confirmatory ones, nor can the reason for the acceleration produced by galactose be dealt with. It is however interesting to note that two such closely allied sugars as glucose and galactose should have such diametrically opposed effects on the action of saponin.

SUMMARY.

1. Certain results are recorded confirming Ponder's results on the inhibition of saponin haemolysis by serum.
2. Glucose and fructose inhibit saponin haemolysis in a manner much like that of serum.
3. Galactose accelerates the action of saponin, and starch has no effect thereon.

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