XXX. THE INHIBITORY EFFECT OF THE SUGARS ON SAPONIN HAEMOLYSIS.

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ONE of us [Kennedy, 1925] has already made a short study on quantitative lines of the effects of glucose, fructose, and galactose on saponin haemolysis. The general effect was found to be an inhibitory one. In view of the more complete analysis of inhibition of haemolysis made in a recent paper [Ponder, 1926], we have thought it a matter of interest to extend the investigations into the inhibitory effect of the sugars, both with a view to enlarging their scope, and of bringing them into line with the results of the last-mentioned paper.

MATERIAL USED.

With the exception of the specimens of glucose, fructose, and galactose, the sugars used in this investigation were those of the "Difco" brand. Each sugar was dissolved in distilled water to make a few cc. of a $M/3.6$ solution, which is isotonic with the cells employed. In each case the p_H of the solution was determined, and the sugar used only if no acidity was found; in several cases we had to reject samples because they were acid.

The red cells used were those of man and of the sheep. A suspension was prepared by suspending the thrice-washed cells from 1 cc. of blood in 20 cc. of 0.85 % NaCl. In the case of the sheep cells, the blood was received into oxalate instead of into citrate as in the case of the human cells, since citrate solutions which are isotonic for the cells of man are not isotonic for the cells of the sheep.

The saponin (quillia saponin, Merck) was dissolved in sufficient quantities of 0.85 % NaCl to give a series of dilutions from 1 in 1000 to 1 in 70,000.

TECHNIQUE.

The methods used were those described in several previous papers, and especially that of Ponder [1926]. In some cases complete curves were worked out and analysed, but in the majority of cases it has been thought enough to ascertain whether the particular sugar, in 5 % solution, is inactive, acceleratory, or inhibitory. This restriction has been necessary owing to the expense attached to a more complete investigation. All experiments were carried out at 25°.

RESULTS.

Glucose. The investigation of the effect of this sugar shows the general procedure adopted. The time-dilution curve for saponin acting on the suspension of human cells, and that of saponin acting on the sheep cells, are first plotted. Saponin solutions, each 2.5 times as concentrated as the concentration desired, are taken, and of each 0.8 cc. is mixed with 0.8 cc. of saline; to the mixture is then added 0.4 cc. of the suspension, precautions being taken as regards the temperature. The curves are shown in Fig. 1, where a different scale is used for the two suspensions; in the case of the sheep cells, the curve lies between saponin dilutions of 1 in 1000 and 1 in 80,000, while in the case of the human cells the curve lies between 1 in 10,000 and 1 in 60,000.

Fig. 1.

The first point which arises is that the two curves are very similar in form, so that we should expect some simple relation between them. This relation is readily made out, and in the following way. We take a point on the curve for sheep cells, say the point (3000; 1.3), or, if the dilution be converted into the number of milligrams of saponin in the 2 cc. contents of the tube, the point (0.66; 1.3). Dropping an ordinate through this point, we get, on the curve for the human cells (27,000; 1.3) or (0.074; 1.3). Dividing the

two concentrations needed to haemolyse in the same time, that is, 0.66 by 0.074, we get the ratio, 8.9. And so with any number of other points. The ratios are:

Sheep cell curve, concentration	0.66	0.5	0.4	0.33	0.286
Ratio	8.9	8.8	8.8	8.6	8.5

Put into words, this means that the sheep cells require 8.8 times the amount of saponin required by the human cells to give lysis in the same time, or, that the sheep cells are 8.8 times as resistant to the action of the saponin as are the human cells. This is a method of analysis which may be used quite freely, and is, in our opinion, the best method of expressing relative resistances.

The time-dilution curve for saponin plus 5 % glucose is now determined by adding to each of the concentrations of saponin, not 0.8 cc. of saline, but 0.8 cc. of 5 % glucose. To the mixture 0.4 cc. of cells is added. Taking the human curve in full, and calling the dilution of saponin used δ_1 , its corresponding concentration c_1 , the dilution on the curve for saponin alone which has the same time for lysis as is observed in the glucose saponin mixture δ_2 , its corresponding concentration c_2 , and the difference between c_1 and c_2 , x , we get the following table:

δ_1	c_1	δ_2	c_2	x
25,000	0.080	31,000	0.065	0.015
30,000	0.066	36,000	0.055	0.011
40,000	0.050	46,000	0.043	0.007
50,000	0.040	56,000	0.035	0.005

Fig. 2.

Plotting x against c_2 we get a very good straight line; we therefore follow the procedure adopted in similar cases [Ponder, 1926] and plot c_1 against c_2 . This also gives a straight line (Fig. 2), which makes an intercept on the c_2 -axis of 0.006, and which is described by the expression $c_1 = 1.4(c_2 - 0.006)$. This linear

relation between c_1 and c_2 is similar to that described in the paper last mentioned in connection with the inhibitory effect of such substances as the alkalis; it corresponds to the general expression for these inhibitions, $c_1 = R(c_2 - k)$. It has already been shown that R measures the resistance of the cells in the cell-saponin-glucose system as compared with that of the cells in the cell-saponin system; under the action of the 5 % glucose this resistance is increased 1.4 times. The quantity of glucose actually present in the system, it may be noted, is 40 mg. We have commented on the significance of the constant k elsewhere; for our present purpose it may be disregarded.

The results previously given by Kennedy in a consideration of the inhibitory action of glucose can be treated in the same way as the above. They give $R = 1.56$, and $k = 0.003$. The action of the glucose was thus to increase the resistance of the cells in the glucose-saponin-cell system 1.56 times. When published, the results were not treated as we treat them now, and the equation applied was one applicable to quite another type of inhibition. The figures, however, are substantially correct, and it will be noted that Kennedy obtained in the expression which he used a value of n which is nearly equal to unity, as required by the above linear relation.

Sucrose. We have found that a $M/3.6$ solution of sucrose has an inhibitory effect on saponin haemolysis; if the results are treated as above they yield $R = 1.55$, $k = 0.005$. These values are not greatly different from those for glucose, and we may therefore conclude that the effect of the two sugars in equimolar solution is very similar.

Lactose. A similar treatment of the inhibitory effect of $M/3.6$ lactose gives the following values: $R = 1.4$, $k = 0.004$. These figures are substantially the same as those for the same concentration of glucose.

The rarer sugars. In the cases of the rarer and less easily obtained sugars, we have contented ourselves with the determination of a single point instead of the entire time-dilution curve. So as to work at the same place on the curve for human cells as for sheep cells, we use a dilution of 1 in 5000 saponin for the latter and a dilution of 1 in 44,000 saponin for the former, the ratio of these dilutions being the same as the ratio of the cell resistances. To 0.8 cc. of the saponin is added 0.8 cc. of the solution of sugar to be examined, and thereafter 0.4 cc. of the proper cell suspension. The quantity of lysin, x , apparently inactivated by the sugar, is found as in the previous instances. A table of the results shows the relative effect of the sugars.

Sugar	Human cells x , mg.	Sheep cells x , mg.
Glucose	0.006	0.07
Mannose	0.008	0.07
Lactose	0.010	0.08
Sucrose	0.013	0.20
Trehalose	0.016	0.04
Maltose	0.013	0.07
Raffinose	0.010	0.10
Arabinose	0.002	inert
Xylose	0.002	inert
Rhamnose	0.004	0.05

From this table several points arise. (1) All the sugars, except arabinose and xylose with sheep cells, are inhibitory. We have been unable to find any instance of acceleration with a sugar, unless the sample was acid in reaction. Kennedy has described acceleration with galactose, but, as the specimen was acid, the observation is to be withdrawn. Samples of inositol, melezitose, and sorbitol which we examined produced acceleration, but these samples were acid, and therefore useless for our purpose. (2) With the human cells, as with the sheep cells, there are considerable differences in the inhibitory effects of the sugars. It is to be remembered that in this table only single points on the curves for the action of the saponin-sugar mixtures are determined, and that we have no values for the respective constants, k . Only if we take it that k is the same in all cases is it correct to take the figures of the table as indicating the respective resistances of the various cell-saponin-sugar systems; k , however, seems to vary little, and so the assumption that it is constant will result in little error. (3) The effects of the sugars on the sheep and on the human cells do not run parallel. If the effect on the former were the same as that on the latter, we should expect the value of x in the latter case to be 8.8 times as great as in the former, for the sheep cells are 8.8 times as resistant as are the human cells. This condition is approximately fulfilled in the cases of glucose, mannose, lactose, arabinose and xylose, and we may therefore take it, provisionally, that there is no great difference between the action of these sugars on the sheep cells and on the human cells. With the other sugars no such assumption can be made.

We do not wish at this stage to offer any explanation as to the mechanism of the inhibition. It is possible that the sugars affect the cells themselves, and we have already dealt with this possibility [Ponder, 1926]. Other possibilities also present themselves; the lysin may be affected, or the reaction between the cells and the saponin may be modified. The method which we have used takes no account of these things; it merely compares the haemolytic systems, irrespective of which component is altered. Nor is it to be lost sight of that when sugar is added to the cell-saponin system, a certain amount of NaCl is of necessity replaced, and the cell-saponin-sugar system is poorer in this salt. This arrangement is necessary in order to maintain the correct tonicity in the system, but the withdrawal of NaCl may in itself create a new haemolytic system which differs from the one taken as a standard. We intend to investigate these points at a later date.

SUMMARY.

- (1) The inhibitory action of glucose on saponin haemolysis is studied quantitatively, and the inhibition shown to conform to a type already described in connection with sucrose, certain alkalis, and certain amino-acids.
- (2) A number of other sugars were found to be inhibitory in different degrees when human cells are employed in the haemolytic system; when sheep

cells are employed, xylose and arabinose were found to be inert. The action of the various sugars on the human and the sheep cells was found to differ in some cases. No sugar which produced acceleration was met with, unless acidity was present in the sample.

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REFERENCES.

Kennedy (1925). *Biochem. J.* **19**, 318.

Ponder (1926). *Proc. Roy. Soc. Lond. B.* **99**, No. 699.