LXXXV. THE INHIBITORY EFFECT OF SUGARS ON HAEMOLYSIS BY SODIUM TAUROCHOLATE.

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THE effect of certain sugars on saponin haemolysis has already been studied by quantitative methods [Ponder and Kennedy, 1926]. This study has shown that glucose, mannose, lactose, sucrose, trehalose, maltose, raffinose, arabinose, and rhamnose are inhibitory in different degrees when human red cells are used in the haemolytic systems, and that the action of these various sugars on the human red cell presents quantitative differences from the action on the cells of the sheep. In no case is the inhibition great, the value of R rarely exceeding 1.5. It has been further shown [Ponder, 1926] that the greater part of the action of the inhibitory sugars is upon the cells themselves rather than upon the saponin contained in the haemolytic system, for 90 % of the inhibitory effect remains after the cells have been treated with the sugar and afterwards subjected to washing. There is also some evidence that the sugars have an effect on the lysis in these systems, although this effect is very small compared with that on the cells themselves. We have no information, however, as to whether this latter inhibition is attributable to the presence of the sugar or to the fact that the addition of sugar to the system involves the removal of NaCl from it.

This investigation is concerned with the much more difficult problem of the inhibitory action of the sugars in systems containing sodium taurocholate as the lysis.

Materials used.

The sugars whose action we have investigated (glucose, sucrose, and lactose) were chemically pure specimens, and were dissolved in water to make $M/3.6$ solutions, such solutions being isotonic with the cells employed. It is desirable that these shall be freshly prepared.

The red cells used were those of man. A suspension was prepared by suspending the thrice washed cells from 1 cc. of blood in 20 cc. of 0.85 % NaCl, the blood being first received into citrate. Such a suspension is identical with that used in our previous investigations.

The sodium taurocholate used in these experiments was a highly purified and carefully dried sample. The use of this salt as a haemolytic agent neces-

sitates the greatest care in the preparation of the series of dilutions. The taurocholate is first weighed out in a dry weighing bottle, and dissolved in sufficient 0.85 % NaCl to make a 2.5 % solution; this is done about half an hour before the experiments are to be commenced. Immediately before a dilution of the substance is required for producing lysis, 1 cc. of this 2.5 % solution is diluted as required, the dilution being carried out in such a way that the final concentration of lysin in the completed system (0.8 cc. of lysin, 0.8 cc. of saline or sugar solution, and 0.4 cc. of suspension) is 1 in 1000, 1 in 2000, etc. In this way the dilute solutions of the lysin are prepared immediately before use, this method of preparation taking advantage of the fact that the salt is stable in concentrated solutions, but reducing to a minimum the effects of the instability which is met with in dilute solutions. If the dilute solutions are prepared and allowed to stand, it will be found impossible to obtain consistent readings.

When it is necessary, as it is in the experiments to be described below, to plot several time-dilution curves for several haemolytic systems which are afterwards to be compared, the plotting of these curves must be proceeded with in a special manner. A dilution of lysin, say 1 in 1000, is prepared, and immediately after the preparation the haemolytic activity of this dilution in each one of the haemolytic systems is ascertained. In this way several points are obtained, each corresponding to a dilution of 1 in 1000, but each lying on a different curve. The next dilution, say 1 in 2000, is then prepared in a similar manner from the 2.5 % stock solution of taurocholate, and its activity measured in each one of the systems; this results in a second series of points, each belonging to a different curve, being obtained: and so on until the curves are completed. This procedure differs from that usually employed when saponin is used as a lysin, for in the latter case each time-dilution curve may be plotted separately, and completed before any points on the time-dilution curves for the related systems are obtained. The special procedure to be used in conjunction with sodium taurocholate is necessary in order to obviate errors arising from instability of the haemolysin. A similar procedure, it may be mentioned, should be used when sodium oleate is employed as a lysin.

The 0.85 % NaCl used in the experiments should be freshly prepared with conductivity water which has not been exposed to the air for any considerable period. If the saline becomes acid through the taking up of CO₂ the acidity alters the activity of the sodium taurocholate. For many purposes it is desirable to use a buffered saline, but it must be remembered that the introduction of the buffers complicates the haemolytic system and may alter some of the properties of the lysins.

Results.

The results of this investigation will be best expressed by commencing with the analysis of two typical experiments.

Exp. 1. Time-dilution curves were plotted for two haemolytic systems at 25°, the first system (Curve A) containing 0.8 cc. of taurocholate (various dilutions), 0.8 cc. of saline, and 0.4 cc. of suspension, and the second system (Curve B) containing 0.8 cc. of taurocholate (various dilutions), 0.8 cc. of *M*/3.6 sucrose, and 0.4 cc. of suspension. The time-dilution curves are shown in Fig. 1, and below are figures for the usual analysis:

c_1	c_2	R
4.0	2.66	1.50
2.0	0.60	3.33
1.0	0.53	1.88
0.66	0.48	1.39
0.50	0.40	1.25
0.40	0.32	1.25

It will be observed, from inspection of the plotted curves, that this result is very different from that obtained when saponin is used as a lysin in conjunction with sucrose as an inhibitor, for the time-dilution curve for the taurocholate-sucrose-cell system shows a bulge in the region of 1 in 1000 to

Fig. 1.

1 in 2000 dilutions of the lysin. This peculiarity is reflected in the variable value of R (equal to c_1/c_2), and it will be recalled that in saponin-sucrose-cell systems the value of R is constant. Were R constant in the case of the system under consideration, the curve for the taurocholate-sucrose-cell system would be that indicated by the dotted line in Fig. 1.

To explain this anomalous result, let us assume that the sucrose has a double action on the system, affecting the cells so as to make them more resistant to the lysin, and at the same time depressing the activity of the lysin itself. Indications of this double action have already been described in the case of saponin-sucrose-cell systems [Ponder, 1926], and the remarkable depression of the activity of the lysin which occurs when either saponin or sodium taurocholate acts in NaCl-free systems is an additional fact in favour of its occurrence. Let us further assume that the activity of the lysin is depressed to a negligible extent in systems in which it is present in asymptotic

concentration. Then the inhibitory effect of the sucrose on the cells of the system will be

$$\frac{c_{1\infty}}{c_{2\infty}} = R_{\infty}$$

which is equal to 1.25 in this particular experiment; *i.e.* the sucrose so affects the cells that it makes them 1.25 times as resistant to the lysin as normally. If we further call the activity of the lysin I , the activity in the absence of any depression due to the sugar being unity, we have

$$\frac{R_{\infty}}{R} = I,$$

where the values of R are those given in the table above, and are determined from the ratio c_1/c_2 . We may now tabulate the values of I , the activity of the lysin, for various concentrations:

c_1	I
4.0	0.83
2.0	0.38
1.0	0.66
0.66	0.90
0.50	1.00
0.40	1.00

Fig. 2.

Plotting these values, we obtain Curve I in Fig. 2. The activity of the lysin is depressed to the greatest extent when the lysin concentration is about 1 in 1000; when the lysin is either very dilute or very concentrated, the activity is scarcely depressed at all.

Exp. 2. In making the above analysis, we have assumed the existence of a double action on the part of the sucrose, the cells being rendered more resistant by exposure to it, and the activity of the lysin being at the same time depressed. There fortunately exists a method by which we can demonstrate these two effects separately [Ponder, 1926], and this experiment is concerned with its application.

Three preparations are made from the standard suspension of cells. Suspension 1 is obtained by diluting 5 cc. of this standard suspension with 10 cc. of saline. Suspension 2 is prepared by adding 10 cc. of $M/3.6$ sucrose to 5 cc. of the suspension, and allowing the mixture to stand about half an hour before use. Suspension 3 is made by adding to 5 cc. of the standard suspension 10 cc. of $M/3.6$ sucrose, and allowing the mixture to stand at 25° for 30 minutes. 10 cc. of the mixture are then transferred to a small volumetric flask; the contents of this are then distributed into two centrifuge tubes, each of 10 cc. capacity, and 5 cc. of saline added to each. The contents of the tubes are centrifuged, the supernatant fluid is pipetted off, and the cells, together with the small amount of fluid which remains, are replaced in the volumetric flask. The cells are washed out with several small additions of saline, and the washings added to the flask. The 10 cc. volume is then made up with saline, the cells thoroughly distributed throughout the fluid, and the suspension which results used in the experiments. It consists of cells which have been treated with sucrose, but at the same time these cells are suspended in saline. It follows from the method of preparation that suspensions 1, 2 and 3 are of the same cell concentration. Further, if we prepare a system containing 0.8 cc. of lysin and 1.2 cc. of either suspension 1, 2 or 3, we have the reacting components in the same concentration as in the system 0.8 cc. of lysin, 0.4 cc. of standard suspension, and 0.8 cc. of saline or sucrose as the case may be.

Three time-dilution curves are now plotted. Curve 1, Fig. 3, is that for taurocholate acting on the untreated cell suspension (suspension 1). Curve 2, Fig. 3, is for taurocholate acting on cells which have been treated with sucrose and then washed, and Curve 3, Fig. 3, is for a taurocholate-sucrose-system (suspension 3), as in the first experiment of this paper. The figures of the analysis are given below:

c_1	Curve 2		Curve 3		I
	c_2	R	c_2	R	
4.00	2.87	1.39	2.50	1.60	0.75
2.00	1.66	1.20	0.87	2.29	0.52
1.33	1.11	1.20	0.64	2.46	0.49
1.00	0.83	1.20	0.57	1.75	0.69
0.66	0.55	1.21	0.51	1.30	0.92
0.50	0.425	1.17	0.42	1.19	1.00

The result of this experiment, in which the action of the sucrose on the cells (Curve 2) is investigated separately from the action on the lysin itself (obtained from the analysis of Curve 3 in terms of Curves 1 and 2), justifies the assumption made in Exp. 1 that the sucrose has a double action, increasing the resistance of the cells on the one hand and depressing the activity of the lysin on the other. In this particular experiment, the resistance of the cells is increased 1.2 times, while the extent to which the activity of the lysin is depressed in various concentrations may be seen from an inspection of the values of I , plotted in Curve II of Fig. 2. It will be observed that the two curves showing the relation of I to the concentration of lysin, and derived

from the two different experiments, are very similar in general appearance; the value of I is at a minimum, and the depression of the activity of the lysin at a maximum, in the neighbourhood of the concentrations 1 in 1000 and 1 in 2000. When the lysin is either very concentrated or very dilute, the activity is little altered by the presence of the sucrose.

The fact that the depression of activity of the lysin is at a maximum in concentrations of 1 in 1000 to 1 in 2000 is a suggestive one, for it is well known that it is in these concentrations that sodium taurocholate is the least stable. This instability in these concentrations is frequently very obvious when time-dilution curves are plotted for the lysin acting on washed red cells, for correct time readings are much more difficult to obtain for the concentrations between 1 in 1000 and 1 in 2000 than for other concentrations. Indeed, if a large number of time readings are made for these and intermediate concentrations, it will be found that the points do not lie on the curve as they

Fig. 3.

do for other concentrations, but that the curve in this region passes through a cluster of points. Some of the points in the cluster lie on the curve, and these are presumably the correct ones, the others being evidence of the instability of the lysin in this region, and of the great difficulty which is experienced in obtaining consistent readings. This same difficulty has been noted by Shattuck [1928], working with sodium taurocholate and chicken cells. When the activity of taurocholate is depressed by sucrose and other sugars, the greatest depression apparently takes place in this very region, *i.e.* in the region where the lysin is most unstable.

This effect is not produced by sucrose alone, but also by glucose and lactose, and probably by all inhibitory sugars. The three sugars investigated are remarkably alike in their action both on the cells of the haemolytic systems and on the haemolysin, and the way in which I varies with the concentration is much the same in the three cases. The maximum depression always occurs in the concentrations from 1 in 1000 to 1 in 2000, and the way in which I varies with the concentration of lysin is very much the same for

each of the three sugars. In the following table are given the results of three experiments which illustrate the similarity. The figures in the columns are values of I :

c	Sucrose $R=1.25$	Glucose $R=1.24$	Lactose $R=1.23$
4.00	—	0.83	0.62
2.00	0.40	0.52	0.62
1.00	0.62	0.55	0.51
0.66	0.77	0.75	0.85
0.50	0.91	0.90	0.95
0.40	0.94	1.00	1.00
0.33	1.00	—	1.00

As a result of these experiments, we are entitled to conclude that the sugars have a double inhibitory action in taurocholate-sugar-cell systems, one part of the action being on the cells, and the other part on the lysin itself. It will, of course, suggest itself that an increase in the resistance of the cells to the lysin, and a diminution of the activity of the lysin itself, are two terms which, regarded from the point of view of the final effect, have the same meaning; the justification for separating the two effects of the inhibitor is, however, that the effects can be separated experimentally in the manner above described. In the case of saponin, with which we have already dealt, the evidence for the double action of the inhibitory sugars is very much less convincing, although it nevertheless exists, for the diminution in the activity of the lysin is one which is both small in degree and independent of the lysin concentration. There seems, however, to be justification for concluding (i) that the inhibitory action of the sugars is a double one both in the case of saponin-sugar-cell systems and taurocholate-sugar-cell systems, (ii) that in the case of the former system the principal effect is on the cells themselves rather than on the activity of the lysin, while in the latter systems the effect on the lysin may be very much greater than that on the cells themselves, and (iii) that the greatest depression of the activity of the lysin in taurocholate-sugar-cell systems occurs when the lysin is in those concentrations in which it is most unstable.

We do not feel justified in offering any suggestions as to the mechanism of the inhibitory effects. As in the case of saponin, we are confronted with the possibility that the inhibition arises not only from the presence of sucrose or other sugars in the system, but also from the fact that the introduction of these substances leaves the system poor in NaCl. It is certainly the case that when NaCl is removed from the systems altogether, as when the cells and lysin are prepared in isotonic sucrose, the haemolytic activity of saponin and of sodium taurocholate is very much reduced. It is probable that this great reduction in the haemolytic activity of the lysins under these circumstances is to be explained as the result of a direct action of the sugar on the lysin itself, as well as upon the cells which the system contains.

SUMMARY.

1. The inhibitory action of sucrose, glucose, and lactose on sodium taurocholate haemolysis is studied quantitatively, and the inhibition shown to be due to a double effect of the added sugar, one effect being to increase the resistance of the cells, and the other effect being to depress the activity of the lysin. These two effects are distinguished because it is possible to distinguish them experimentally.

2. The activity of the lysin is most depressed in those concentrations in which it is commonly found to be most unstable.

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