XLV. THE BEHAVIOUR OF WHOLE BLOOD TOWARDS MALTOSE *IN VITRO*.

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MALTOSE is the only disaccharide which, on subcutaneous injection, has been found capable of alleviating the symptoms caused by insulin. As has been shown by Herring, Irvine and Macleod [1924], its action, in this respect, in the case of mice is almost as efficient as that of glucose or mannose, but with larger animals, such as rabbits, cats or dogs, it is less effective. Even with mice, it exercises its action more slowly than glucose and this would suggest that maltose requires to undergo hydrolysis before it can counteract the action of insulin. Further, the inequality in the rates of hydrolysis of maltose might account for the difference in the results obtained in different species of animals. Support for this view seemed to be furnished by the results of investigations on the maltase content of blood-serum reported by Compton [1921]. This worker found that the maltase content of the blood was not the same in all animals and he divided mammals into two groups, according to whether they had or had not maltase in their blood. Unfortunately, Compton did not include the mouse amongst the animals he investigated, and the experiments now recorded were undertaken, initially at least, to determine whether the greater efficiency of maltose in relieving mice from the effects of an injection of insulin could be correlated with a greater maltase content of the blood in this animal than in larger animals such as the rabbit or dog.

EXPERIMENTAL.

Our experimental procedure was very similar to that described by Compton except that whole blood was used in place of serum, and Wood-Ost's method for the sugar determinations was employed instead of Bertrand's. One of us (A. H.) is responsible for the animal experiments necessary for the research, while the other (M. G. M.) carried out the greater part of the chemical analyses. All the apparatus used (flasks, pipettes, etc.) was sterilised, and the sugar solutions (4-6 %) were prepared with water previously saturated with toluene. Blood from the following eight species of animals, namely, mouse, rat, guinea-pig, rabbit, kitten, pig, sheep and ox, was examined. The smaller animals, mouse, rat, and guinea-pig, were chloroformed and bled from the neck: the kitten was also anaesthetised and blood taken from either the carotid artery or jugular vein; the rabbit's blood was obtained from the marginal ear vein, while blood of the pig, sheep and ox was procured directly from the

slaughter-house. In the case of mice, a number of animals, usually 5 or 6, had to be used for each experiment so as to obtain a sufficient quantity of blood. A small amount of potassium oxalate was added to prevent the blood samples from clotting. One cc. of blood and 5 cc. of sugar solution were used for each experiment, the blood, in every case, being added to the sugar solution in from 2 to 5 minutes after its removal from the animal. In our earlier experiments, the reaction mixtures were incubated at body temperature, 37°, but the majority were carried out at 50°, which approaches more closely to the optimum temperature for the action of maltase [Compton, 1915].

After 5 cc. of the maltose solution and 1 cc. of blood had been incubated at 50° for 24 hours, 43 cc. saturated sodium sulphate solution were added and the mixture brought to the boil. After cooling, 1 cc. of "dialysed iron" was added to complete the precipitation of the blood proteins. Ten cc. portions of the resulting clear filtrate, which is exactly ten times more dilute than the original maltose solution, were used to determine the sugar content. Two control experiments were always carried out simultaneously. In one, 5 cc. of the maltose solution and 1 cc. of blood were mixed and immediately heated to boiling to inactivate all enzymes present. Thereafter the mixture was incubated at 50° for 24 hours and the sugar estimated as described above. In the other, 5 cc. of an approximately equivalent glucose solution and 1 cc. of blood were incubated at 50° for 24 hours, and the glucose content then determined and compared with the theoretical amount.

The experimental results obtained are summarised in the following table, in which there is also indicated the condition of the animal used.

Table of Results.

A=initial reducing power expressed as glucose in reaction mixture.

B=reducing power expressed as glucose after 24 hr. at 50° with blood.

C=reducing power expressed as glucose after 24 hr. at 50° with "boiled" blood.

D=initial concentration of glucose in control reaction mixture.

E=concentration of glucose after 24 hr. at 50° with blood.

Animal				Temp.	A	B	C	D	E
				°	%	%	%	%	%
1.	Rat (normal)	...	...	37	·616	·629	·646	—	—
2.	"	...	...	"	·616	·635	·618	—	—
3.	"	...	...	50	·612	·632	·629	·608	·584
4.	"	...	...	"	·612	·620	·616	·495	·466
5.	Guinea-pig (normal)	...	...	"	·612	·560	·616	·495	·460
6.	"	...	...	"	·612	·568	·600	·495	·461
7.	Mouse (high carbohydrate diet)	...	...	"	·328	·352	·336	·388	·388
8.	" (low carbohydrate diet)	...	...	"	·328	·352	·352	·388	·396
9.	Rabbit (normal)	...	...	"	·593	·593	·600	·495	·455
10.	" (after daily intravenous in- jection of maltose for a week)	...	...	"	·593	·584	·585	·388	·379
11.	Kitten (excessive carbohydrate diet)	...	...	"	·616	·623	·616	·577	·583
12.	" (" protein diet)	...	...	"	·616	·632	·638	·577	·585
13.	" (" fat diet)	...	...	"	·616	·633	·637	·577	·584
14.	Ox (normal)	...	...	"	·609	·625	·610	·516	·525
15.	"	...	...	"	·609	·615	·612	·516	·536
16.	Sheep (normal)	...	...	"	·595	·552	·565	·516	·519
17.	"	...	...	"	·595	·591	·578	·516	·523
18.	Pig (normal)	...	...	"	·365	·724	·355	·445	·446
19.	"	...	...	37	·365	·558	·364	·445	·428

Complete hydrolysis of the filtrates obtained in the maltose experiments with both blood and "boiled" blood was also carried out as follows. To 20 cc. of filtrate 2 cc. concentrated hydrochloric acid were added, and the mixture boiled gently under a reflux condenser for half an hour. The solution was then neutralised with the *exact* amount of sodium carbonate solution, the total volume adjusted to 50 cc., and portions used for analysis. The results of these experiments need not be reported in detail, as in every case values approximating closely to the theoretical were obtained.

Further control experiments were undertaken in which 1 cc. of an extract of commercial maltase was used in place of blood. In the first of these experiments 1 cc. of extract was added to 5 cc. of the maltose solution: in the second a trace of potassium oxalate was also added to the mixture, while the third was heated to boiling immediately on mixing the maltose solution with the enzyme extract. All three were then incubated for 24 hours at 50°, and examined exactly as described for the experiments with blood. The following results were obtained.

		Reducing power expressed as glucose		% of maltose hydrolysed
		Initial	After 24 hr. at 50°	
1.	Maltose + enzyme extract ...	0.616 %	1.071 %	87 %
2.	" + " + oxalate ...	"	1.045	85
3.	" + boiled enzyme extract ...	"	0.694	—

The p_H of the above three mixtures as determined by the colorimetric method was identical, namely 6.7. As this method could not be applied directly to the experiments in which blood was used, the p_H of a mixture containing 5 volumes of the maltose solution and 1 volume of a phosphate buffer of p_H 7.4 was determined and found to be 6.9.

DISCUSSION.

From the results tabulated above, it is evident that during the incubation no appreciable loss of glucose took place in any of our experiments, as a close agreement exists in every case between the values in columns D and E. Consequently the glycolytic action of the blood was a negligible factor in these experiments. Further, the values in columns A, B and C also exhibit a close agreement in the case of all the animals used except the pig. Maltase would thus appear to be absent from the blood of these animals, or only present in amounts too small to be detected by the method employed. At the same time, it is to be noted that, under identical experimental conditions, in the case of the pig, while the value for C (0.355 %) agreed with that of A (0.365 %), B was increased to an amount (0.724 %) corresponding to almost 100 % hydrolysis of the maltose used. This result is in agreement with that of other workers as to the high maltase activity of pig's blood, and also indicates that our experimental conditions were suitable for the enzyme action to proceed.

Although the p_H of the reaction mixtures was not determined, it was assumed, in view of the control experiment with the phosphate buffer referred to above, that the p_H of each mixture would be in the neighbourhood of 6.7–6.9, and as the experiments were conducted at 50°, the conditions employed were, according to the researches of Compton [1915, 1924], the optimum both as regards temperature and reaction. Additional proof of this is afforded by the result of the experiments in which an extract of a commercial sample of maltase was used. In this case 87 % of the maltose was hydrolysed. As with the addition of a trace of potassium oxalate 85 % was hydrolysed, the oxalate used to prevent clotting in the experiments with blood would exert no inhibiting action on maltase.

Consequently, the negative results obtained in the case of the mouse, rat, guinea-pig, rabbit, kitten, ox and sheep indicate that maltase is lacking from the blood of these animals. This is not altogether in agreement with the observations of other workers. Thus, while the rabbit, guinea-pig and kitten fall in his "non-maltase" group, the rat and sheep belong to Compton's "maltase" group, but Doxiades [1911], on the other hand, using a polarimetric method, gives figures indicating that rabbit's serum has a maltase activity as great as that of the sheep or dog. Bial [1893], also, at an earlier date, claimed to have demonstrated the presence of maltase in human blood by the isolation of glucose phenylosazone, although it is to be noted that no change in the polarimetric or reducing value of the solutions was detected. The discrepancy between the results of other workers and those now recorded does not appear to be due to the substitution of whole blood for blood-serum, for an experiment with pig's serum gave a much lower percentage of maltose hydrolysed (50 %) than a similar experiment with whole blood (100 %). It seems likely that the discordant results depend partly on different methods being adopted to detect the enzyme action and partly on a larger proportion of serum being employed by earlier workers. Taking the results as a whole, they do not support Compton's classification.

In our experiments we failed to detect the presence of maltase in the blood of any of the animals employed except the pig: and we did not succeed in our attempts to affect the content of blood maltase by different diets in the case of mice and kittens or by a daily intravenous injection of maltose in the case of a rabbit. Our results point to there being no distinct difference in the activity of maltase in the blood of the mouse, as compared with that of the rabbit. Consequently it follows that if maltose counteracts the effects of insulin only after hydrolysis, such hydrolysis does not occur in the blood. As it is known that an insulinised mouse responds more readily to an injection of maltose than does an insulinised rabbit, it is possible that hydrolysis is effected by an enzyme present in the tissues.

On the other hand, it may be noted that maltose, being a glucose α -glucoside, differs from other sugars (save glucose) so far employed to alleviate the symptoms caused by insulin, in having in its molecule, at one and the same

time, a *reducing group* and a *complete glucose configuration*. As the combined effect of these two factors may account for the efficiency of a compound sugar in counteracting the effect of insulin, experiments with cellobiose and gentiobiose in this connection would be of great interest. However, until further work can be carried out, it must remain an open question as to whether the efficiency of maltose in alleviating the symptoms resulting from insulin administration depends on the constitution of the compound or on its hydrolysis to glucose. In the latter case, the splitting up of the disaccharide is not accomplished by the blood maltase, but probably takes place in the tissue cells.

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