CXXXV. THE EFFICIENCY OF VARIOUS SUGARS AND THEIR DERIVATIVES IN RELIEVING THE SYMPTOMS CAUSED BY INSULIN IN MICE.

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PART I.

EXPERIMENTAL OBSERVATIONS.

THE object of the experiments recorded in this paper was primarily to ascertain the effects of alterations in the molecular constitution of glucose upon the efficiency of the parent sugar in alleviating the symptoms caused by insulin. The work was initiated at the suggestion, and under the direction, of Professor Macleod, and was rendered possible by the collaboration of Principal Irvine, who supplied the necessary chemical compounds. Some of these were available in small quantities only, and as the tests had to be made upon a sufficient number of animals, mice were chosen for the purpose. The results obtained rendered it advisable to extend the observations to the effects of other carbohydrates, some of which have already been investigated by Noble and Macleod [1923] in rabbits.

The mice were healthy young adults, varying in weight from 16 to 35 g., and were of both sexes. The animals were well fed, and no preliminary starvation or other procedure for diminishing their glycogen content was adopted.

The insulin employed was that of Eli Lilly and Company, and contained 20 units per cc. The amount injected hypodermically was intended to cause convulsions without being fatal, but experience shows that the attainment

¹ The experimental part of the work was carried out in the Physiology Department of the University of St Andrews, being part of the work done by P. T. Herring for the Medical Research Council.

of such a standard is impossible, great variations occurring in different animals as the result of the same dosage. The standard solution of insulin was diluted to one in twenty-five with 0.05 % HCl, and the dosage of the latter varied between 3 and 5 minims.

The mice were selected in batches of three of as nearly the same weight as possible, but the three were usually of different colours to facilitate recognition when in convulsions. Each mouse of the batch received a hypodermic injection of the same amount of insulin. The animals were then placed in open boxes in a large incubator which was kept at a temperature between 20° and 30°. The observations were as far as possible conducted in a warm atmosphere throughout.

The results of the injection were very variable in the mice of the same batch. Not infrequently one or more of the animals showed no symptoms even when they were severe in another. Occasionally all were affected alike. The most severe symptoms might occur in the heaviest animal. The colours of the mice employed were white, black and white, black, fawn, fawn and white, and brown. No one colour appeared to be associated with a greater or a less resistance to the effects of the insulin.

When symptoms had definitely developed the animal was carefully watched for a time and then a hypodermic injection of the test substance was given. This was frequently repeated after an interval and the results noted.

The symptoms produced in mice by an injection of insulin vary somewhat in detail, but are, on the whole, very much alike. Their onset usually occurs about three-quarters to one hour and a half after the injection, but may be delayed for two hours or longer. The animal becomes irritable and restless. The tail stiffens and may be held arching over the back or pointing upwards and backwards. A slight tap on the box or sudden noise makes the mouse jump. This stage is quickly followed by convulsions, of which there are two types. In the more common form the mouse springs into the air and runs about wildly, or rolls over and over sideways, the muscular contractions being of a clonic character. In the other, and not infrequent form, the mouse has a tonic spasm affecting all its muscles. The back is arched, the limbs extended, and head retracted. The spasm lasts for a few seconds and the animal falls over on its side. In the intervals between the convulsions the animal is limp and lies with legs extended in the prone position. Weakness of the postural muscles is very prominent, and the sprawling thus occasioned is a prominent phenomenon after the first stage of irritability is over. Occasionally the sprawling precedes the convulsions. At this stage handling the animal usually sets up convulsions. Voluntary movements gradually become weaker, but the mouse, if placed on its back, regains the prone position and may drag itself about with its hind limbs extended and abducted. The convulsive stage may be prolonged or may be very short. If the amount of insulin injected has been sufficiently large the mouse gradually loses all power of movement and passes into the stage of collapse and coma. The animal now lies in whatever position

it is placed, and makes no attempt to right itself. The body is limp, the eyes protrude, and the pupils are widely dilated. Respirations become slower and of a convulsive inspiratory type. Death soon follows unless the mouse is resuscitated by the hypodermic injection of an active sugar.

Occasionally convulsions do not occur or are so slight that they are not noticed, and the animal passes rapidly into the comatose state.

If the dose of insulin administered has not been too large spontaneous recovery from the convulsive stage is usual but takes place slowly with frequent relapses. This fact has to be borne in mind in drawing conclusions as to the efficiency of substances tested for their capability of relieving symptoms and effecting a permanent cure. It is known that the hypodermic injection of glucose relieves the symptoms of insulin very promptly, and is effective even in the late stages of insulin coma. Moreover glucose effects a permanent cure if given in sufficient amount. Noble and Macleod have pointed out that certain substances, e.g. fructose and galactose, are capable of alleviating the symptoms of insulin in rabbits, but are incapable of effecting a complete recovery, the animal showing relapses after temporary improvement. In claiming for a substance an efficiency in relieving the condition produced by insulin which is comparable with that of glucose it is therefore necessary to show that it can produce not only temporary relief but can also effect a permanent recovery. For this purpose a more than lethal dose of insulin is required, and the substance tested must be capable of rapidly producing a complete cure.

In the following experiments the action of substances tested has been recorded on mice which were subject to insulin symptoms of various degrees of severity. Where any doubt existed as to the efficiency of the substance tested the experiments have been more numerous. Where the results were decidedly negative a few experiments have been deemed sufficient.

The substances tested were made up in distilled water and were of such concentration as to be as nearly equivalent as possible to that of the standard, i.e. glucose, 15 %. In some cases the solubility of the substance did not admit of this concentration, but in these and other variations below the standard the dose administered by hypodermic injection was correspondingly increased.

SUBSTANCES INVESTIGATED.

I. *Unsubstituted Reducing Sugars.*

1. *Glucose.* Three minims of a 15 % solution of glucose was usually effective in resuscitating a mouse even from deep insulin coma. The injection of glucose failed only in a few cases, and in all of these the animals were moribund at the time of the injection, death occurring before the glucose had time to be absorbed. Recovery to glucose was usually very rapid, a few minutes sufficing to restore the animal from deep coma to an apparently normal condition. Relapse may occur even after glucose, and if the dose of insulin has been a

large one the tendency to relapse lasts for some hours, but the condition is at once relieved by a further injection.

In recovery from insulin coma one of the first changes noticeable is the reduced protuberance of the eyeballs and the occurrence of winking movements by the eyelids. This is sometimes followed by convulsive movements which may be similar to those of the pre-coma stage but are of short duration only. Such convulsions do not always take place in recovery. The convulsive movements in recovery are more often those of a spasmodic contraction of the flexor muscles and differ from the earlier convulsions in which contraction of the extensors predominates. The fore limbs are tightly flexed against the chest, the toes strongly flexed, and the body curled up. The mouse soon struggles into a prone position, the irritability disappears, as does also the weakness of the postural muscles. In a very short time the animal resumes a normal posture, and, except for some lethargy from which it is easily aroused, appears to be fully restored.

2. *Mannose*. A 15 % solution of this sugar was used in place of glucose to resuscitate animals in which other substances had failed. Mannose was used on nine occasions. In two of these the mice were moribund in the comatose state with convulsive inspirations, a condition which is rapidly fatal if untreated. 5 minims of the mannose solution in each case brought about a complete recovery in 3 minutes which was permanent. In three mice the irritability and sprawling which had not been relieved by fructose were at once permanently abolished by 4 minims of mannose. In the remaining four mice the convulsive stage was at once abolished and permanent cure effected by similar doses of mannose after failure by galactose in three cases and glucosan in one.

The mannose was uniformly successful and appeared to be no less efficient than glucose.

3. *Fructose*. This sugar has been used in a 15 % solution in 20 mice. In nine of these its action was tested after other substances had failed to produce any effect upon the insulin symptoms. In the other 11 mice fructose was the first substance employed. The sugar was tested upon mice showing insulin symptoms of various degrees of severity. The results show that fructose is not always successful in relieving the symptoms, but that it does so in a majority of instances, though not so quickly as glucose or mannose. Larger doses are necessary and the beneficial effects are not so complete and permanent. In three mice fructose had no apparent effect but recovery was immediate and permanent to a small dose of glucose.

In one of these, black mouse, weight 29 g., a 4 minim dose of fructose failed to induce any sign of recovery from the comatose state, but 6 minutes later complete recovery was rapidly produced by the injection of 2 minims glucose. Another mouse of the same batch, white, 27 g., received the same dosage of insulin, and passed through the convulsive stage to that of coma. A dose of 5 minims fructose failed to produce any improvement, and the

mouse was resuscitated three minutes later by the injection of 2 minims glucose solution. Both mice recovered very rapidly to glucose and the recovery was permanent in each case. Fructose in a dose which was double or more than that of glucose failed to induce any recovery in 6 minutes in the first mouse and 3 minutes in the second.

Definite improvement to fructose took place in 17 mice, but in 10 of them an injection of glucose was eventually necessary to make the recovery complete. The remaining seven recovered without further treatment, but as several mice receiving the same dose of insulin and showing the same symptoms also recovered without even fructose it cannot be claimed that the seven mice owed their recovery to the fructose. The temporary alleviation often given by an injection of fructose may, however, be the determining factor in recovery when the dose of insulin has not been too great.

The experiments show that fructose may have a temporary alleviating action, but fails in itself to produce a complete recovery. The results obtained in mice with fructose are similar to those found in rabbits by Noble and Macleod.

4. *Galactose*. A 15 % solution of this sugar was employed in 18 mice. No improvement was noted in eight, and in three of these death occurred after doses of galactose amounting to 13 minims in one, 10 minims in another, and 5 minims in the third. The other five were resuscitated, two by glucose 4 and 5 minims respectively, and the remaining three by 5 minims maltose each. Nine mice showed definite improvement after the injection of galactose, but the improvement was of short duration. Improvement was often manifested to repeated doses, but relapse always followed, and the animals were eventually resuscitated by small doses of glucose, mannose or maltose. Only one mouse showed a gradual and permanent recovery after a dose of 5 minims galactose, and it would probably have recovered without treatment.

The temporary cessation of convulsions after an injection of galactose was a striking phenomenon in many cases, but relapse occurred within a few minutes. From 10 to 16 minims of galactose were given in some instances in two or more doses, and while producing a temporary short alleviation even these amounts were incapable of effecting a complete recovery. In two mice, after the failure of galactose, two doses each of 5 minims fructose were successful in effecting a complete recovery. These mice had not had large doses of insulin, and fructose proved to be more efficient than galactose in alleviating the symptoms.

Galactose is not a sugar which is capable of effecting a complete recovery from insulin symptoms in mice. It is not always successful in alleviating the symptoms even, and when it does so its action is evanescent. The temporary beneficial action of galactose is best shown in mice which are in the convulsive stage and have not received a large dose of insulin. It fails to resuscitate mice which have passed into the comatose state, and is less effective than fructose. Noble and Macleod found that galactose, like fructose, had a temporary

alleviating action in rabbits. The results of these experiments on mice show that galactose acts in a way like fructose but is much less efficient. Galactose cannot take the place of glucose or mannose.

5. *Maltose*. The maltose employed was of exceptional purity and made up in a 15 % solution. 16 mice were injected, the dosage being 5 minims in each case, the same amount being repeated in three only. Six of the mice received maltose alone, the other ten were treated with maltose after other substances had failed to give any relief from the symptoms. The maltose effected a complete and permanent cure in 15 of the mice, but failed in one in which death occurred 3 minutes after the injection. This mouse was moribund at the time of injection and had previously received two doses of 5 minims each of sucrose. An injection of glucose or mannose might have been effective. There is some indication that the action of maltose is somewhat slower than that of glucose or mannose.

The conclusion is reached that maltose is an effective sugar and can rank with glucose and mannose though its efficiency is less. Larger doses have to be employed and there is often some delay in its action. Noble and Macleod found maltose to be somewhat similar in its action to fructose and galactose in rabbits, and that it could not take the place of glucose. The experiments now done on mice appear to show that in these animals maltose is not much less efficient than glucose.

6. *Lactose*. A 15 % solution of this sugar was used in four mice only. Three of them received 10 minims in two doses of 5 minims each, the fourth had three doses of 5 minims each. In no case was there the slightest effect upon the symptoms. Three were eventually resuscitated by small doses of mannose, and one recovered slowly after a dose of fructose.

Lactose is incapable of giving any relief in mice to the symptoms produced by insulin. The results agree with those of Noble and Macleod in rabbits.

II. *Substituted Reducing Sugars.*

7. *Tetra-acetylfructose*. This sugar was available in a 5 % solution and was tested upon 10 mice, the total dosage in each mouse varying from 9 to 19 minims. The tetra-acetylfructose was ineffective in relieving symptoms in six cases, but appeared to produce a temporary alleviation, as indicated by a suspension of the convulsions, in three. One mouse, receiving a single dose of 17 minims, gradually recovered without further treatment, but it was one of a batch of three in which one had no symptoms, and the other, which had mild symptoms similar to those of the one injected, recovered without any treatment in much the same way. All the other nine were subsequently treated with glucose, whether temporary relief or no relief at all had resulted from the injections of tetra-acetylfructose.

In one mouse 16 minims of tetra-acetylfructose were injected. Temporary improvement followed the second dose, but it may not have been due to the action of the sugar injected, although the flexor spasms noted after its admini-

stration suggest that it had a beneficial effect. In other two mice, as already mentioned, the tetra-acetylfructose appeared to have a temporary beneficial effect. Fructose itself has been shown to be capable of alleviating symptoms but not of producing complete cure. The tetra-acetylfructose was only one-third of the strength of the fructose solution, and in the doses given could not be expected to have much effect. It is possible that the acetylation of fructose does not altogether render it incapable of action, but from the results obtained it is clear that this modified sugar is an ineffective one.

8. 2:3:5:6-*Tetramethylglucose*. An 11.4 % solution of this sugar was used in 11 mice. In none of these did any apparent result follow the injection. The sugar had no effect in relieving symptoms, whether slight or severe, neither did it seem to do any harm. All the mice were eventually treated with glucose and made complete recoveries.

9. 2:3:5-*Trimethylglucose*. A 10 % solution was employed in five mice. The symptoms varied from slight to severe. One mouse died after receiving 17 minims in three doses at intervals of 11 and 15 minutes between. In none did any apparent benefit ensue, and the four mice which survived were resuscitated by glucose, 4 minims in each case.

III. *Glucosidic Compounds.*

10. *Sucrose*. A 15 % solution was tested in seven mice. The dosage varied from 5 to 10 minims in each. Two of the mice died after receiving 5-minim doses without showing any improvement. One received 10 minims in two doses of 5 minims each, and died 11 minutes after the first dose, 5 minutes after the second dose. In the latter animal an injection of 5 minims maltose 3 minutes before death failed to elicit any improvement. The other four mice showed no improvement to two doses of sucrose in each case, and were finally resuscitated by maltose.

The results confirm the observations of Noble and Macleod in rabbits that sucrose is incapable of alleviating the symptoms due to insulin.

11 and 12. α - and β -*Methylglucoside*. A 10 % solution of α -methylglucoside was tested upon 11 mice, and a 10 % solution of β -methylglucoside upon two mice. Neither solution had any apparent effect upon the insulin symptoms, although used in a dosage up to 10 minims. One mouse died after an initial dose of 2 minims α -methylglucoside. The other 10, which showed symptoms of various degrees of severity, were not visibly affected in any way by repeated injections of larger doses, but each of them was eventually resuscitated by a small dose of glucose. Of the two mice injected with β -methylglucoside one showed no change to 4 minims of the solution and later required glucose. The other had slight symptoms only, which were not affected by 2 minims, and recovered without further treatment.

This compound is an important one, for in it the glucose molecule is altered by substitution in the least possible manner, but neither the α - nor the β -form showed the slightest sign of activity.

13. *Tetramethyl- β -methylglucoside*. This substance was used in a 10 % solution in six mice in doses of from 3 to 10 minims. In no case was there any alleviation of the symptoms noted. One mouse died, and the others were resuscitated by small doses of glucose.

The compound is inactive.

14. *Tetramethyl- γ -methylglucoside*. A 10 % solution was tested upon five mice in doses of from 5 to 10 minims. It had no appreciable effect upon the symptoms. One mouse, with slight symptoms only, recovered slowly after a dose of 5 minims. Another showed no change to 9 minims and recovered after two doses of fructose. The other three mice received doses of 10 minims each, and gradually becoming worse were resuscitated by small doses of glucose.

The compound is inactive.

15. *Glucose monoacetone*. A 12 % solution was tested upon four mice in doses of 8 to 10 minims. Two mice recovered gradually without further treatment, but their symptoms were slight and the injection made no apparent difference. The other two showed no change to 10 minims each, and gradually becoming worse were resuscitated by glucose. Glucose monoacetone is inactive.

16. *Salicin*. A solution of 14 % was employed, but had to be kept at body temperature and the syringe heated to prevent crystallisation at this strength. The solution was tested upon seven mice. The results obtained with this material are somewhat difficult of interpretation. The mice employed were small, 15 to 23 g., and not more than 3 minims of the solution was injected as a single dose. This amount was repeated once in each of three mice. Slight improvement appeared to result from the first dose in four instances, but none from the second dose. Two mice recovered slowly after a single dose of 3 minims, but as the other mice of the same batches also recovered without any treatment it cannot be inferred that their recovery resulted from the injection of salicin. The other five mice were all subsequently treated with glucose, but one which had received two doses of salicin without any improvement was in a moribund condition at the time it received glucose. It made a rapid recovery but died 10 minutes later with haemorrhage from the lungs. In all the mice which had severe symptoms a second dose of salicin was followed by an intensification of the symptoms, and the recovery resulting from 3 minims glucose was comparatively slow. The impression was formed that the salicin had actually done harm. The salicin was certainly not capable of effecting a complete recovery, and it is doubtful if it had any beneficial effect in temporarily alleviating symptoms.

IV. *Alcohols related to the Sugars.*

17. *Mannitol*. A 15 % solution was tested upon four mice. None of them showed the slightest improvement to doses of 3 minims. Two which had slight symptoms only recovered slowly after a single dose of 3 minims each, their

controls also recovered in the same way without any treatment. The other two mice were rapidly resuscitated by a single dose each of 2 minims glucose. Mannitol is inactive.

18. *Dulcitol*. A 15 % solution was tested upon four mice in doses of 3 minims each, once repeated in two of them. No change in the severity of the symptoms was occasioned. Two mice, showing slight symptoms only, recovered slowly without further treatment, and the other two made a rapid and complete recovery to a dosage of 2 minims glucose.

Dulcitol is inactive.

V. *Anhydro-sugars*.

19. *β -Glucosan*. This material was tested in a 10 % solution upon three mice. One had two doses of 5 minims each with an interval of 10 minutes between. It showed no improvement but was rapidly relieved and permanently restored by a dose of 4 minims mannose. The second mouse had a total quantity of 14 minims injected at intervals of 13 and 6 minutes, and died 4 minutes after the last dose. The third mouse received in all 17 minims in three doses, showed no improvement, and was eventually resuscitated by mannose.

β -Glucosan is inactive.

A few experiments were made to ascertain if a mixture of two substances, one of them having a partial action, and the other no action when separately tested, was capable of exercising a greater influence upon the insulin symptoms. Equal parts of a 10 % solution of galactose and a 10 % solution of α -methyl-glucoside were mixed and used as the test because the results obtained from this mixture have some bearing upon the chemical problem. The experiments show that the mixture has no greater effect than that of the galactose alone.

SUMMARY.

Of the unsubstituted sugars tested, glucose and mannose are found to be equally efficient in alleviating the symptoms and bringing about the complete recovery of mice suffering from insulin convulsions or coma. The hypodermic injection of from 2 to 5 minims of a 15 % solution of either of these sugars suffices in most instances, though its repetition is sometimes necessary.

Fructose is much less effective than glucose or mannose. It usually induces a temporary alleviation of the symptoms, more especially of the convulsive stage, and, if the dose of insulin has not been too large, the injection of fructose may enable the animal to recover. Relapses are more frequent after the use of fructose, larger doses are required, and there is not the same rapid relief; even after the convulsive stage is recovered from, weakness of the muscles persists after fructose, which is not the case when glucose or mannose is employed. The conclusion is reached, confirmatory of Noble and Macleod in rabbits, that fructose cannot take the place of glucose.

Galactose has an action similar to that of fructose, but is even less efficient. It does not always produce alleviation of the symptoms and when it does

effect a cessation of the convulsions, such relief is of very short duration. Galactose is incapable of effecting a complete cure, and cannot take the place of glucose. (Confirmatory of Noble and Macleod.)

Maltose is an active sugar, but is inferior to glucose and mannose. It can relieve symptoms and effect a permanent cure, but is somewhat slower in action, and the dosage required is larger than that of glucose or mannose. With respect to this sugar its action on mice appears to be much more efficient than was found to be the case by Noble and Macleod in rabbits.

Lactose is inactive. (Confirmatory of Noble and Macleod.)

Three substituted reducing sugars have been tested. Of these tetra-acetylfructose alone showed signs of activity. It appeared to produce a temporary alleviation of convulsions in a few instances, but its action in this respect is doubtful. Tetra-acetylfructose is incapable of effecting recovery from insulin symptoms. The other two compounds tested, tetramethylglucose and trimethylglucose, are devoid of activity.

Seven glucosidic compounds were tested. Sucrose, α - and β -methylglucoside, tetramethyl- β -methylglucoside, tetramethyl- γ -methylglucoside, and glucose monoacetone are inactive. The results obtained from salicin suggest that this compound may actually do harm. It alone of the glucosidic compounds gave any indication of being able to exercise an action. It is possible that salicin undergoes hydrolysis in the body; a slight beneficial action was sometimes apparent after a first dose, but none after a second. The compound is incapable of effecting a cure of the insulin condition.

Two alcohols related to the sugars were tested, mannitol and dulcitol. Neither alcohol had any apparent activity.

One anhydro-sugar, β -glucosan, was tested and found to be inactive.

None of the substances tested appeared to have any deleterious action with the possible exception of salicin.

A mixture of equal parts of galactose and α -methylglucoside was tested to ascertain if the partial action of galactose is enhanced by the presence of a glucose molecule in which the reducing group is the only one substituted. The action of the mixture was found to be no greater than that of the galactose alone.

PART II.

DISCUSSION OF RESULTS IN TERMS OF CARBOHYDRATE STRUCTURE.

The sugars and related compounds, the effects of which were studied at St Andrews, may be classified as under:

I. UNSUBSTITUTED REDUCING SUGARS.

Glucose. This was used chiefly to establish the standard against which other results might be compared.

Mannose, Fructose, Galactose, Maltose, Lactose. The use of the above compounds determined the effect of the reducing group in conjunction with different asymmetric systems.

II. SUBSTITUTED REDUCING SUGARS.

Tetra-acetylfructose, 2:3:5:6-Tetramethylglucose, 2:3:5-Trimethylglucose.

The above compounds were employed so as to ascertain the effect of the reducing group alone.

III. GLUCOSIDIC COMPOUNDS.

Sucrose, α -Methylglucoside, β -Methylglucoside, Tetramethyl- β -methylglucoside, Tetramethyl- γ -methylglucoside, Glucose monoacetone, Salicin.

IV. ALCOHOLS RELATED TO THE SUGARS.

Mannitol, Dulcitol.

V. ANHYDRO-SUGAR.

β -Glucosan.

The compounds classified in Sections III, IV and V were employed to test the effect of eliminating the reducing group in different ways, six varieties being introduced.

In order to make the discussion intelligible the structural formulae assigned to each of the test substances employed are now given:

I. Unsubstituted Reducing Sugars.

II. *Substituted Reducing Sugars.* III. *Glucosidic Compounds.*

IV. *Alcohols related to the Sugars.* V. *Anhydro-sugar.*

Another method of displaying the nature of the test compounds is to refer them by means of a skeleton formula to the glucose molecule, and indicate by a numerical method the positions which are substituted or modified in each of the derivatives used. If the carbon atoms of the glucose molecule be indexed by the numbers 1 to 6 as below:

then the following table shows how the glucose molecule has been varied in each test compound employed:

Position 1 substituted	...	...	...	...	α - and β -Methylglucosides
					Sucrose
					Salicin
Position 2 modified	...	...	...	...	Mannose
Position 4 modified	...	...	...	...	Galactose
Position 5 substituted	...	...	...	...	Lactose
Position 6 substituted	...	...	...	...	Maltose
Positions 1 and 2 modified	...	...	...	...	Fructose; mannitol
Positions 1 and 2 substituted	...	...	...	...	Glucose monoacetone
Positions 1 and 4 modified	...	...	...	...	Dulcitol
Positions 1 and 6 substituted	...	...	...	...	β -Glucosan
Positions 2, 3, 5 substituted	...	...	...	...	Trimethylglucose
Positions 1, 3, 4, 6 substituted and 2 modified	...	...	...	...	Tetra-acetylfructose
Positions 2, 3, 5, 6 substituted	...	...	...	...	Tetramethylglucose
Positions 1, 2, 3, 5, 6 substituted	...	...	...	...	Tetramethyl- β -methylglucoside
Positions 1, 2, 3, 4, 5 substituted	...	...	...	...	Tetramethyl- γ -methylglucoside

The twenty test compounds thus represent fourteen structurally defined variations in the glucose molecule. It is important to remark that the purity of the materials was the highest obtainable, the actual specimens employed being those upon which the physical constants have been standardised. This remark has special significance in the case of maltose, average specimens of which are notoriously variable in composition, the impurities being generally dextrans, isomaltose and free glucose.

The effects of the various sugars may be classified as under, the compounds being arranged in order of efficiency:

Class I.

Complete positive results, *i.e.* abolition of irritability and convulsions, rapidly followed by complete and permanent recovery.

Glucose }
Mannose } practically equal in effect.

Class II.

Complete positive results as above, but more slowly produced.

Maltose.

Class III.

Partial positive results, *i.e.* immediate abolition of irritability and convulsions, followed very soon by recurrence of the same symptoms; no permanent recovery.

Fructose.

Galactose.

Tetra-acetylfructose.

Class IV.

Entirely negative results, but with the possible exception of salicin, no toxic symptoms.

All the remaining compounds mentioned in the list.

Taking the foregoing results in conjunction with the facts already known that arabinose, xylose, glycerol and the mixture of glyceric aldehyde and dihydroxyacetone are without effect, a number of generalisations are justified. Of these, the most obvious is that the presence of a reducing group is an essential factor. This is shown by the entirely negative results obtained with all non-reducing compounds, and in this connection the use of α - and β -methylglucosides is instructive. In these compounds the reducing group of the parent glucose is modified to the minimum extent possible, yet the specific action of the glucose is eliminated entirely. Moreover, it is important to notice that the combined evidence points strongly to the idea that the functional sugars react not as aldehydes but as cyclic structures. If this were not the case, arabinose should behave similarly to glucose.

The second conclusion is that certain asymmetric systems of the sugar chain are necessary before a positive result is obtained and that others are not. Thus, whereas in glucose position 2 is $\text{H}-\text{C}-\text{OH}$ the reverse arrangement $\text{HO}-\text{C}-\text{H}$ is present in mannose yet the sugars function equally. Considering the profound effect on the reactions of sugars occasioned by alteration in the stereochemical position of the hydroxyl groups, it is highly improbable that the above system is operative in the curative reaction. It would also appear that the primary alcohol group is not involved, as although position 6 is unsubstituted in the majority of the test substances it is definitely replaced in maltose where the effect is positive. Positions 2 and 6 can thus be eliminated from the discussion, which is now focussed on 3, 4 and 5. In glucose these systems are formulated as under:

and the arrangement also exists in mannose and maltose. There is no direct evidence bearing on the function of 3, but 5 is of evident importance. Substitution of 5, as is the case with lactose, or ring-formation through this position as in arabinose destroys the curative action. Incidentally it may be remarked that this position is involved in the ring structure of the molecules of cellulose, of the amylo-pectin constituent of starch, and of glycogen. It is of course possible that the effect of maltose may be explained by hydrolysis to glucose as a result of enzyme action. The specimen used was free from either glucose or soluble dextrans yet a very decided positive result was obtained, although somewhat more slowly than by glucose or mannose. This suggests that the disaccharide was undergoing hydrolysis. It is, of course, impossible to verify or refute this suggestion, but as maltose is 6-glucosido-glucose it is evident that this particular sugar is the only reducing disaccharide in which the second hexose residue is not attached directly to any of the asymmetric carbon atoms. Irrespective of its mechanism, the result actually obtained is, therefore, consistent with the generalisation.

With regard to position 4 it may be noted that displacement of the oxygen ring has a pronounced effect. When the ring is formed on the opposite side of the chain of carbon atoms (e.g. one form of galactose) the positive reaction is diminished. Under some circumstances galactose may react in another form in which the ring is displaced to position 5, thus substituting a group which is evidently essential. It is important to emphasise that the presence of a reducing group alone is insufficient and the asymmetric systems of positions 3, 4 and 5 are ineffective in the absence of a reducing group. This is shown by

the negative behaviour of arabinose, xylose and lactose, all of which are reducing aldoses, but do not possess the complete asymmetric systems referred to. A more striking case leading to the same conclusion is presented by the tetra- and tri-methylglucoses. Here, although in each case the reducing group is free and the glucose configuration is preserved, the compounds are ineffective as the substituting methyl groups are stable and permanently mask the hydroxyl groups. Further, in both mannitol and sucrose the necessary asymmetric system is present, but there is no reducing group and the action is negative. The point now under discussion is best emphasised by the observation that a mixture of galactose (which contains a normal reducing group) and methyl glucoside (where the necessary asymmetry is present) was ineffective. Both conditions must therefore be satisfied in one molecule.

A review of the combined results shows no exception, which cannot be adequately explained, to the generalisation expressed in the statement that *the type of carbohydrate molecule functional in eliminating the convulsion symptoms occasioned by the administration of insulin is:*

where either *X* or *Y* represents a reducing group.

The asymmetric system formulated above will, of course, be affected to some extent by the nature of *X* and *Y*, and this suggests that *X* and *Y* may undergo rearrangement to give a common form.

It may further be claimed that if the above general formula be substituted in any respect, a necessary condition for the development of a positive result is that the substituting groups are removable either by enzymes or by dilute alkali. The case of glucose monoacetone is specially instructive as this compound has recently been proved to be a derivative of γ -glucose probably possessing the structure

Despite the fact that the compound is hydrolysed with extreme ease through the agency of very dilute acids, being in this respect one of the most unstable

organic compounds so far known, it is devoid of effect. This may be taken as a test case proving that glucosidic derivatives of γ -glucose play no part in the changes now under discussion. The behaviour of sucrose shows that derivatives of γ -fructose can also be ruled out.

It is at this stage premature to offer any suggestions as to the chemical reactions which accompany or account for a positive result, but a review of all the evidence points to the idea that two series of reactions are involved and that they proceed consecutively or take place simultaneously at different speeds. Consideration of the curious behaviour of galactose affords some support to this view and indicates that the first reaction is an attack on the reducing group of the sugar, thus making possible a further change in which the asymmetric chain of CHOH groups participates. This opinion is also supported by the general experience that specific reactions of sugars take place in definite steps the first of which involves the reducing group, either alone or in conjunction with one other hydroxyl group. The remaining hydroxyl groups then react singly or in pairs according to their stereochemical position. In other words it is not the sugar as a whole which reacts but definite sections of the molecule.

Although the generalisations now put forward seem to exclude from the list of functional compounds the anhydro-glucoses, it was nevertheless deemed advisable to conduct experiments designed to test the effect of such molecules. The question at once arises if glycogen can replace glucose as a corrective carbohydrate, but the solubilities of the polysaccharide are inconvenient for this purpose. As an alternative, the simplest possible molecule possessing the structure $C_6H_{10}O_5$ was selected for trial and β -glucosan

was therefore employed. This compound failed to show any positive effect in relieving the symptoms produced by insulin, and the reasonable deduction is that glycogen as such plays no part as a corrective carbohydrate and can do so only if it undergoes hydrolysis to give glucose.

PART III.

COMMENTS.

Although my participation in the foregoing experiments has been limited to a few preliminary observations in conjunction with the other two authors, I am requested by them to add a few comments, particularly with regard to the physiological aspects of the work. In so far as these relate to the same

sugars there is a general agreement with the results obtained by Noble and myself, such differences as exist being possibly accounted for by the fact that animals of different species were used in the two series of observations. In both of these, glucose and mannose were found to be by far the most efficient antidotes. In the experiments on rabbits, next in order, and of about equal efficiency, came maltose, fructose and galactose, but in those on mice, maltose was found to be decidedly more efficient than the other two sugars of this group. In the experiments on mice this sugar appeared to be almost as effective an antidote as glucose and mannose, whereas in those on rabbits it was decidedly less so. A conspicuous feature of the action of maltose in the experiments on rabbits was that temporary recovery often occurred shortly after the injections, followed by relapses in which the symptoms were either convulsions alone or convulsions, coma and ultimate respiratory failure. In some cases also the symptoms ended in death, although the blood sugar (as measured by the reducing power) had risen to over twice the normal level. It is possible that maltose cannot act as an antidote until after it has become hydrolysed to glucose and, if this be the case, inequality in the rates of this process in mice and rabbits may account for the difference in the results obtained in the two groups of experiments. It has not been possible to find in the literature any very definite information bearing on this aspect of the question. To judge from the results published by Bial [1893], to whose and to Hamburger's [1895] researches we owe the demonstration that maltase does actually exist in blood, there is no distinct difference in the activity of this enzyme in the blood of the ox, as compared with that of the dog or man, but it is unknown whether the blood of the mouse is of the same potency as that of the rabbit.

Since the conclusion, that the primary alcohol group of the glucose molecule is not essential, depends on the positive results obtained with maltose, N. B. Taylor, at my request, has performed a few experiments comparing the antidoting effects of maltose (commercial) and glucose on the hypoglycaemic symptoms in cats and dogs; and we have also repeated them on rabbits. In a cat showing the hypoglycaemic symptoms characteristic for this animal (profuse salivation, ataxia, pupillary dilatation and mewing followed by a semi-comatose state with muscular twitching and inability to stand), 2.4 g. per kilo. body weight of maltose caused no visible improvement in 15 minutes, but in 35 minutes the pupils were seen to be less dilated and the animal was able to stand. It was not until about 1 hour after administration of the maltose, however, that recovery could be considered in any way complete and the animal was able immediately to gain its equilibrium when dropped from a height of 4 feet. In the same cat, two days later, injection of 1.2 g. of glucose per kilo. body weight was followed by a similar degree of recovery in 16 minutes although the hypoglycaemic symptoms were more marked (deep coma). In another cat in which the most prominent of the symptoms were convulsions, 1.3 g. of maltose (per kilo.) brought about a corresponding degree of recovery in 30 minutes. In a third animal, in deep coma which had been

preceded by convulsive symptoms, 0.6 g. of maltose (per kilo.) caused a doubtful improvement in 20 minutes but this did not become more evident as time went on so that another similar amount was injected in 45 minutes. One hour after this second injection consciousness had returned but the animal was unable to stand.

The conclusion is that maltose is usually decidedly inferior to glucose in removing the symptoms of hypoglycaemia caused by insulin in the cat.

In a dog in which symptoms of moderately deep coma had existed for a little over one hour, 0.6 g. maltose per kilo. had absolutely no effect in 20 minutes, after which the same dose was repeated. Although the symptoms became somewhat less marked, another injection of 1.2 g. per kilo. was given; after 10 minutes there was some improvement and in 35 minutes the animal sat up although it could not stand erect. This improvement did not last for long, however, for 10 minutes later the coma had again become much more intense, and the animal unable to hold up the head. The condition did not improve during the next 90 minutes, after which 0.6 g. glucose was injected, followed by 1.2 g. 10 minutes later, with the result that the animal was able to stand and to walk a few steps in 20 minutes after the last injection.

In this case there is no doubt that maltose was inferior to glucose as a restorative.

In another dog in which convulsions were a prominent feature of the symptoms, although coma was also prominent, 0.6 g. of glucose brought about decided improvement in 3 minutes (convulsions disappeared and animal sat up) and consciousness very quickly returned, so that the animal was practically normal within 7 minutes of the injection of glucose. This did not last for long, however, for in 22 minutes after the first sugar injection the coma had again become profound, indicating a relapse of the hypoglycaemic symptoms due no doubt to the using up of all the previously injected glucose, so that a further 0.6 g. glucose was injected. Since no improvement was observed after 13 minutes a further quantity of 1.2 g. per kilo. was injected. This caused decided improvement within 7 minutes and thereafter the animal gradually improved and became normal in 37 minutes after the second injection.

In numerous other observations in this laboratory in which glucose has been used to remove the hypoglycaemic symptoms in dogs it has been noted that relapses are liable to occur and it is our practice to make the injections at intervals. There can be no doubt, however, that maltose is decidedly inferior to glucose as an antidote in this animal.

In three rabbits exhibiting convulsions recently injected with maltose one recovered as promptly as would have been the case with glucose and did not relapse, but in two, although there was prompt improvement, the convulsions returned within half an hour. One of these animals had ultimately to be given glucose to restore it.

As Irvine points out, it is impossible to say whether or not hydrolysis to glucose must precede the antidoting action of maltose. It seemed to act more

efficiently in the experiments on mice than in those on larger animals but it must be pointed out that the maltose used in Toronto (Schuchardt's), unlike that in St Andrews, was not chemically pure and may therefore have contained considerable quantities of dextrin which would be very slowly, if at all, hydrolysed in the blood.

It is of interest to compare the results of these two groups of observations on the hypoglycaemia due to insulin with those obtained by Mann and Magath [1921, 1922] on that following removal of the liver from dogs. These observers found that the hypoglycaemic symptoms were rapidly removed by the injection of 0.25 to 0.5 g. glucose per kilo. body weight and that a certain benefit was also obtained by the injection of maltose, mannose, dextrin and galactose. With regard to maltose it was found that when first injected its influence in removing the symptoms was only slowly displayed, whereas the second injection acted much more quickly. This may have some relationship to the maltase content of the blood. Mannose acted with about the same efficiency as maltose, which would place it at a lower value than we have judged it to be. The effect of dextrin is described as being very slowly developed and slight in degree, and of galactose as being even less definite than that of dextrin. Curiously enough fructose was found to have no restorative effect. With regard to other particulars, the results obtained by Mann and Magath agree exactly with those obtained in the experiments in which insulin was used.

It is significant that the sugars which act best as antidotes for the hypoglycaemic symptoms are those that are directly fermentable by yeast: glucose, fructose and mannose. The interesting point also comes to light that galactose, which we have seen to be so decidedly inferior as a restorative in insulin hypoglycaemia, is also much less fermentable than any of the other three hexoses [E. F. Armstrong, 1905]. It seems to be agreed that glucose and fructose are fermented at the same rate both by normal yeasts and by yeasts the action of which has been crippled in various ways, whereas mannose is fermented at varying rates according to the variety and the condition of the yeasts. On the other hand, the antidoting value of mannose in hypoglycaemia is at least as great as that of fructose.

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