CXLV. THE EFFECT OF CERTAIN SUGAR DERIVATIVES ON INSULINISED MICE.

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IN their researches on the efficiency of various sugars and their derivatives in relieving the symptoms caused by insulin in mice, Herring, Irvine and Macleod [1924] made no use of nitrogenous derivatives. Consequently, early in the spring of 1925, a series of experiments was carried out to test the effect of such compounds, but the results then obtained were not reported owing to their negative character. However, as, recently, other workers have published researches covering the same field, the following results are now communicated.

As a large number of the better known nitrogenous sugar derivatives contain their nitrogen in a group which is foreign and toxic to the animal organism, the compounds investigated were limited to glucose derivatives, in which the nitrogen was present either as an NH_2 -group or in the form of urea.

In carrying out the experiments the technique adopted was identical with that described by Herring and co-workers, except that control experiments with normal mice were carried out with each of the compounds tested. These were: (1) glucosimine, (2) glucose ureide, (3) glucosamine hydrochloride, and (4) chitose. A 15–20 % solution of each was employed, 5–10 minims being used for an injection.

(1) d-Glucosimine.

The glucosimine used for the first experiments was prepared by Lobry de Bruyn's method [1895], and, as the melting point of the specimen agreed with that quoted ($127\text{--}128^\circ$ with decomposition), the material was taken as sufficiently pure for use. Its injection into mice rendered comatose by insulin brought about slight and temporary relief, but this was subsequently shown to be due in all probability to a slight contamination with glucose since a specimen of glucosimine (M.P. 129°), prepared by Ling and Nanji's method [1922] and then recrystallised from methyl alcohol, not only was incapable of producing any relief, but seemed to exert a toxic action.

The specific rotation of the first specimen of glucosimine was found to be $+23.61^\circ$ ($c = 2.287$), that of the purified glucosimine was $+19.87^\circ$ ($c = 1.928$), which agrees with that quoted by Lobry de Bruyn, namely $+19.5^\circ$. Consequently, a specimen of glucosimine, although possessing the required melting point, cannot be taken as reliable unless it is known that the $[\alpha]_D$ is less than $+20^\circ$.

(2) *d-Glucose ureide*.

The glucose ureide employed was perfectly pure (m.p. 207°, $[\alpha]_D = -23.44^\circ$) [Hynd, 1926]. This compound had no effect on either normal or insulinised mice.

(3) *d-Glucosamine hydrochloride*.

No symptoms were detected on injecting the pure salt into normal mice. Four mice suffering from insulin convulsions were in no way benefited by injection of this compound, but recovered slowly after the administration of glucose. Moschini [1924] has recorded similar findings. As this worker found that a previous injection of this compound diminished and delayed the effect of insulin on rabbits, he concluded that glucosamine is transformed slowly into glucose *in vivo*. This appears to be opposed to the generally accepted view as to the behaviour of glucosamine in the animal organism, a summary of which is given by Levene [1925]. An alternative explanation is that glucosamine hydrochloride, just like ammonium chloride, would tend to produce an acidosis, which would render the rabbit somewhat more resistant to the action of insulin [Abderhalden and Wertheimer, 1924; Hetényi, 1926].

(4) *Chitose*.

Chitose syrup was tested on one normal and on six insulinised mice. It was quite inactive.

DISCUSSION.

Despite the negative character of the above results, they are not without interest when the close relationship of all four compounds to glucose is considered. In both glucosimine and glucosamine, the glucose molecule is altered only by the substitution of an NH_2 -group for an OH-group. Yet this is sufficient to render the molecule inactive.

As all the evidence points to the reducing group being essential, its substitution in the case of glucose ureide and glucosimine made it unlikely that these derivatives would have any efficiency. However, these two compounds differ from those hitherto examined with the reducing group substituted, in that they are relatively unstable in alkaline solutions, and might, therefore, have been expected to produce a positive result under the conditions obtaining in the animal body.

The third nitrogenous compound employed, namely glucosamine, conforms to the general formula arrived at by Irvine for a compound which should alleviate the symptoms produced by insulin, as the only alteration is at the second carbon atom. The groups at this position have so far been regarded as immaterial, but it is evident that while it is permissible to have inversion of the OH-group at the second carbon atom, this OH-group cannot be substituted without destroying the efficiency of the compound.

The fourth compound examined, namely chitose, is nitrogen-free, but again closely related as it is obtained by the deamination of glucosamine,

and may be regarded as 2.5-anhydro-glucose (or -mannose). Chitose is thus the third example of an anhydro-sugar which has been tested as to its efficiency for relieving insulin symptoms. Winter [1926] carried out experiments with α -glucosan, while the action of β -glucosan was investigated at an earlier date by Herring, Irvine and Macleod [1924]. Negative results were obtained in both these cases, presumably because the reducing group was absent. The inactivity of chitose cannot be explained in this way, as the reducing group is free. Further, as the correct configuration is preserved, the lack of efficiency must be referred to the ring structure which is present in the compound.

It is clear that glucose is by far the most efficient sugar for counteracting the action of an excessive amount of insulin, and any alteration of its molecule, except changes at the second carbon atom to give either mannose or glucal, which is converted readily into mannose [Winter, 1926], lowers the efficiency. Though changes at the second carbon atom are involved in glucosamine and chitose, the non-activity of these two compounds is not surprising. Accordingly it may be concluded that the introduction of nitrogen (*e.g.* glucosimine, glucosamine and glucose ureide) or of a second ring structure (*e.g.* chitose, α -glucosan and β -glucosan) into the glucose molecule completely destroys the power of relieving insulin hypoglycæmia.

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