VII. STUDIES IN CARBOHYDRATE METABOLISM.

II. INFLUENCE OF METHYLGLYOXAL AND OTHER POSSIBLE INTERMEDIARIES UPON INSULIN HYPOGLYCAEMIA.

By WILLIAM OGILVY KERMACK, CHARLES GEORGE LAMBIE
(*Beit Memorial Research Fellow*), AND ROBERT HENRY SLATER.

From the Department of Therapeutics, Edinburgh University, and the Research Laboratory of the Royal College of Physicians, Edinburgh.

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IN a previous communication [Kermack, Lambie and Slater, 1926], it has been shown that dihydroxyacetone when administered subcutaneously causes rabbits and mice to recover from insulin hypoglycaemia. It seemed to us that no significant difference could be discovered between the time taken for recovery after injection of dihydroxyacetone as compared with that taken after injection of glucose. In a paper by Campbell and Hepburn [1926], it has likewise been demonstrated that dihydroxyacetone causes prompt recovery from the symptoms of insulin poisoning, but these workers are of the opinion that glucose effects recovery more rapidly than does dihydroxyacetone, at least when both substances are injected intravenously. On the other hand, Hewitt and Reeves [1926], who have also confirmed the fact that dihydroxyacetone cures insulin hypoglycaemia, state that after administration of dihydroxyacetone recovery takes place in a manner identical with that seen when glucose is injected. They, like ourselves, injected the carbohydrate subcutaneously. We have now carried out further experiments to discover whether the discrepancy between our results together with those of Hewitt and Reeves on the one hand, and those of Campbell and Hepburn on the other, was due to the method of administration. We have not been able to convince ourselves that with intravenous injection there is any significant difference in the time taken for recovery. Naturally, without using very large numbers of animals it would be impossible to determine this point conclusively owing to the varying response of different animals or even of the same animal at different times.

In the paper by Hewitt and Reeves, referred to above, it is shown that glyceric aldehyde is not able to effect recovery from insulin hypoglycaemia.

We have prepared methylglyoxal by a method described by Fischer and Taube [1924, 1926] and we have tested this substance in a similar way. Briefly, we may say that the results were entirely negative and we even

received the impression that the substance possessed some toxicity. In all, seven animals were tested and in none of them did recovery take place. In each case the substance was injected subcutaneously, and usually several doses were administered. The rabbits were allowed to become completely comatose before injection of the test substance in order to minimise the risk of fallacies due to spontaneous recovery.

The following is a typical experiment.

Rabbit, female, weight 2320 g., starved 24 hours:

- 12.0 noon. 10 units insulin per kg.
- 2.50 p.m. Convulsions followed by recovery.
- 3.45 „ Convulsions with imperfect recovery.
- 4.0 „ Convulsions and coma.
- 4.18 „ Methylglyoxal 0.7 g. per kg. subcutaneously.
- 4.21 „ Violent convulsions.
- 4.32 „ Sprawling.
- 4.41 „ Convulsion, deeply comatose.
- 4.44 „ 0.7 g. methylglyoxal per kg.
- 4.48 „ Deeply comatose, lying on side apparently moribund, rapid respiration.
- 4.55 „ Violent convulsion.
- 5.5 „ Dihydroxyacetone 0.7 g. per kg. subcutaneously.
- 5.10 „ Sitting up in normal position.

Recovery complete and permanent.

Three of the animals to which large quantities of methylglyoxal were given, failed to recover even after subsequent injection of glucose or dihydroxyacetone. It is because of this and the peculiar deep breathing observed in these hypoglycaemic rabbits after repeated large doses of methylglyoxal, that we are led to believe that it possesses some toxic action.

A note may be added here on the preparation of methylglyoxal from dihydroxyacetone.

Experiments carried out according to the instructions of Fischer and Taube in which dihydroxyacetone (5.0 g.) and phosphorus pentoxide (15.0 g.) were thoroughly mixed and very gently heated until the reaction proceeded spontaneously, the product being condensed by liquid air or by a good freezing mixture, did not give yields as good as those claimed by these authors. The product, the yield of which varied from 1–2 g., polymerised very rapidly and became insoluble in water and so had to be mixed with an equal volume of water very soon after the distillation. If the quantity of phosphorus pentoxide was reduced, the yield became greater (up to 2.6 g.), but the product obtained was less pure, as shown by the yield of di-semicarbazone (M.P. 253°), obtained from a weighed quantity.

For instance, the products from two experiments using the larger quantity of phosphorus pentoxide gave 96 % and 99 % respectively of the theoretical yield of semicarbazone, whilst with the smaller quantity only 60.5 % of the

theoretical yield was obtained. The material obtained by the use of the smaller quantity of phosphorus pentoxide did not become vitreous as the result of polymerisation as did the other, but only thickened to a very viscous consistency.

Since either dihydroxyacetone or methylglyoxal might conceivably form lactic acid in the animal body, while the latter might either undergo complete combustion or form glucose, it was considered of interest to investigate further the influence of this acid upon insulin hypoglycaemia. We entirely failed to effect recovery or to prevent convulsions, either by subcutaneous or by intravenous injection of the substance. Our experiments therefore confirm the impression gained by Noble and Macleod [1923] who, while not coming to any final conclusion, did not obtain evidence of recovery.

Glycerol also failed to produce recovery or to prevent convulsions, even when large quantities were injected intravenously. This agrees with the results of Noble and Macleod [1923], although it should be noted that Voegtlin, Dunn and Thompson [1924, 1925], claim that glycerol administered intraperitoneally or by mouth protects mice from insulin hypoglycaemia and causes a rise in the blood-sugar of fasting rabbits. If it is active at all, it is certainly, in our experience, in no way comparable to either dihydroxyacetone or glucose.

Sodium pyruvate, like methylglyoxal, so far from removing the symptoms of hypoglycaemia, appeared to have a distinct toxic action, which, in some animals interfered with the recovery by glucose or dihydroxyacetone.

During the present work, sodium citrate and rhamnose were also tested, in each case with negative results.

DISCUSSION.

From the above, it is evident that there can be no comparison between dihydroxyacetone and any of the other assumed intermediaries in carbohydrate metabolism containing three carbon atoms, namely, methylglyoxal, glyceric aldehyde, glycerol, lactic acid and sodium pyruvate.

In our previous communication we left it an open question as to whether dihydroxyacetone acted by being converted into glucose or whether it could be directly oxidised, although we came to the conclusion that the balance of evidence was in favour of the latter assumption. Since this communication was written, a paper has appeared by Campbell, Fletcher, Hepburn and Markowitz [1926] in which the conclusion is reached, from a number of different lines of investigation, that dihydroxyacetone is apparently completely converted into glucose as the first step in its metabolism in the animal body. Nevertheless, we consider that such a definite conclusion is not justified. There is, we think, a considerable body of evidence to show that dihydroxyacetone may be metabolised more rapidly than glucose. Firstly, the blood-sugar curves reported by Isaac and Adler [1924], Rabinowitch [1925, 1, 2], Mason [1926, 1] and ourselves all indicate that dihydroxyacetone does not

cause an increase in the blood-sugar comparable to that produced by an equal amount of glucose. Even Campbell [1926] found very little rise in the blood-sugar curve after dihydroxyacetone although, curiously, he got very little rise in his control experiments with glucose. Secondly, Isaac and Adler [1924] have brought evidence to show that dihydroxyacetone can form glycogen with greater ease than can glucose. Thirdly, the respiratory metabolism experiments of Mason [1926, 2] indicate that dihydroxyacetone is oxidised more easily and more completely than is glucose in the normal individual and to a less extent in the diabetic¹. Fourthly, in the perfusion experiment upon the eviscerated and decerebrate cat reported in our first communication, it was shown that dihydroxyacetone disappears from the blood more rapidly than glucose when perfused at the same rate, even although the liver is out of the circulation.

The problem is a difficult one, but it is not easy to reconcile these observations with the conclusions reached by Campbell, Fletcher, Hepburn and Markowitz. Particularly when we consider the greater ease with which dihydroxyacetone can be oxidised *in vitro* as compared with glucose does it seem probable that it can undergo, to some extent at least, direct oxidation. Campbell, Fletcher *et al.* found that in the completely depancreatized dog dihydroxyacetone was not only converted into glucose and quantitatively excreted as such, but that the elimination was as rapid as if an equivalent quantity of glucose had been administered. We have examined two cases of complete diabetes in the human subject, that is to say, patients who were excreting more sugar than they were taking in. In such clinical experiments it is difficult to obtain clear cut results but in general our conclusion was that the dihydroxyacetone administered was almost quantitatively excreted as glucose. Incidentally, it was confirmed by polariscopic examination that the reducing substance in the urine was actually dextrose.

It seems then that in extreme diabetes dihydroxyacetone may be converted rapidly into glucose, whereas, where insulin is available, it is more easily utilised than glucose. This conclusion is confirmed by the observations of Mason [1926, 2], who found that in diabetics dihydroxyacetone did not exhibit as great a difference in its rate of metabolism when compared with glucose as was found in the case of normal individuals.

In order to explain this result we venture to suggest the hypothesis that an equilibrium exists in the body between dihydroxyacetone and glucose (similar to that which appears to exist *in vitro* in weak alkaline solution), such that in diabetes or in the absence of insulin the tendency is in the direction dihydroxyacetone to glucose and in the opposite direction when there are inadequate supplies of insulin. Administration of excess of dihydroxy-

¹ [Note added January 1, 1927.] Since the present paper was submitted for publication, the results of Mason have been amply confirmed by one of us (C. G. L.) and it has further been shown, by intravenous injection, that greater and more rapid increase in metabolism after dihydroxyacetone, as compared with glucose, is not due to its more rapid absorption. These results will be published fully elsewhere.

acetone, even in the presence of insulin, would by mass action give rise to a transient hyperglycaemia.

Dihydroxyacetone will naturally be in equilibrium with glycerol and with methylglyoxal, and through the latter with lactic acid and alanine. If dihydroxyacetone is being rapidly converted into glucose, as in the diabetic organism, not only will all these substances tend to produce glucose, but also compounds such as propionic acid and fatty acids containing an odd number of carbon atoms as well as certain amino-acids which ultimately on degradation give rise to fragments containing three carbon atoms. On the other hand, after administration of dihydroxyacetone, an increase in the excretion of lactic acid is to be expected, as this is a comparatively stable product in equilibrium with dihydroxyacetone through methylglyoxal, and it has been shown, in fact, by Isaac and Adler [1924] and by Mason [1926, 1, 2] that there is an increase in the production of lactic acid under these conditions. Again, it has been shown by Smedley MacLean and Hoffert [1926] that in yeast the probability is that fat is produced directly from at least three glucose molecules and, if this applies to the animal organism, the tendency often observed in early diabetics to the deposition of fat would be simply due to the mass effect of the large amount of glucose formed and its non-conversion into dihydroxyacetone. Naturally, the fat deposits disappear when the organism becomes increasingly dependent upon these stores of fat as a source of energy. The glycerol formed from their catabolism would then go through dihydroxyacetone into glucose and, of course, it is well known that glycerol yields glucose quantitatively in the complete diabetic.

It at first seems an important objection to this view that dihydroxyacetone exists, if it exists at all, in the blood stream in a concentration of at any rate less than 1 in 20,000 (human), to 1 in 10,000 (rabbit). However, if dihydroxyacetone is extraordinarily easily oxidised and if the conversion of glucose into dihydroxyacetone under the influence of insulin only takes place comparatively slowly as compared with the rate of oxidation of dihydroxyacetone, and further when it is remembered that the site of the action of insulin is not in the blood but in the tissues [Eadie, Macleod and Noble, 1923; Burn and Dale, 1925; Lambie, 1926], such a low concentration of dihydroxyacetone in the blood stream is only to be expected.

If the above hypothesis were true, the effect of dihydroxyacetone in causing animals to recover from insulin hypoglycaemia would be that it is directly oxidised, thereby supplying energy for the activity of the nerve cells. The absence of effect in the case of any of the other supposed intermediaries would be due to the fact that they can neither be themselves oxidised in such a way that their energy can be utilised in cellular metabolism nor converted into dihydroxyacetone at a sufficient rate. Presumably the only substances which could be so converted would be glucose, fructose and the few substances which cause recovery from insulin hypoglycaemia.

That glucose and dihydroxyacetone appear to effect recovery in the same

time may be due to the fact that the limiting factor is not simply the rate at which a sufficient quantity of dihydroxyacetone is brought in contact with the tissues, whether from glucose or directly, but the time taken for the nerve and muscle cells to recover from the secondary effects of the hypoglycaemic state.

SUMMARY.

(1) No significant difference could be observed in the time taken to recover from insulin hypoglycaemia following intravenous dihydroxyacetone administration as compared with glucose.

(2) Methylglyoxal and sodium pyruvate fail to cause animals to recover from insulin hypoglycaemia and appear to have a toxic action.

(3) Negative results were also obtained with sodium lactate, glycerol, sodium citrate and rhamnose.

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