XLVII. STUDIES IN CARBOHYDRATE METABOLISM.

IV. ACTION OF HYDROXYMETHYLGLYOXAL UPON NORMAL AND HYPOGLYCAEMIC ANIMALS.

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THREE compounds containing three carbon atoms have frequently been considered as possible intermediaries in carbohydrate metabolism: glyceraldehyde, dihydroxyacetone, and methylglyoxal. If the formula of hydroxymethylglyoxal, $\text{CH}_2\text{OH}.\text{CO}.\text{CHO}$, is compared with those of these three compounds it is seen that it bears a close relationship to all of them, and may, in fact, be regarded as an oxidation product of any of them. It was therefore considered of some interest to ascertain the action of this compound on the animal organism. This appeared the more desirable since evidence has been advanced by Hynd [1927] and Herring and Hynd [1928] which, it is claimed, supports the view that glucosone, $\text{CH}_2\text{OH}.\text{(CH}.\text{OH)}_3.\text{CO}.\text{CHO}$, is amongst the products formed in the animal body by the oxidation of dextrose under the action of insulin. In particular, they have found that the administration of glucosone to various animals causes the appearance of symptoms similar to those associated with insulin hypoglycaemia in the same species. The suggestion is that glucosone is the compound directly responsible for the appearance of these symptoms. Hydroxymethylglyoxal may be regarded as the simplest compound analogous to glucosone, bearing in fact the same relation to the triose glyceraldehyde that glucosone bears to dextrose. It was therefore of interest to determine whether hydroxymethylglyoxal causes the appearance of toxic symptoms similar to those produced by glucosone.

It may be noted that White and Willaman [1928] have found that hydroxymethylglyoxal is readily fermented by *Fusarium lini*, the only other compounds showing an equal ease of degradation by this mould being dihydroxyacetone and the fermentable hexoses.

EXPERIMENTAL.

The hydroxymethylglyoxal used in the following experiments was prepared by the method of Evans and Waring [1926], with one slight modification. These authors obtained good yields of the compound by oxidising dihydroxyacetone with copper acetate, allowing the mixture to stand for 10 days at ordinary laboratory temperature. We have been unable to obtain a crystalline product under these conditions, but found that the syrup produced rapidly crystallised if the oxidation had been allowed to continue for 30 days at room temperature or for 25 days at 25°. This difference is probably due to the fact that the laboratory temperature was considerably below that which prevails in American laboratories. With this modification we found that the reaction proceeds and the product crystallises just as described by Evans and Waring. It may be mentioned here that these authors found that when solid hydroxymethylglyoxal is dissolved in cold water it exists in a dimeric form, but when heated for 5 minutes at 100° it changes into the monomeric state.

Effect of hydroxymethylglyoxal on normal mice.

Experiments were first of all carried out to ascertain the effect of hydroxymethylglyoxal on normal mice, first with a freshly prepared solution of hydroxymethylglyoxal which had not been heated. The results are summarised in the following table.

Table I. *Effect of injections of unheated hydroxymethylglyoxal solution on mice.*

No. of exp.	Colour of mouse	Vol. of solution injected cc.	Concn. of hydroxymethylglyoxal %	Wt. of hydroxymethylglyoxal g.	Remarks
1	Albino	0.4	15	0.06	Sprawling in 1 minute then lying on side comatose. Breathing shallow, then gasping. Death in 3½ minutes
2	Albino	0.2	15	0.03	Arrest of respirations, convulsions and death in 2 minutes
3	Albino	0.4	7.5	0.03	Convulsions and death in 3 minutes
4	Black and white	0.3	7.5	0.0225	Convulsions, coma and death in 3 minutes
5	Black and white	0.2	7.5	0.015	Drowsiness, tail erected, paddling movements, coma, followed by recovery
6	Black	0.2	7.5	0.015	Erection of tail, convulsions, running movements, coma and death in 3 minutes
7	Black and white	0.2	7.5	0.015	Drowsiness, sprawling, paddling movements. Recovery in 2 hours
8	Albino	0.2	7.5	0.015	Convulsions, coma and death in 4 minutes
9	Black	0.2	7.5	0.015	Convulsions, coma and death in 4 minutes
10	Black	0.2	7.5	0.015	Drowsiness and sprawling, no convulsions. Recovery
11	Black	0.2	7.5	0.015	Drowsiness and sprawling, no convulsions. Recovery
12	Black and white	0.4	0.75	0.003	No symptoms

It will be seen that the lethal dose for a mouse is approximately 0.015 g. In those experiments in which death did not occur rapidly the animals exhibited a train of symptoms resembling those resulting from insulin hypoglycaemia. Thus in mouse No. 5, after a preliminary period during which the respirations were accelerated, the animal became drowsy for a time, then later the respirations became shallow and irregular, the tail was erected and the animal showed running movements. It then became comatose and lay on its side, respiration being barely perceptible, and in fact it was to all appearances moribund. After 20 minutes however, it showed signs of recovery, the respirations increased and the animal sat up unsteadily, the legs sprawling and the tail showing the peculiar stiffening commonly noted in the mouse during insulin hypoglycaemia. It remained in a languid condition for 3 to 4 hours and ultimately recovered completely.

The second series of experiments was carried out with a solution of hydroxymethylglyoxal which had previously been heated to 100° for 10 minutes. The results are summarised in Table II.

Table II. *Effect on mice of injections of hydroxymethylglyoxal solution, heated to 100° for 10 minutes.*

No. of exp.	Colour of mouse	Vol. of solution injected cc.	Concn. of hydroxy-methyl-glyoxal %	Wt. of hydroxy-methyl-glyoxal g.	Remarks
1	Brown	0.2	7.5	0.015	No symptoms
2	Albino	0.4	7.5	0.03	No symptoms
3	Black	0.2	15	0.03	Temporary excitement. No other symptoms
4	Albino	0.2	15	0.03	Transient muscular weakness; sprawling. No other symptoms
5	Albino	0.2	15	0.03	Became inactive for a short time. No other symptoms
6	Albino	0.4	15	0.06	After 8 minutes weakness of extremities. Moved about with difficulty. No convulsions. No coma. Breathing gradually became shallower. Death in 3 hours

It will be seen that the lethal dose of monomeric hydroxymethylglyoxal is 0.06 g., which is about four times the lethal dose of the unheated material. It appears, therefore, that the depolymerisation considerably reduces the toxicity of hydroxymethylglyoxal. Further, the depolymerised substance when administered in sublethal doses does not appear to cause the appearance of symptoms similar to those of insulin hypoglycaemia.

In the preparation of hydroxymethylglyoxal, it appears very difficult to eliminate the last traces of hydrogen sulphide and colloidal sulphur. The sulphur partially separates when the solution is heated to 100°. It seems, however, that hydrogen sulphide plays no part in the production of the toxic symptoms, since no symptoms were produced in control experiments

in which much larger quantities of hydrogen sulphide were employed than were contained in the unheated solution of hydroxymethylglyoxal. Further, the very small precipitate formed by heating the solution had no toxic effects.

Effect of hydroxymethylglyoxal on hypoglycaemic mice.

Ten mice were brought into a hypoglycaemic condition as the result of the administration of 0.2 cc. of standard insulin solution (20 units per cc.), diluted 1 in 25. To five of these mice sublethal doses of an unheated solution of hydroxymethylglyoxal were administered, and to the other five sublethal doses of a solution of hydroxymethylglyoxal previously heated to 100° for 15 minutes. In no case did recovery take place, but the animals recovered promptly after the administration of glucose. Hydroxymethylglyoxal is evidently without effect on insulin hypoglycaemia. It may be added that dextrose is without effect on the toxic symptoms produced by hydroxymethylglyoxal.

Effect of hydroxymethylglyoxal on rabbits.

The above results have all been confirmed by experiments on rabbits. These animals do not recover from hypoglycaemia as the result of the administration of hydroxymethylglyoxal, but rather are made worse, and death frequently follows in 10 or 15 minutes when the unheated material is used. This fatal result was observed even with doses which, when administered subcutaneously to normal animals, caused no obvious symptoms. In this respect the result is analogous to that previously observed with methylglyoxal [Kermack, Lambie and Slater, 1927]. As much as 9 cc. of a 25 % solution of hydroxymethylglyoxal, administered subcutaneously, produced no effect even when the solution had not been heated. When unheated hydroxymethylglyoxal was administered intravenously, 0.25 g. was sufficient to cause asphyxial convulsions and cessation of respiration following a momentary stimulation of the breathing. After generalised convulsions the animal lay on its side, showing running movements and intermittent gasping respirations. Artificial respiration was performed and the animal gradually recovered. On the other hand intravenous administration of 1.25 g. of a solution of hydroxymethylglyoxal, which had previously been heated to 100° for 10 minutes, produced no toxic symptoms. In relation to the results of Hynd, which have been mentioned above, an experiment may be reported here in which enormous doses of insulin (up to 200 units), were administered to rabbits and at the same time very large quantities of dextrose, to prevent reduction in blood-sugar. As expected, no hypoglycaemic symptoms occurred. If insulin produced hypoglycaemic convulsions as the result of the conversion of dextrose into glucosone it appears probable that such symptoms would have been observed in these experiments.

DISCUSSION.

The above experiments demonstrate the highly toxic nature of hydroxymethylglyoxal in its dimeric form. Herring and Hynd [1928] state that 2.4 mg. of glucosone per g. body weight is sublethal for a mouse. In the case of dimeric hydroxymethylglyoxal subcutaneous administration of 0.75 mg. per g. body weight is sufficient to cause death. The symptoms produced by dimeric hydroxymethylglyoxal are similar to those described by Hynd and Herring as being produced by glucosone. On the other hand, 3 mg. of the monomeric form of hydroxymethylglyoxal per g. body weight were necessary to cause death in the mouse and the animal did not exhibit the characteristic train of symptoms above noted. It is surprising to find that the monomeric form, which gives the Schiff's reaction more readily than the dimeric form, and of which the aldehyde groups are therefore in a more active condition, is the less toxic. As pointed out by Olmsted and Logan [1923], the symptoms of insulin hypoglycaemia resemble those produced by asphyxia in the rabbit and cat. It would appear that many substances which interfere with the normal oxidative processes in cells (*e.g.* such as cyanide poisoning, cf. Hess [1921], carbon monoxide poisoning, etc.), tend in the lower animals to produce a train of symptoms which have many features in common, especially within the same species. Among these symptoms are muscular weakness (as shown by sprawling of the limbs or drooping of the head), head retraction, weakness, drowsiness, convulsive seizures with paddling and running movements, and ultimately coma. In the absence of other evidence, it would appear particularly hazardous to conclude that glucosone plays a part in the production of the symptoms of insulin hypoglycaemia because of the resemblance of these symptoms to those produced by insulin. The above experiments show that almost identical effects are produced even more readily by hydroxymethylglyoxal. It is also difficult to explain the non-appearance of such symptoms after the administration of enormous doses of insulin, provided that sufficient dextrose is given to prevent a fall in blood-sugar below the normal. Reference may also be made to the fact that in cases of diabetic coma large quantities of insulin are often given without harmful effect as long as the blood-sugar is not allowed to fall unduly. Even Herring and Hynd [1928] find it necessary to suggest that the sensitiveness of the animal to glucosone depends on the blood-sugar being abnormally low; and so in the absence of independent evidence as to the existence of glucosone in the animal body it does not seem to simplify matters to assume the development of a specific poison to explain the symptoms. It must also be remembered that the symptoms of glucosone or hydroxymethylglyoxal poisoning are not relieved by the administration of dextrose.

In its inability to relieve the symptoms of insulin hypoglycaemia, hydroxymethylglyoxal resembles the majority of compounds containing three carbon atoms of which, so far, only one, namely dihydroxyacetone, has been found to possess the power of bringing about rapid recovery.

SUMMARY.

1. Hydroxymethylglyoxal in its dimeric form is highly toxic to mice and rabbits and in sublethal doses produces symptoms similar to those of insulin hypoglycaemia.

2. In its monomeric form the toxicity is reduced by 75 % and the same train of symptoms is not produced.

3. Hydroxymethylglyoxal, either in the dimeric or monomeric form, is unable to cause recovery from insulin hypoglycaemia.

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