L. IS AVITAMINOSIS B_1 AN INTOXICATION BY METHYLGLYOXAL?

GLYOXALASE—CO-ENZYME RATIO IN EXPERIMENTAL BERIBERI.

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In 1921 Findlay [1921] reported that "the glyoxalase content of the liver in pigeons with beriberi is less than that in control pigeons," and "the administration of vitamin B to a beriberic pigeon is followed by an increase in the glyoxalase content of the liver," but "vitamin B does not act as a co-enzyme of glyoxalase." Findlay drew no conclusion concerning the nature of the toxic substance supposed to come into existence during carbohydrate metabolism in the absence of the antineuritic vitamin. Since Findlay's discovery important investigations have been made both with respect to vitamin B and to the conditions of dismutation of methylglyoxal (and phenylglyoxal).

The complex nature of water-soluble vitamin B, *viz.* its resolution into the components B_1 (antineuritic and growth-promoting vitamin) and B_2 (P.P. factor, growth-promoting and pellagra-preventing vitamin) is now a well-known fact; it is possible that more components are contained in the vitamin B complex, but as yet this has not been definitely elucidated.

According to our present knowledge vitamin B_1 seems to be closely related to carbohydrate metabolism [Evans and Lepkovsky, 1929–30] and, through the latter, possibly also to protein metabolism. As to the importance of vitamin B_1 for fat metabolism it can only be said to be minimal; as yet the importance of vitamin B_2 in metabolism is unknown.

Several investigators have spoken in favour of the toxic pathogenesis of beriberi, none of them, however, stating the nature of the toxic substance with certainty; it was only supposed to come into existence during carbohydrate metabolism in the absence of vitamin B_1. The hypothesis most recently advanced [Peters, 1930], associated avitaminosis B_1 with an increase of the amount of lactic acid in the organism, especially of the brain. Peters believes that vitamin B_1 is concerned with the removal of lactic acid in the Meyerhof carbohydrate cycle.

As far as I know the literature does not comprise any work associating beriberi with an intoxication by methylglyoxal.

As demonstrated by Toenniessen and Fischer [1926], Ariyama [1928, 1, 2] and Vogt [1929] methylglyoxal is, also in animal glycolysis, an intermediary substance in carbohydrate catabolism, a dismutation of methylglyoxal into lactic acid eventually taking place.

Formerly methylglyoxal was considered a non-toxic substance [Dakin and Dudley, 1913, 4] but this view proved to be erroneous; it is a toxic substance as shown by Sjolleme and Seekles [1926], Fischler [1927], Kermack, Lambie and Slater [1927], and Herring and Hynd [1928].

Normally methylglyoxal cannot be demonstrated in the organism, a dismutation of the substance by means of glyoxalase (ketonealdehydemutase) taking place in the presence of the co-enzyme (co-mutase). Neuberg and Kobel [1928, 1929, 1, 2] noticed that well-washed lactobacillus or yeast, which was capable of converting hexosediphosphate to methylglyoxal but incapable of forming lactic acid from methylglyoxal, acquired the ability of producing lactic acid when boiled yeast juice was added to it. This was interpreted by them as indicating that co-enzyme was indispensable for the enzymic production of lactic acid from methylglyoxal. It was also demonstrated by Vogt [1929] in the case of liver (and other organic) enzyme systems that no dismutation of methylglyoxal will take place in the absence of the co-enzyme; under these conditions methylglyoxal is stabilised as such, and dismutation will only take place when co-enzyme is added to the enzyme system. Glyoxalase (a much better, because more logical, term would be ketonealdehydemutase as suggested by Neuberg [1913, 1, 2]) is widely distributed in nearly all tissues, more especially in the liver and muscles; and besides its rôle in the metabolism of sugar it also plays a part in the conversion of *d*-alanine to lactic acid. The presence of glyoxalase in the tissues is thus of importance in relation to the metabolism of carbohydrates and proteins. The wide distribution of glyoxalase in the plant and animal worlds seems to afford a strong indirect support for the methylglyoxal theory.

In my experiments mice and pigeons were used and three different methods employed. (1) The technique of Dakin and Dudley [1913, 1, 2, 3] and Neuberg [1913, 1, 2] which consists essentially in allowing a 20 % watery extract of the tissues to act on methylglyoxal (phenylglyoxal), and when the methylglyoxal (phenylglyoxal) is converted into lactic acid (mandelic acid), extracting the acid and estimating it by titration in terms of $N/10$ NaOH. (2) The technique of Vogt (1929) which consists in precipitation of methylglyoxal with 2 : 4-dinitrophenylhydrazine as bis-hydrazone. (3) The colorimetric method introduced by Ariyama [1928, 1] which is based upon the fact that glyoxals acquire, by the addition of cyanide, such a great increase of reducing intensity in alkaline solution that they cause the colour development of Benedict's arsenophosphotungstic reagent. None of the possible intermediate products of sugar metabolism, such as formic, acetic, glycollic, and glyceric aldehydes,

lactic and pyruvic acids or free hexose, interferes with the determination by developing the blue colour, with the exception of dihydroxyacetone, which gives about 20 % of the colour of methylglyoxal of the same concentration.

I thus succeeded in proving that methylglyoxal cannot be demonstrated in hexosephosphate + liver enzyme systems in starved animals any more than in normal animals, but that this substance can be demonstrated in hexosephosphate + liver extract from animals that do not get vitamin B_1, *i.e.* in animals otherwise getting a complete diet, comprising vitamins A + D (cod-liver oil), B_2 (autoclaved yeast), and E (wheat germ oil). I succeeded in demonstrating that the ingestion of vitamin B_1 (tikitiki-extract or Peter's concentrate) by the animals experimented upon apparently removed the difficulty attending the dismutation of methylglyoxal into lactic acid, and I also succeeded in demonstrating that in liver enzyme systems from animals suffering from avitaminosis B_1 methylglyoxal (and phenylglyoxal) is only converted into lactic acid (mandelic acid) to a small extent, whilst it was impossible in liver enzyme systems originating from normal (or starved) animals to demonstrate the presence of added methylglyoxal after the mixture had been allowed to stand for 24 hours. According to our present knowledge the enzyme is present in the liver tissue of animals suffering from avitaminosis B_1, whilst it seems to be the co-enzyme that is lacking, as a boiled aqueous extract of a normal animal liver, when added to the enzyme system originating from animals suffering from avitaminosis B_1, brings about the dismutation. This proof however is not conclusive when considered in relation to Vogt's [1929] investigations, because aqueous boiled extract of liver is also capable, although to a small extent, of bringing about a dismutation of methylglyoxal, the more so as the experiments with co-enzyme originating from yeast did not give completely satisfactory results (compare Findlay's results [1921]). As yet it cannot be decided with certainty whether it is the enzyme or, more probably, the co-enzyme that is lacking; perhaps there is a change of concentration in the enzyme/co-enzyme ratio of the organs of animals suffering from beriberi.

The fact that there is a possibility of accumulating methylglyoxal in animals suffering from avitaminosis B_1 is the more interesting as the symptoms of methylglyoxal intoxication in many respects bear a resemblance to those of experimental beriberi. Possibly the low blood-sugar level in the terminal stage of avitaminosis B_1 plays a role in regard to the sensitiveness of the animal to methylglyoxal; it is interesting in this connection to note that polyneuritic symptoms in the mouse resemble insulin symptoms in the same animal, especially interesting because methylglyoxal possibly plays a part in the production of the symptoms of insulin hypoglycaemia.

The whole of the experimental material forming the basis of this preliminary communication will soon be published.

SUMMARY.

The hypothesis is set up that the symptoms of avitaminosis B_1 are caused, wholly or partly, by an intoxication by methylglyoxal occurring on account of a failure of the dismutation of methylglyoxal, the tissue being deprived of the co-enzyme (not glyoxalase itself), or at least of a greater quantity of co-enzyme in comparison with glyoxalase.

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