CLXVII. ON THE GLYOXALASE-CO-ENZYME RATIO IN LIVER TISSUE IN INANITION¹.

By PAUL VOGT-MØLLER.

From the County and City Hospital, Medical Department, Odense, Denmark.

(Received August 7th, 1931.)

IN a previous communication [Vogt-Møller, 1931] on the glyoxalase-co-enzyme ratio in liver tissue in avitaminosis B₁ + B₄ [Reader, 1930], *i.e.* in experimental beriberi in mice, it was stated that starved animals, from which food was withdrawn, but which were allowed to take water, differed from animals suffering from avitaminosis B₁ + B₄, whose allowance of food was complete in every respect with the exception of vitamins B₁ and B₄, in such a manner that the liver tissue of the former animals (and probably the whole organism except the pancreas, in which special conditions prevail) in contradistinction to that of the latter contained more co-enzyme (co-mutase), though in both cases less than the normal amount was present. Under the conditions of the experiments, therefore, methylglyoxal was partly stabilised in avitaminous ferment systems.

When the technique that was then used is employed, *viz.* the determination of acidity described by Neuberg [1913, 1, 2] and by Dakin and Dudley [1913, 1, 2, 3], the precipitation of methylglyoxal employed by Vogt [1929], either as *p*-nitrophenylosazone or as *bis*-2 : 4 dinitrophenylhydrazone (described by Neuberg and Kobel [1928]), and the colorimetric method described by Ariyama [1928], all the methods being employed after 24 (48) hours' incubation of the ferment system at 37°, and when the technique used by me for the institution of inanition is used, *i.e.* the food but not the water is removed from the cage, the floor of which is covered with wood-wool and sawdust, the facts previously stated still hold good, provided the inanition is not carried too far.

Having begun to use Ariyama's method, but only with 20 minutes' incubation, and letting the animals starve in glass cages devoid of shavings instead of in the manner referred to above, I have attained results which deviate somewhat from those previously communicated, for which reason they are now briefly published.

As to the quantitative (colorimetric) determination of methylglyoxal I refer to Ariyama's communication. The incubation time was only 20 minutes at 37°, and by determining the amount of the added methylglyoxal which has been used during this period a measure of the glyoxalase + co-enzyme

¹ Supplementary note to my article in *Biochem. J.* (1931), 25, 418.

content of the organ (in this case of the liver) is obtained. As glyoxalase + co-enzyme preparation I used a 20 % preparation of ground liver tissue suspended in distilled water after it had been allowed to stand for 1 hour at room temperature after evisceration and after filtration of the suspended tissue. As co-enzyme preparation I used a 10 or 20 % boiled (5 minutes) preparation of suspended liver tissue prepared as stated above. Co-enzyme-free preparations were prepared by means of 6 hours' dialysis of the glyoxalase + co-enzyme preparations.

Using this method it appeared that an inanition of short duration (42 hours in the case of mice) involved a very considerable loss of the co-enzyme content of the liver tissue. It is a well-known though remarkable fact that the co-enzyme is used up during the fermentation process *in vitro*; the same thing may also occur *in vivo*. In contradistinction to this fact Ariyama found that after 10 days' inanition the amount of glyoxalase (*i.e.* glyoxalase + co-enzyme) in liver tissue (rabbit) "remained unchanged," whilst Sakuma [1930] found that 10 days' inanition of a rabbit caused a considerable decrease of the amount of co-enzyme.

In relation to what has been communicated previously, *viz.* the abnormal conditions of carbohydrate metabolism in avitaminosis B₁, I tried, in relation to the glucose cure [Kinnersley *et al.* 1928], the possible co-enzyme-forming or co-enzyme-stimulating power of glucose (saccharum amylaceum puriss. anhydric. U.S.P.X.) and found, like Sakuma, that weak solutions of glucose *in vitro* furthered the dismutation of methylglyoxal in dialysed, *i.e.* co-enzyme-free, ferment systems, and similar conditions were also found in the case of weak solutions of sodium nucleinate (originating partly from yeast, partly from the thymus gland). This fact in view of Euler and Myrbäck's [1929] supposition that co-enzyme (co-mutase) is "ein Adenin-nucleotid besonderer Art" brings the co-enzyme in a rather close relation to vitamin B₁ or perhaps especially vitamin B₄, without, however, allowing us to identify these two substances. One might suppose that the vitamin contained constituents necessary for the composition or formation of the co-enzyme. This much is certain: in inanition and in avitaminosis B₁ + B₄ there is a decrease of the amount of co-enzyme, a decrease that is not found in the controls used (paired-feeding method). It is possible that the cause of the decrease of the co-enzyme concentration in avitaminosis B₁ + B₄ should be looked for in the anorexia, always engendered by the avitaminosis, although this is not very probable when the conditions of the controls are considered, but it is now at least within our reach to find out the substances (nutritives, chemical compounds) causing the formation of co-enzyme *in vivo*. Possibly the toxic effect of glucose injected parenterally in excess is best understood on the ground that by the catabolism of glucose the tissue is deprived of the co-enzyme.

SUMMARY.

Information is given about the decrease of the co-enzyme concentration of liver tissue in inanition and about the co-enzymic properties of glucose and sodium nucleinate. Attention is drawn to the fact that it is now possible to examine the co-enzyme formation *in vivo*.

REFERENCES.

- Ariyama (1928). *J. Biol. Chem.* **77**, 359.
Dakin and Dudley (1913, 1). *J. Biol. Chem.* **14**, 155.
—— — (1913, 2). *J. Biol. Chem.* **14**, 423.
—— — (1913, 3). *J. Biol. Chem.* **15**, 463.
Euler and Myrbäck (1929). *Z. physiol. Chem.* **184**, 163.
Kinnersley, Peters and Reader (1928). *Biochem. J.* **22**, 276.
Neuberg (1913, 1). *Biochem. Z.* **49**, 502.
—— (1913, 2). *Biochem. Z.* **51**, 484.
—— and Kobel (1928). *Biochem. Z.* **203**, 463.
Reader (1930). *Biochem J.* **24**, 1827.
Sakuma (1930). *J. Biochem.* **12**, 247.
Vogt (1929). *Biochem. Z.* **211**, 17.
Vogt-Møller (1931). *Biochem. J.* **25**, 418.