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SYNTHESIS OF FAT FROM CARBOHYDRATE IN THIAMINE DEFICIENCY

by

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A number of papers published by MC HENRY and his collaborators[1,2,3] have led to a wide-spread acceptance of the view that the synthesis of fat from carbohydrate is impaired in thiamine deficiency. The evidence put forward by these authors to substantiate this claim is, however, inconclusive. In most of their experiments the groups of rats and pigeons compared had consumed different amounts of food and only one experiment is published in which paired feeding of a fat-free diet was applied to rats (Ref. 1, p. 294). As only the mean results of this experiment are stated it is impossible to decide whether or not the small difference observed between the means is statistically significant.

BOXER AND STETTEN[4], using the incorporation of deuterium into the body fatty acids of rats as a measure of fat synthesis, arrived at the conclusion that the failure of synthesis and deposition of fatty acids in thiamine deficient rats "is attributable chiefly to the diminished food intake rather than to any specific action of thiamine". Whether any specific action of thiamine exists at all cannot be decided as the amount of food consumed was the limiting factor in these experiments.

Therefore we have carried out experiments on pigeons with forced feeding of large amounts of carbohydrate. In a first experiment 30 pigeons received 2 g thiamine-free casein and 18 g sucrose daily, supplemented with a salt and vitamin mixture including thiamine. After 18 days ten animals were sacrificed and their fat contents determined (Group I); half of the remaining number continued on this food (Group II), while the others received the same food, but with omission of thiamine (Group III). When, after 14 days, symptoms of thiamine deficiency appeared in the animals of Group III, all animals of Groups II and III were sacrificed. The mean figures for the fat contents found are 36.9 g (9.3% of body weight) for Group I, 44.6 g (10.3%) for Group II, and 43.6 g (11.5%) for the thiamine deficient Group III. The differences between these figures are not statistically significant. Presuming that the carbohydrate ingested is at least partly metabolized via fat[5,6], one should conclude that the synthesis of fat from carbohydrate is not impaired in thiamine deficiency.

In a second experiment with 24 pigeons, which had been subjected to semi-starvation (in order to exhaust their fat deposits) and to partial depletion of the thiamine pyrophosphate stores of their tissues, a direct proof of unimpaired fat synthesis with a net increase of fat content in thiamine-deficient pigeons could be given. The pigeons were divided in three groups of eight pigeons each, numbered IV, V and VI. Group IV was sacrificed after the starvation and depletion period; Group V received 3 g casein and 24 g sucrose daily with salts and vitamins, including thiamine in abundance. Group VI received the same food without thiamine. When, after six days, the animals of Group VI showed grave symptoms of thiamine deficiency all animals were sacrificed. Thiamine pyrophosphate determinations in the hearts showed that during this period Group VI had a moderate to grave

thiamine deficiency, while Group V was normal. The fat contents were 2.1 ± 0.18*% for Group IV; 4.3 ± 0.93% for Group V and 3.7 ± 0.44% for Group VI. These figures demonstrate that both Groups V and VI had synthesized fat from carbohydrate (P values for differences with Group IV < 0.05 and < 0.01 respectively).

Their mutual difference has no statistical significance whatever (P > 0.5).

Hence neither of these experiments has given the slightest indication that thiamine deficiency causes an impairment of the synthesis of fat from carbohydrate.

This work forms part of investigations on the metabolism and physiological function of thiamine carried out by H. G. K. WESTENBRINK and collaborators. Full details and discussion will appear shortly[7].

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* Standard deviation of the mean.

A RAPID DETERMINATION OF GLYCOGEN IN TISSUES

by

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A quick method for the determination of glycogen in tissues has been elaborated by combining a rapid procedure of extraction, viz. grinding the tissue sample with sand and trichloroacetic acid, with the very simple spectrophotometric method of glucose determination according to MENDEL AND HOOGLAND[1]. The latter makes use of a pink colour which develops when concentrated sulphuric acid is added to a glucose solution and the mixture is placed in a boiling waterbath for a few minutes. The glycogen need not be hydrolyzed before performing the glucose determination as 7 minutes' heating with the only slightly diluted sulphuric acid is sufficient to bring about hydrolysis of the glycogen as well as development of the colour.

The procedure is described below for the determination in rat liver and muscle tissue. The practically negligible amounts of free glucose present are included in the determination.

1 g of liver is ground in a mortar with 1 g quartz sand (purified by boiling with potassium bichromate and sulphuric acid) and 10 ml of a solution of 5% trichloroacetic acid and 0.1% silver sulphate, until a very fine suspension is obtained (this point is reached in about 2 minutes). The suspension is transferred to a volumetric flask of 50 ml, the mortar is rinsed with 10 ml of the trichloroacetic acid-silver sulphate solution and the volume is made up with water to 50 ml. Quartz sand and precipitate are removed by centrifuging.

According to MENDEL AND HOOGLAND the glucose determination is carried out by adding 1 ml glucose solution to 3 ml concentrated sulphuric acid, placing the mixture in a boiling waterbath for 7 minutes and measuring the extinction at 515 mμ. As the most suitable concentrations for a determination are situated between 100 and 200 γ glucose/ml, the glycogen determination can be carried out in 1 ml of the trichloroacetic acid-silver sulphate extract, when the glycogen concentration of the tissue does not exceed 1%. Extracts with higher contents must be diluted.

For muscle the following modifications are introduced in the above-mentioned procedure: 2 g of quartz sand should be used instead of 1 g; in order to obtain a sufficiently fine suspension it is