

PYRUVATE CONTENT OF BLOOD AND PHOSPHORYLATION OF THIAMINE IN DIABETIC COMA

by

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According to MARKEES AND MEYER¹ an increased content of pyruvate is found in the blood of patients in diabetic coma. It decreased by intravenously injecting thiamine pyrophosphate, but not, however, by injecting thiamine. The authors concluded that in this condition phosphorylation is inhibited. We have found, however, that in these investigations the pyruvate determinations, which were performed according to the method of FRIEDEMANN AND HAUGEN², have been carried out in such a way that pyruvate was determined together with acetyl acetate. Overnight storage of the deproteinized blood in the refrigerator, as prescribed by FRIEDEMANN AND HAUGEN and as had been done by MARKEES AND MEYER, did not appear to be efficacious for removing the acetyl

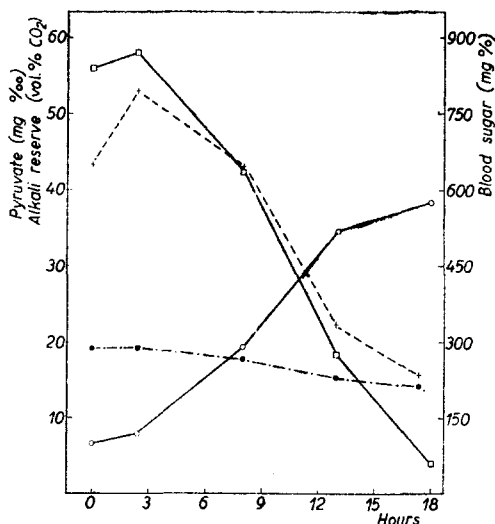


Fig. 1. Diabetic coma treated with several insulin injections.

Abscissa: hours after first injection.

- × - - - - × "pyruvate", without heating in boiling water bath
- - · - · - ● pyruvate, after 5 min in boiling water bath
- - - - - □ blood sugar
- - - - - ○ alkali reserve

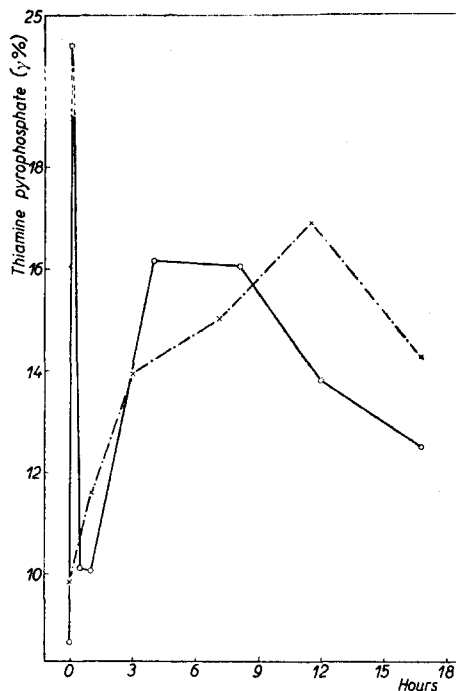


Fig. 2. Thiamine pyrophosphate in blood.

Abscissa: hours after injection.

- - - - - ○ injection of 100 mg thiamine pyrophosphate in normal subject
- × - - - - × injection of 60 mg thiamine in patient in diabetic coma

acetate. All acetyl acetate is decomposed, however, by placing the deproteinized blood in a boiling-water bath for 5 minutes. The pyruvate content is not affected by this treatment. After the conclusion of our experiments a note by MARKEES³ appeared, showing that also he had independently arrived at the same results.

Fig. 1 shows the results of the treatment with insulin of a patient in diabetic coma. In this experiment FRIEDEMANN AND HAUGEN's method was each time applied to two samples of deproteinized blood, one of which had been stored for 24 hours in the refrigerator, while the other had been placed in a boiling-water bath for 5 minutes. This figure proves that the pyruvate content of the blood was not higher than would have been found in a normal subject in the same state of muscular activity.

The question of the diminished phosphorylation remains. We found the thiamine pyrophosphate content of the blood in diabetic coma had not decreased. When thiamine was administered to a patient in diabetic coma by intravenous injection the thiamine pyrophosphate content of the blood slowly increases, just as happens in normal subjects, attaining a maximum several hours after the injection. Injected thiamine pyrophosphate disappears from the blood of normal persons as well as from that of the patients in 30 to 60 minutes. This initial rapid decrease of the thiamine pyrophosphate content was in both cases followed by a slow increase, similar to the increase obtained after thiamine injection (Fig. 2). The explanation can obviously be found in the rapid decomposition of injected thiamine pyrophosphate by the plasma phosphatase, followed by phosphorylation of the thiamine, thus formed, by the blood corpuscles⁴.

Hence we believe that in diabetes neither the breakdown of pyruvate, nor the phosphorylation of thiamine are affected.

Full details will be published.

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THE ENZYMIC BREAKDOWN OF DEOXYRIBOSENUCLEIC ACIDS

by

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From recent work on deoxyribonucleic acids (DNA), including the isolation of nucleoside-5-phosphates by successive treatment of DNA with deoxyribonuclease and phosphodiesterase^{1,2}, it seems probable that the majority of the nucleotides are joined through phosphate ester links on carbon atoms 3' and 5'.

Deoxyribonuclease depolymerises DNA, liberating some fragments which readily dialyse through Cellophane, and others which have been believed to be non-dialysable. Electrometric titration has shown that about 1 secondary phosphoryl dissociating group is liberated for every 4 nucleotide residues³. No free phosphate is formed. Some of the polynucleotide products must therefore be small, but while their partial separation has been effected by electrophoresis⁴ the analyses obtained on these preparations were not adequate to permit the identification of the constituents with any certainty. Using the paper chromatographic and electrophoretic techniques which we have developed for the separation of polyribonucleotides⁵, we have isolated and identified some of the enzymic breakdown products from DNA.

Herring sperm DNA⁶ (20 mg/ml) was digested with deoxyribonuclease⁷ (20 µg/ml) in 0.005 M MgSO₄ at pH 7 for 18 h. A trace of CHCl₃ was present to prevent bacterial growth. This digest was dialysed into water, allowing some of the smaller fragments to escape, and the dialysate concentrated *in vacuo*.

Preliminary fractionation of the dialysate was possible by paper chromatography in 70% iso-propanol-water (v/v), with NH₃ in the vapour phase, but separation into sharp bands was difficult.