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 corpuscles[4].

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

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From recent work on deoxyribosenucleic 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 residues[3]. No free phosphate is formed. Some of the polynucleotide products must therefore be small, but while their partial separation has been effected by electrophoresis[4] 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 polyribonucleotides[5], we have isolated and identified some of the enzymic breakdown products from DNA.

Herring sperm DNA[6] (20 mg/ml) was digested with deoxyribonuclease[7] (20 $\mu$g/ml) in 0.005 *M* $MgSO_4$ at pH 7 for 18 h. A trace of $CHCl_3$ 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 $NH_3$ in the vapour phase, but separation into sharp bands was difficult.

Consequently, to enhance the relative differences in numbers of ionising groups carried by the polynucleotides, their terminal phosphoryl groups were removed by treatment of the dialysate with prostate phosphomonoesterase. Dinucleotides would thus be converted to dinucleoside monophosphates, and trinucleotides to dinucleotide esters of nucleosides. It will be noted that besides facilitating the chromatographic and electrophoretic separations, this removal of the phosphate groups enables one to determine the molecular size from an analysis of bases and phosphorus.

The digest was run on a paper chromatogram in the *iso*propanol-$NH_3$ solvent and the constituents, detected by ultraviolet light photography[8], were resolved into 7 bands. The lowest three bands (nos. 5, 6 and 7) were eluted, dried at 55°, and run on paper electrophoresis in 0.05 *M* ammonium formate buffer, pH 3.5. Each then gave several distinct bands which were eluted, dried and analysed for purines and pyrimidines by chromatography[6], and for phosphate[9].

A number of dinucleoside monophosphates were identified. As an example we shall take deoxyadenosine, deoxycytidine monophosphate diester (A–p–C). This is found in band 5 of the *iso*propanol $NH_3$ chromatogram, which on electrophoresis at pH 3.5 gives 4 bands, including a substance with a movement towards the anode of 2 cm in 2 h at 20 v/cm. Analysis showed this to contain very nearly equimolar proportions of adenine, cytosine and phosphorus. Its mobility is about that calculated for A–p–C. Confirmation of the existence of A–p–C has been obtained from the isolation by similar methods from the original deoxyribonuclease digest, before phosphomonoesterase treatment, of the dinucleotide of deoxyadenylic and deoxycytidylic acids (AC). AC was found to have an electrophoretic movement of 8.3 cm in 2 h at 20 v/cm (calculated 8.9 cm[5]) and contained 0.98 moles adenine/1.00 moles cytosine/1.98 moles P (theoretical 1/1/2). Phosphomonoesterase converted this entirely to free phosphate and a substance which moved 2.7 cm in 2 h at 20 v/cm and contained 1.01 moles adenine/1.00 moles cytosine/1.01 moles P. The latter substance which is identical with that isolated from band 5 is therefore A–p–C. By similar means we have recognised five other dinucleoside phosphates (Table I). Such substances must have been derived from dinucleotides in the original digest by removal of the terminal phosphoryl groups with the phosphomonoesterase treatment. We have not found any mononucleotides in any of the digests.

With a view to elucidating the action of deoxyribonuclease we are investigating the structure of the larger fragments liberated including the so-called non-dialysable core, which we have found consists of a mixture of polynucleotides with an average chain length of 5–6 nucleotides, and is under appropriate conditions readily dialysable.

TABLE I

THE PROPERTIES OF SOME DINUCLEOSIDE MONOPHOSPHATE DIESTERS FROM DNA

A = deoxyadenosine, G = deoxyguanosine, C = deoxycytidine, T = thymidine and p indicates a phosphoric acid residue joining two nucleosides.

| *Substance* | *Molar ratio of bases found* | *Position on chromatogram* | *Electrophoretic mobility (cm/2 h at 20 V/cm)* |
|---|---|---|---|
| A–p–C | A/C 0.97 | Band 5 | 2.0 cm |
| A–p–T | A/T 0.97 | Band 6 | 6.2 |
| G–p–T | G/T 0.99 | Band 5 | 9.0 |
| C–p–T | C/T 1.05 | Band 6 | 3.9 |
| T–p–T | | Band 7 | 10.9 |
| C–p–C | | Band 5 | 0 |

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