This method has also been applied to the degradation of the dinucleoside monophosphates obtained from guanylyl uridylic acid, adenylyl cytidylic acid and adenylyl uridylic acid, and in each case the dialdehyde derivative yielded, on mild alkaline treatment, either guanylic acid *b* or adenylic acid *b*. Thus both purine nucleotides occur in the dinucleotides (and presumably in ribonucleic acid) as the *b* isomers, which are probably the nucleoside 3′-phosphates.

The possibilities of this procedure, by means of which the phosphate terminal nucleotide of a dinucleotide may be removed, are at once apparent. The conditions under which the experiment is carried out are so mild that any polynucleotide would be quite stable, and by a repetition of the procedure it should be possible to determine the nucleotide sequence in a polynucleotide chain. As the next step we intend to apply the method to the investigation of trinucleotides.

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P. R. Whitfeld
Roy Markham

Agricultural Research Council
Plant Virus Research Unit,
Molteno Institute,
Cambridge.
April 10.

[1] Carter, C. A., *J. Amer. Chem. Soc.*, **72**, 1466 (1950).
[2] Cohn, W. E., *J. Amer. Chem. Soc.*, **72**, 2811 (1950).
[3] Brown, D. M., and Todd, A. R., *J. Chem. Soc.*, 44 (1952).
[4] Markham, R., and Smith, J. D., *Biochem. J.*, **52**, 552 (1952).
[5] Cohn, W. E., *J. Cell. Comp. Physiol.*, **38**, supp. 1, 21 (1951).
[6] Markham, R., and Smith, J. D., *Biochem. J.*, **52**, 558 (1952).

Enzymatic Splitting of Purine Internucleotide Linkages

The nature of the phospho diester internucleotide linkages involving purine residues of ribonucleic acid has not been previously demonstrated. In the preceding communication, it was decided that purine nucleotides occur in ribonucleic acid as the *b* isomers. We have reached the same conclusion from enzymatic studies with a spleen fraction. Volkin and Cohn[1] had already isolated *b* nucleotides of all the bases after digestion of ribonucleic acid with a crude spleen extract[2]. However, this did not establish the position of the phosphoryl group (2′- or 3′-) in the original diester, since the occurrence of nucleoside 2′ : 3′-phosphates as intermediates was not ruled out. These cyclic compounds, if formed in the course of hydrolysis, would allow the possibility of phosphoryl migration. In the present investigation, we have found that guanylic acid *b* and adenylic acid *b* resulted from the digestion of the ribonuclease-resistant 'core' with a fraction from spleen, and no cyclic compounds were observed at any time. Furthermore, it has been shown that the cyclic mononucleotides were quite slowly attacked by the enzyme and therefore would have accumulated, had they been intermediates.

The enzyme preparation was one of several fractions previously separated from calf spleen[3]. Fresh tissue was homogenized in cold 8·5 per cent sucrose, adjusted to *p*H 5 and centrifuged. The precipitate was washed with 8·5 per cent sucrose and then with acetone (— 10°). From the dry acetone powder it was possible to extract the enzyme activity in soluble form with 0·2 *N* acetate buffer, *p*H 6. The extract was fractionated with ammonium sulphate at *p*H 5 and again at *p*H 8.

This fraction was incubated with 'core' prepared from ribonucleic acid by exhaustive digestion with ribonuclease followed by dialysis against tap water. Both yeast and turnip yellow mosaic virus were used as sources of ribonucleic acid. At least 90 per cent of the purine residues of 'core' were liberated as *b* nucleotides, as determined by paper chromatography[4]. No cyclic intermediates were found at any time during the digestion.

Now it was necessary to establish that our failure to observe cyclic mononucleotides was not due to their being rapidly hydrolysed. Accordingly, we compared the rate of formation of purine mononucleotides from 'core' with the rate of hydrolysis of cyclic guanylic and adenylic acids. The incubations were carried out at 37° in a total volume of 1 ml., containing 80 μmoles of potassium phosphate buffer, *p*H 7·4, 0·3 mgm. of spleen fraction and substrate. As an example, 9·6 μmoles of guanylic acid *b* were derived from 5 mgm. of 'core' in 1 hr. During this time, 1·8 μmoles of guanosine 2′ : 3′-phosphate were hydrolysed to the extent of only 16 per cent, and 0·3 μmole was split to the extent of 70 per cent. Clearly, the guanylic acid was not formed by way of guanosine 2′ : 3′-phosphate. Under these conditions, the cyclic compound would be expected to accumulate because of its slow rate of hydrolysis, but none was detected. In parallel experiments 0·3 and 0·6 μmole of guanosine 2′ : 3′-phosphate was actually added to a mixture of 'core' and enzyme. Hydrolysis of 'core' proceeded, and most of the added cyclic compound survived an hour's incubation. Cyclic adenylic acid was somewhat more rapidly split by the spleen preparation, but its accumulation could also be expected, had it been formed as an intermediate.

Similar observations were made with the dinucleotides of guanine and cytosine, guanine and uracil, adenine and uracil, and adenine and cytosine. Preparation of these compounds is described elsewhere[5]. All were split to give *b* nucleotides of purine bases, without the appearance of cyclic mononucleotides. Again, as an example, 1·25 μmoles of the dinucleotide of guanine and cytosine were hydrolysed to the extent of 85 per cent in 1 hr., with no accumulation of guanosine 2′ : 3′-phosphate. Under similar conditions, 1·3 μmoles of guanosine 2′ : 3′-phosphate were incubated with spleen fraction and 50 per cent still remained after 3 hr.

From these findings we conclude that the purine nucleotide residues in ribonucleic acid occur largely in the *b* form.

One of us (L. A. H.) is on leave from the National Institutes of Health, Bethesda, Maryland.

Leon A. Heppel
Roy Markham

Agricultural Research Council
Plant Virus Research Unit,
Molteno Institute, Cambridge.

R. J. Hilmoe

National Institutes of Health,
Bethesda, Maryland, U.S.A.
April 10.

[1] Volkin, E., and Cohn, W. E., *Fed. Proc.*, **11**, 303 (1952).
[2] Maver, M. E., and Greco, A. E., *J. Biol. Chem.*, **181**, 861 (1949).
[3] Heppel, L. A., and Hilmoe, R. J., *Fed. Proc.*, **12**, 217 (1953).
[4] Markham, R., and Smith, J. D., *Biochem. J.*, **49**, 401 (1951).
[5] Markham, R., and Smith, J. D., *Biochem. J.*, **52**, 558 (1952).