

fundamentals, and showed by adapting the equations of Lagrange that in certain systems the part played by energy in linear systems is played by areas under a curve in the case of non-linear systems.

Manual control as distinct from automatic control was not forgotten. The paper on this subject by J. D. North included a thorough analysis of a 'model' that may provide a useful approximation for the response of hand to eye in some simple situations such as target tracking or car driving.

The discussions throughout the conference were vigorous and sustained. It appeared to be the opinion of all who attended that the conference has helped to lay foundations on which much further progress may now be based.

The papers presented, together with selected material from the discussions during the conference, will be published in due course as a single volume by Butterworths Scientific Publications, Ltd.

A. TUSTIN

## STRUCTURE OF RIBONUCLEIC ACID

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IN the past few years the basic constitution of several ribonucleic acids has been established<sup>1-4</sup>, but so far there has been little evidence as to the way in which the constituent nucleotides are arranged in the molecules. Hydrolysis of ribonucleic acids by alkali and by cold dilute acids gives rise to isomeric nucleotides which are probably 2' and 3' nucleoside phosphates<sup>5-7</sup>, and Cohn and Volkin<sup>8</sup> have recently also identified 5' nucleoside phosphates in ribonucleic acids digested successively with both ribonuclease and alkaline phosphatase. In the work which we propose to describe, we have attempted to isolate the larger fragments produced by ribonuclease action, and we have succeeded in establishing the structure of certain of the dinucleotides which were formed.

### Anhydrides of the Pyrimidine Nucleotides

In our investigation of the products of digestion of ribonucleic acids, our attention was drawn at once to the presence of two substances which we have called *C* and *U*<sup>9</sup>. These substances are digested slowly by ribonuclease to give free cytidylic and uridylic acids respectively, and each contains one atom of phosphorus per pyrimidine residue. They differ from the nucleotides in that they have higher  $R_F$  values in 70 per cent v/v isopropanol-water-ammonia (solvent *a*), and lower  $R_F$  values in 80 per cent saturated ammonium sulphate in water, 2 per cent isopropanol, 18 per cent *M* sodium acetate (v/v/v) (solvent *b*). These properties led us to believe that they contained less free hydrophylic groups than exist in the corresponding nucleotides, possibly because they were dinucleotides or dinucleoside pyrophosphates. When we were doing this work, we were fortunate in having a discussion with Prof. A. R. Todd and Dr. D. M. Brown, and they were kind enough to disclose their theory of the formation of the isomeric nucleotides of Carter and Cohn. This theory<sup>7</sup> necessitates the existence as

intermediates during the chemical hydrolysis of ribonucleic acids of cyclic anhydrides of the nucleotides, which have the phosphoric acid residue esterifying the ribose at positions 2' and 3'. Such substances would have the properties of our *C* and *U*, and, in addition, would have the same electrophoretic mobilities as the corresponding nucleotides at pH 3.5, as do *C* and *U*, while, as we will show, the electrophoretic mobility of a dinucleotide should be about 1.54 times that of a mononucleotide having the same constituents. Synthetic samples of the anhydrides of cytidylic and uridylic acids prepared by Mr. D. Magrath were chromatographed by us in several solvent systems and could not be distinguished from *C* and *U* in any of these, while they too were digested slowly by ribonuclease to give the free nucleotides. It seems very likely that *C* and *U* are the 2', 3' anhydrides of cytidylic and uridylic acids respectively. The analogous adenine derivative was not digested by ribonuclease, and it seems probable that the guanine derivative is also resistant to digestion.

### Free Nucleotides and Polynucleotides

For preparing these substances we have digested purified yeast ribonucleic acids with ribonuclease in dialysis sacs at 20° and also at 4° with stirring, so that any fragments liberated should have a chance to escape further digestion. After concentration *in vacuo*, the dialysate has been chromatographed in solvent *a* on Whatman No. 3 paper in bands. The developed chromatograph shows a residue at the top (band 0) and six main bands numbered 1-6 in order of their  $R_F$  values. Band 6 contains *C* and *U* only. Bands 1, 2 and 3 contain compounds of all four bases, while bands 4 and 5 have no guanine derivatives. Band 4 contains only adenylic acids *a* and *b*, cytidylic and uridylic acids. Free guanylic acids *a* and *b* are found in band 2. Bands 3 and 5 contain substances having properties similar to *C* and *U*; that is, they are digested by ribonuclease to give substances running faster in solvent *b* and slower in solvent *a*, but without changing their gross analytical composition. The analyses, however, show that each band contains several substances, as the purine and pyrimidine bases are not present in simple proportions.

### Electrophoretic Separation

After trying without success a number of solvent systems for separating the substances in each band, we decided to try electrophoresis on filter paper strips, immersing the wet filter paper in carbon tetrachloride to cool it and to prevent evaporation. Strips of Whatman No. 3 paper 56 cm. long and 8 cm. wide were used, and the material was applied about 12 cm. from one end. A voltage gradient of 20-25 V./cm. was used and the average run was two hours (Fig. 1).

Before attempting the separation, we calculated the probable relative electrophoretic mobilities of all mono-, di-, and some tri-nucleotides at several pH values, using the dissociation constants of Levene and Simms<sup>10</sup>, assuming that the mobilities were proportional to the net charge on the molecule, and inversely proportional to the cube root of the molecular volumes, a relationship which should hold to a close approximation in the case of such asymmetrical molecules, and which has been found to hold with some minor deviations in practice. The

CALCULATED ELECTROPHORETIC MOBILITIES TOWARDS THE POSITIVE ELECTRODE OF ALL THE MONO- AND DI-NUCLEOTIDES AND SOME OF THE TRI-NUCLEOTIDES AT pH 3.5, RELATIVE TO THAT OF URIDYLIC ACID. The symbols *A*, *G*, *C* and *U* signify the presence in the compound of one residue of adenylic, guanylic, cytidylic and uridylic acid respectively. The order in which they appear is not significant.

Mononucleotides	Dinucleotides	Trinucleotides
<i>C</i> 0.16	<i>CC</i> 0.25	<i>ACC</i> 0.54
<i>A</i> 0.46	<i>AC</i> 0.48	<i>AAC</i> 0.75
<i>G</i> 0.95	<i>AA</i> 0.71	<i>GCC</i> 0.88
<i>U</i> 1.00	<i>GC</i> 0.85	<i>ACG</i> 1.09
	<i>CU</i> 0.89	<i>ACU</i> 1.13
	<i>AG</i> 1.08	<i>AAG</i> 1.30
	<i>AU</i> 1.13	<i>AUA</i> 1.33
	<i>GG</i> 1.46	<i>AGG</i> 1.84
	<i>GU</i> 1.50	<i>GCU</i> 1.67
	<i>UU</i> 1.54	<i>AUU</i> 1.71

order of movement that we calculated for pH 3.5 (*M*/20 ammonium formate buffer)—the value which we selected as optimum for our purposes—is shown in the accompanying table. At this pH the mobility is decided entirely by the primary phosphoric acid —OH<sup>-</sup> groups and the —NH<sub>2</sub><sup>+</sup> groups of the bases, and therefore anhydrides of the type of *C* and *U* and their derivatives will move at approximately the same speed as the corresponding nucleotides and their derivatives.

On electrophoresis, bands 1, 2, 3 and 5 are each found to contain at least three or four major constituents, most of which have been identified provisionally, while several of these substances have been submitted to analysis and have been found to have simple compositions. Among the latter are the dinucleotides of adenine and guanine, adenine and cytosine, guanine and uracil, and guanine and cytosine. These substances are found in bands 1 and 2 and are not attacked by ribonuclease; but there are also substances having the same composition with regard to the bases as the last three of these dinucleotides, but occurring in bands 3 and 5. The latter are digested by ribonuclease to give the dinucleotides only. As an example of this we can take the guanine-uracil dinucleotide of band 1. This substance can be isolated in a pure state from band 1 by electrophoresis, and is the fastest moving component of this band, having a gross mobility of  $1.1 \times 10^{-4}$  cm.<sup>2</sup> sec.<sup>-1</sup> volt<sup>-1</sup>. In band 3 there is a substance which also has one equivalent of guanine to one of uracil and has a mobility of  $1.3 \times 10^{-4}$  cm.<sup>2</sup> sec.<sup>-1</sup> volt<sup>-1</sup> (this difference is not significant). The substance from band 3 is converted into the

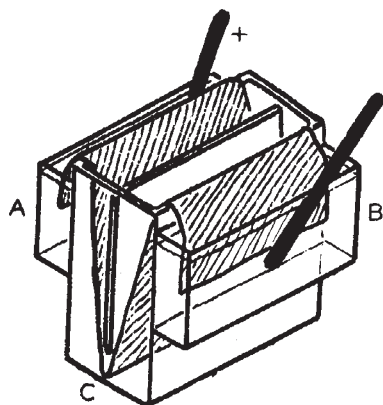


Fig. 1. Electrophoresis apparatus. Vessels *A* and *B* contain the buffer solution. Vessel *C* contains carbon tetrachloride, and in use is covered with a piece of plate glass. The wet paper is spread in vessel *C* by a piece of celluloid. The electrodes are arc carbons

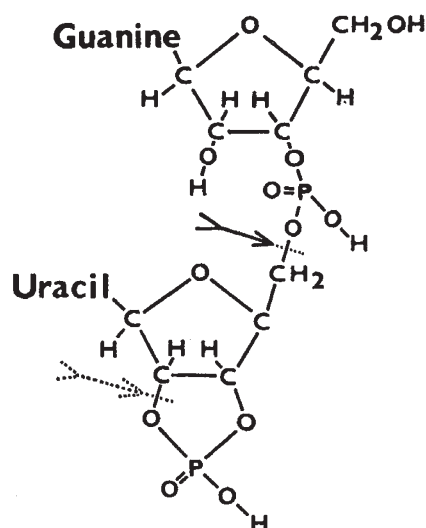


Fig. 2. Probable structure of the guanine-uracil dinucleotide anhydride. The arrows show the alkali labile bonds, while the dotted arrow indicates the bond which is also broken by ribonuclease

dinucleotide of band 1 slowly by ribonuclease digestion. This digestion is not splitting a tetranucleotide, as such a compound would have a mobility of about  $2 \times 10^{-4}$  cm.<sup>2</sup> sec.<sup>-1</sup> volt<sup>-1</sup>, nor, of course, is any phosphorus liberated. Similarly, the adenine-cytosine dinucleotide of band 5 is converted by ribonuclease to that in band 2, as is the adenine-uracil dinucleotide of band 5; and all these digestions also cause the resulting dinucleotides to have higher *R<sub>F</sub>* values in solvent *b*.

Now these properties parallel exactly the behaviour of the pyrimidine nucleotide 2', 3' anhydrides, so it would seem that the dinucleotides of bands 3 and 5 contain the latter joined at position 5' as one half of the molecule.

The purine nucleotides derived from these dinucleotides by alkaline hydrolysis are of the 2' and 3' types and the dinucleotides do not react with periodate, so that the internucleotide link must be of the 3' (or 2'), 5' type. The only likely formula for the guanine-uracil dinucleotide anhydride is given in Fig. 2 (it being understood that at the moment it is not possible to distinguish between a 2' and a 3' nucleotide residue in such a compound, as hydrolysis invariably results in the formation of roughly equal amounts of the 2' and 3' nucleotides).

### Discussion

It will be seen from these results that a large number of the internucleotide linkages in yeast ribonucleic acid are of the 3' (or 2'), 5' type and also that some at least of the alkali labile bonds of ribonucleic acid are situated at the 5' sugar carbon atoms. From this it would appear that the linkages broken by ribonuclease are similar to those broken by alkali, with the important exception that ribonuclease distinguishes between purine and pyrimidine nucleotides. We have found that, by cautious alkaline hydrolysis of ribonucleic acids, using barium carbonate at 100°, corresponding to a pH of just over 9, the anhydrides of the pyrimidine nucleotides are released, showing the analogy of the action of alkali and ribonuclease.

The general nature of the products of ribonuclease digestion suggests that, as in alkaline hydrolysis, it

is necessary for a cyclic intermediate to exist, and that this intermediate must also be a pyrimidine nucleotide derivative. This would explain the rapid formation of the large amounts of the anhydrides *C* and *U*, as they would be chopped out of the ribonucleic acid wherever two or more adjacent pyrimidine nucleotides having either a free 2' or 3' —OH group were to be found in the molecule. The existence in the digests of the free 2'- and 3'-guanylic and adenylic acids is difficult to account for by this theory, unless they were joined to pyrimidine nucleotides by a 2', 3' link (or the equivalent), as this link would allow the formation of the intermediate anhydride.

A study of the trinucleotides of band I and the larger products of ribonuclease digestion is now being made in an attempt to confirm this theory, and we are also hoping to be able to make quantitative estimates of the amount of the various constituents on the well-characterized and undegraded turnip yellow mosaic virus nucleic acid<sup>4</sup>.

It is a pleasure to thank Prof. D. Keilin and Dr. K. M. Smith for their interest in this work. We are also greatly indebted to Prof. A. R. Todd and his colleagues, notably Drs. Brown and Dekker, for discussing problems of mutual interest.

[August 7.

<sup>1</sup> Chargaff, E., Magasanik, B., Vischer, E., Green, C., Doniger, R., and Elson, D., *J. Biol. Chem.*, **186**, 51 (1950).

<sup>2</sup> Smith, J. D., and Markham, R., *Biochem. J.*, **46**, 509 (1950).

<sup>3</sup> Markham, R., and Smith, J. D., *Biochem. J.*, **46**, 513 (1950).

<sup>4</sup> Markham, R., and Smith, J. D., *Biochem. J.*, **49**, 401 (1951a).

<sup>5</sup> Carter, C. E., *J. Amer. Chem. Soc.*, **72**, 1466 (1950).

<sup>6</sup> Cohn, W. E., *J. Amer. Chem. Soc.*, **72**, 2811 (1950).

<sup>7</sup> Todd, A. R., and Brown, D. M., *J. Chem. Soc.* (in the press).

<sup>8</sup> Cohn, W. E., and Volkin, E., *Nature*, **167**, 484 (1951).

<sup>9</sup> Markham, R., and Smith, J. D., *Research*, **4**, 344 (1951b).

<sup>10</sup> Levene, P. A., and Simms, H. S., *J. Biol. Chem.*, **65**, 519 (1925).

## OBITUARIES

### Prof. A. J. Allmand, F.R.S.

PROF. A. J. ALLMAND, emeritus professor of chemistry in the University of London, died in hospital in London on August 11 after an operation.

He was the son of Frank Allmand and was born at Wrexham in 1885. He was educated at Alley'n's School, Dulwich, at Grove Park School, Wrexham, and at the University of Liverpool. He also studied at the technical high schools of Karlsruhe and Dresden. He took his D.Sc. in Liverpool in 1910 and then returned to study in Germany as a Science Research Scholar of the Exhibition of 1851. At Karlsruhe he worked under Prof. F. Haber on the measurement of radiation from flames, and at Dresden he worked under Prof. Luther on the primary products of photochemical change. He then took the post of assistant lecturer and demonstrator at Liverpool, where he was one of the group of chemists inspired by Donnan, whom he always regarded with gratitude and affection. At this period his work was naturally concerned with electrochemistry, and in 1912 he published his well-known text-book on the "Principles of Applied Electrochemistry", of which a revised edition was published in 1925.

During the First World War, Allmand was called upon to bring his knowledge and experience to bear upon the new problems of chemical warfare. He became chemical adviser to the Fourth, and later to

the Second, Army H.Q., with the rank of major, and during this period he was awarded the Military Cross. His Army service left him with an enduring enthusiasm for military life and history which is somewhat unusual among men of science, and in his library the official history of the war jostled with the *Proceedings (A) of the Royal Society*, and was studied with equal diligence and zest.

In 1919 Allmand was appointed professor of chemistry at King's College, London, where he spent the remainder of his academic life until his retirement in 1950. While maintaining his interest in electrochemistry and developing the Drumm transport battery, he worked mainly in two other fields, namely, in the adsorption of gases and vapours by charcoal, and in photochemistry. In the latter field he was specially interested in photolysis and the photochemical reaction between hydrogen and chlorine. In both these fields he carried out a large amount of accurate experimental work which played an important part in future developments.

In 1938 he succeeded Prof. S. Smiles as Daniell professor and head of the Department of Chemistry at King's College. He was a member of the Senate of the University of London from 1929 until 1932, and he acted as assistant principal of King's College during 1937–43. He was elected a Fellow of the Royal Society in 1929 and made a Fellow of King's College in 1932.

During the Second World War, Allmand was again called to serve his country, this time as superintendent of the Explosives Research Department in the Ministry of Supply—an exacting task which he combined with almost nightly fire-watching duties at King's College. After the War his research interests returned again to electrochemistry, and his most recent publication dealt with the simultaneous electrodeposition of zinc and copper from solution. It was fitting that his last official position was that of president of the Faraday Society, a post which he held during 1947–48.

Although Allmand was a great teacher and a very distinguished physical chemist, it is more by his extraordinary personal influence that he will be remembered rather than by his purely scientific contributions. He was one of the most loved and revered members of the Common Room at King's College. His Department was singularly happy and harmonious. He was always greatly interested in the work and welfare of all his students, both while they were at College and afterwards when they occupied posts in universities or industry. They in turn regarded him with warm affection, and, at his farewell dinner, every year since his appointment was represented by a group of former students.

He was a man of complete moral integrity, whose whole life was inspired by spiritual purposes. His advice and judgment were always sound, selfless and rigidly based on principle. He was severe and ascetic with himself, although never censorious of others. On the rare occasions when he expressed disapproval, he was decisive and final; but he will be remembered more readily for the warm encouragement of his approval and the frequent generosity of his help. He was one of the most modest and self-effacing men in the College; but he has left a deep and enduring influence and a noble example.

He leaves a wife, a son and a daughter. His elder son, Michael, was wounded in the Burma campaign and was posthumously awarded the Victoria Cross for great gallantry in action. G. TEMPLE