73. *The Constitution of Yeast Ribonucleic Acid. Part XV.* The Mode of Union of the Nucleotides.*

By A. S. Anderson, G. R. Barker, (the late) J. Masson Gulland, and M. V. Lock.

Yeast ribonucleic acid has been methylated by a method which is believed to avoid degradation of the molecule. From the behaviour of related nucleosides towards the methylating agent it is concluded that the extent of methylation of the carbohydrate groups is in agreement with the existence of internucleotide linkages between ribose and phosphoryl residues. Positive evidence for this has been previously lacking. After hydrolysis of the methylated polynucleotide, ribose, monomethyl ribose, and dimethyl ribose have been identified by paper chromatography in quantities which indicate a high degree of branching of the polynucleotide chain. At the sites of branching, ribose residues are believed to be triply phosphorylated, and in the terminal nucleotides of branches, they are singly esterified.

It has long been established that the nucleotides which constitute the yeast ribonucleic acid molecule are joined together through linkages involving the phosphoric acid residue of one nucleotide and a functional group of the next (for a discussion of the earlier literature, see Tipson, *Adv. Carbohydrate Chem.*, 1945, **1**, 193). No positive evidence has been obtained, however, regarding the nature of these functional groups concerned in the internucleotide linkages although it is known that they are not phosphoric acid residues (*i.e.*, the internucleotide linkage is not a pyrophosphoric ester grouping) and it is unlikely that amino- or enolic hydroxyl groups of the nitrogenous residues are the points of attachment, since they have been shown by electrometric titration to be unsubstituted (Levene and Simms, *J. Biol. Chem.*, 1926, **70**, 327; Fletcher, Gulland, and Jordan, *J.*, 1944, 33). Furthermore, destruction of the primary amino-groups causes no reduction in molecular weight (Fletcher, Gulland, Jordan, and Dibben, *J.*, 1944, 30). These facts have led to the acceptance of the assumption (Levene and Simms, *loc. cit.*) that the phosphoric acid residue of one nucleotide is esterified with a carbohydrate hydroxyl group of the next.

The only carbohydrate phosphoric ester linkages which can be said, with any degree of certainty, to exist in the polynucleotide are those which survive hydrolysis to simple

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nucleotides. It is now known that yeast ribonucleic acid can yield mixtures of isomeric nucleotides (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811; Loring, Luthy, Bortner, and Levy, *ibid.*, p. 2811; Lipkin and McElheny, *ibid.*, p. 2287), which, in spite of some evidence to the contrary (Doherty, Abstr. Papers, 118th Meeting, Amer. Chem. Soc., 1950, 56c), probably differ in the location of the phosphoryl residue on the sugar. More recently, Cohn and Volkin (*Nature*, 1951, **167**, 483) have demonstrated in the products of enzymic degradation of calf liver ribonucleic acid the presence of 5′-phosphorylated nucleosides differing from the isomers previously found. All these results are in accordance with the formulation of a ribopolynucleotide as a series of nucleosides which are united by double esterification with phosphoric acid and, on hydrolysis to monophosphoric esters, yield isomeric products by fission of one or other of the ester linkages. This type of structure cannot be regarded as proved unless first it can be shown that doubly esterified ribose residues are present in the nucleic acid molecule. In the work now to be described, uncombined ribose hydroxyl groups have been labelled by methylation and the number of hydroxyl groups originally phosphorylated has been determined by examination of the carbohydrates obtained by hydrolysis of the methylated nucleic acid.

Methylation of Yeast Ribonucleic Acid.—Of the techniques available for methylating sugars, that due to Purdie and Irvine (*J.*, 1903, 1021) using methyl iodide and silver oxide seemed the most suitable for the present purpose, but Levene and Jacobs record (Levene and Bass, "The Nucleic Acids," Chem. Catalog Co., New York, 1931, p. 208) that this reagent decomposes nucleosides and nucleotides faster than it methylates them, owing to the insolubility of the materials in organic solvents. It has now been found, however, that yeast ribonucleic acid can be methylated by vigorous shaking at 25° with methyl alcohol, methyl iodide, and silver oxide (Anderson, Barker, and Farrar, *Nature*, 1949, **163**, 445). The product resembled the original nucleic acid and contained approximately two methoxyl groups per nucleotide residue, one of which is presumed to be attached to phosphorus since it is removed by hot aqueous sodium hydroxide. In order to determine whether this method of methylation could give a true indication of the state of combination of the carbohydrate residues in the nucleic acid it was necessary to decide whether the treatment was accompanied by any degradation which might result in methylation of hydroxyl groups originally phosphorylated; and, conversely, whether the technique employed achieved methylation of all the uncombined carbohydrate hydroxyl groups. Determination of the diffusion coefficients of the methylated nucleic acid and of the starting material indicated that the nucleic acid had not suffered appreciable reduction in molecular size during methylation. As far as the authors are aware, carbohydrates have not previously been methylated under such mild conditions and, in order to determine the efficiency of the method and also the behaviour of nitrogenous systems towards the reagents, the nucleosides guanosine, adenosine, and uridine have been subjected to the same treatment.

Methylation of Nucleosides.—After repeated methylation, guanosine and adenosine yielded hygroscopic glassy products of rather indefinite composition which approximated to pentamethyl derivatives. After hydrolysis, 2 : 3 : 5-trimethyl D-ribose, identical with synthetic material (Barker, *J.*, 1948, 2035), was obtained in each case and the two methylated nucleosides yielded respectively a dimethylguanine and a dimethyladenine. Derivatives of these purines had melting points 6—7° higher than those recorded for corresponding compounds obtained after methylation of guanosine and adenosine with methyl sulphate (Bredereck, Müller, and Berger, *Ber.*, 1940, **73**, 1058). No evidence was obtained on which to base structures for the methylated purines but it is considered probable that they are identical with those prepared by the German workers and that the two methylating agents behave similarly towards the nucleosides. In no case was a monomethylguanine or monomethyladenine obtained as described by Bredereck, Haas, and Martini (*Ber.*, 1948, **81**, 307).

Methylation of uridine gave crystalline 1-methyl-3-(2 : 3 : 5-trimethyl D-ribosyl)uracil which, after hydrogenation and hydrolysis, yielded 2 : 3 : 5-trimethyl ribose and 4 : 5-dihydro-1-methyluracil. In none of the above methylated nucleosides was any partly methylated ribose present in sufficient quantity to be observed during fractional distillation

of the hydrolysis product. However, since in subsequent experiments with the methylated nucleic acid it was envisaged that the more sensitive techniques of partition chromatography would be used, an estimate of the completeness of methylation was made by examining the carbohydrate residues from the crude methylated nucleosides by this method. This showed in each case intensely staining spots corresponding to 2 : 3 : 5-trimethyl ribose. Other spots of lower R_F value were observed, which are believed to correspond to partly methylated riboses, but these more slowly moving components gave such faint spots that it was concluded that methylation was virtually complete and that incompleteness of methylation to this small extent could in no way influence the interpretation of results obtained with the methylated nucleic acid.

Since neither methylated guanosine nor methylated uridine possesses a methoxyl group in the nitrogenous part of the molecule, it is reasonable to assume that, of the methoxyl groups present in the methylated nucleic acid, those which are not removed by alkaline hydrolysis are located on the ribose residues. Therefore, since the initial rapid methylation stopped abruptly when an average of approximately one methoxyl group per ribose residue had been introduced into the polynucleotide, it is concluded that, on the average, two carbohydrate hydroxyl groups per residue are esterified with phosphoric acid. This is also in agreement with the approximate equality of the percentage of ether-methoxyl and ester-methoxyl groups. As far as is known, this constitutes the first positive evidence of the existence of multiple esterification of carbohydrate residues in yeast ribonucleic acid.

Examination of the Carbohydrate Residues of Methylated Yeast Ribonucleic Acid.—In order to facilitate the liberation of the pyrimidine-bound ribose, the methylated nucleic acid was hydrogenated until the absorption of the solution between 260 and 270 mμ was constant at approximately half its original value. The resulting material was hydrolysed with N/10-sulphuric acid at 100° for two hours, by which time the reducing power of the solution had become constant. Examination of the resulting mixture of sugars on the paper chromatogram revealed three fractions having the same R_F values as ribose, 5-methyl ribose (Levene and Stiller, *J. Biol. Chem.*, 1934, **104**, 299), and 2 : 3-dimethyl ribose (Barker and Lock, *J.*, 1950, 23) respectively. There was also an intensely reducing spot on the starting line, which contained organically bound phosphoric acid. This was unexpected since it has been shown (Levene and Jorpes, *J. Biol. Chem.*, 1929, **81**, 575) that hydrogenation of the pyrimidine ring reduces the stability towards acid, not only of the glycosidic linkage, but also of the phosphoryl group of cytidylic acid. It was found, however, that whereas acid hydrolysis of yeast ribonucleic acid results in a rapid mineralisation of the phosphoric acid residues of the purine nucleotides, followed by a slow hydrolysis of the pyrimidine nucleotides, no such discontinuity in the hydrolysis of the methylated polynucleotide could be discerned. Furthermore, fission of the carbohydrate–phosphoric acid linkage took place at approximately the same rate as the hydrolysis of the ester-methoxyl groups since examination of the products on the paper chromatogram after varying periods of hydrolysis, indicated that phosphorus-containing reducing substances were present on the starting line so long as mineralisation of the phosphoric acid was incomplete. For this reason it was necessary to carry out a prolonged hydrolysis in order to achieve complete liberation of the carbohydrate. This introduced the further difficulty that the liberated sugars suffered considerable decomposition which resulted in " streaking " on the chromatogram. The conclusion was reached that under no conditions could full recovery of the carbohydrates be achieved. It was therefore decided to investigate the manner in which the proportions of the three fractions travelling down the chromatogram varied with time of hydrolysis.

In order to apply the method of Hirst, Hough, and Jones (*J.*, 1949, 928) to the determination of carbohydrates in eluates from the chromatograms, it was necessary to remove purine bases which reacted with the hypoiodite ion under the conditions used. The hydrolysates were neutralised and concentrated and the residues extracted with methyl alcohol. Chromatography on paper of the resultant solution indicated that interfering substances had been largely removed. The carbohydrate fractions were eluted from the chromatogram, transferred to a second chromatogram, and developed again either with the same or with a different solvent. Eluates from transverse strips of the paper were

titrated with hypoiodite. Except where the aniline hydrogen phthalate spraying reagent indicated carbohydrate material, reducing substances were present on the chromatogram in only small concentration.

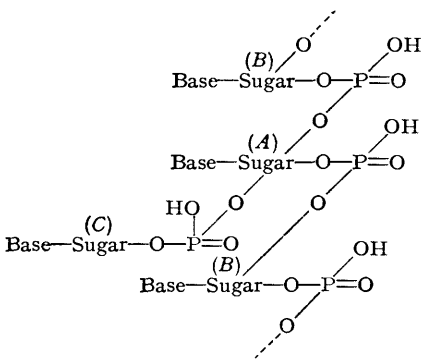
In the annexed table the proportions of what are believed, on the basis of their R_F values, to be ribose, monomethyl ribose and dimethyl ribose are seen to vary with the time of hydrolysis. Since it is not feasible to allow hydrolysis to proceed to completion, an absolute value for the ratios of the three fractions cannot be obtained. However, it is clear that, within the limits of the accuracy of the methods employed, the ratio of ribose

Time of hydrolysis (hours)	Molecular proportion of		
	ribose	monomethyl ribose	dimethyl ribose
3·5	0·95	0·6	1
60	1·1	1·35	1

to dimethyl ribose remains constant at about unity, whereas the proportion of monomethyl ribose increases with time of hydrolysis. Since test experiments indicated that demethylation during hydrolysis is negligible, it is reasonable to assume that the ratio of ribose to dimethyl ribose residues in the methylated polynucleotide is approximately unity, and that the molecular proportion of monomethyl ribose is something in excess of 1·4. After hydrolysis for 60 hours, approximately 70% of the phosphoric acid has been mineralised. Even if it is assumed, therefore, that further mineralisation would yield only monomethyl ribose, the proportion of this fraction would only rise to 2·8. Thus, although it is impossible to state with a high degree of certainty the proportions of the three carbohydrate fractions in the methylated polynucleotide, without attributing to the results greater accuracy than is warranted, it may be concluded that each fraction forms a major constituent of the mixture.

Discussion

From the results summarised above, it appears that triply, doubly, and singly phosphorylated ribose residues are present in the molecule of yeast ribonucleic acid. This conclusion involves three assumptions. First, that the unsubstituted ribose does not arise through failure to methylate all available hydroxyl groups; secondly, that the dimethyl ribose is not produced from simple nucleotides formed by degradation of the polynucleotide; thirdly, that the mixture of carbohydrates analysed is representative of the carbohydrate residues in the methylated polynucleotide. The first two assumptions have been discussed above and are considered to be justified. In every experiment, methylation resulted in the introduction of an average of one methoxyl group per ribose



residue. This means that the molecular ratio of ribose to dimethyl ribose should be unity, and this is found experimentally. Furthermore, the proportion of monomethyl ribose has been found to vary only with time of hydrolysis and to be otherwise reproducible. It is concluded, therefore, that incomplete recovery of carbohydrate incurred in working up the hydrolysate involves loss of each fraction to approximately the same extent. Thus the third assumption is also believed to be justified. On the basis of the results now presented, it is suggested that the ribonucleic acid of yeast consists of a branched chain

of nucleotides of the annexed type. In such an arrangement of nucleotides, ribose, monomethyl ribose, and dimethyl ribose would arise from residues marked (*A*), (*B*), and (*C*) respectively. On the basis of the quantitative results quoted above, branching is believed to occur on the average at every 2·4—3·8 nucleotides along a straight chain. Nothing can be said regarding the size of the branches or whether further ramification exists within them, since these factors would not affect the proportions of the carbohydrate fractions. Branching at phosphoric acid centres has previously been suggested by Fletcher, Gulland, and Jordan (*loc. cit.*), although doubts as to the validity of the conclusions have been expressed (Schmidt, *Ann. Rev. Biochem.*, 1950, **19**, 149). It must be emphasised, however, that branching at carbohydrate centres as is now postulated does not preclude the occurrence of branching also at phosphoric acid centres. On the contrary, no conclusion may be drawn from the present results concerning the numbers of primary and secondary phosphoric acid dissociations, in spite of what has been inferred by Schmidt (*loc. cit.*) and by Baddiley (*Ann. Reports*, 1950, **47**, 253) from preliminary publications of our results (Barker, Abstr. 1st Intern. Congr. Biochem., 1949, p. 216; Anderson, Barker, and Farrar, *loc. cit.*). Although no supporting evidence was produced, and no indication of its possible nature given, a branched structure has been suggested by Carter and Cohn (*J. Amer. Chem. Soc.*, 1950, **72**, 2604) to explain the preferential removal of pyrimidine nucleotides from yeast ribonucleic acid by ribonuclease. In the present studies it has not been possible to determine whether for instance the pyrimidine nucleotides are present largely in the side chains, owing to the stability of the ester groups of the methylated nucleic acid towards hydrolysis. However, it is proposed in the future to determine the degree of branching in various oligonucleotides.

The dimethyl ribose fraction, on prolonged chromatography, remains as a single spot, but the R_F value is found to be slightly different from that of 2 : 3-dimethyl ribose. Also, since periodate oxidation of the material on the paper (Buchanan, Dekker, and Long, *J.*, 1950, 3162) produces no formic acid, it is concluded that 2 : 5-dimethyl ribose alone is present. It is therefore tentatively suggested that the end nucleotides of branches are esterified at position 3′. If partition chromatography of the monomethyl ribose fraction is continued for a prolonged period, the spot exhibits a waist, the front part coinciding with the position of 5-methyl ribose. It appears, therefore, that the monomethyl ribose fraction contains at least two isomers, one of which may be 5-methyl ribose. In the unbranched parts of the molecule ester groups may therefore be present at any or all of the three possible positions. A comprehensive examination of the monomethyl and dimethyl ribose fractions is being undertaken but in any event, since at the sites of branching the ribose residues are triply esterified, the present results are in accordance with the isolation of isomeric nucleotides as reported by Cohn (*loc. cit.*). It is considered desirable now to record the results obtained so far, in view of the postulation of the new branched structure and the bearing which the results may have on researches in progress in other laboratories.

Experimental

Guanosine and adenosine were prepared by the method of Bredereck, Martini, and Richter (*Ber.*, 1941, **74**, 694), and uridine by that of Harris and Thomas (*J.*, 1948, 1936). Yeast ribonucleic acid (Pharmaco-Chemical Products Co.) was purified by the method of Sevag, Lackman, and Smolens (*J. Biol. Chem.*, 1938, **124**, 425).

Methylation Technique.—The following procedure was found to yield products from nucleosides and nucleic acid, the methoxyl content of which was not appreciably altered by further treatments. The dried material (2·8 g.), suspended in methyl alcohol (dried by the method of Lund and Bjerrum, *Ber.*, 1931, **64**, 210) (15 c.c.), was vigorously shaken at 25° for 24 hours with silver oxide (2·5 g.) and methyl iodide (2·5 c.c.). Four further additions of silver oxide (2·5 g.) and methyl iodide (2·5 c.c.) were made at intervals of 12 hours and shaking was then continued for a further 12 hours. Silver salts were removed by filtration and extracted three times with hot methyl alcohol (60 c.c.). Solvent was removed below 35° from the combined filtrates, leaving a pale straw-coloured syrup.

Methylated Purine Nucleosides.—The syrupy methylated product was repeatedly rubbed with ether, and the solvent removed under reduced pressure leaving, as a hygroscopic brittle

glass, methylated guanosine (Found: C, 49·9; H, 6·5; N, 17·7; OMe, 24·5. Calc. for $C_{15}H_{23}O_5N_5$: C, 51·0; H, 6·5; N, 19·8; OMe, 26·4%), or methylated adenosine (Found: C, 49·5; H, 6·4; N, 19·3; OMe, 28·5. Calc. for $C_{14}H_{21}O_4N_5$: C, 53·4; H, 6·8; N, 20·8; OMe, 27·6%).

Methylated Uridine.—The crude methylated product had b. p. 132°/10^{-4} mm. and from the distillate 1-*methyl*-3-(2 : 3 : 5-*trimethyl* D-*ribosyl*)*uracil* separated in colourless needles which, after recrystallisation from light petroleum (b. p. 60—80°), had m. p. 96—98° (Found: C, 52·4; H, 6·6; N, 9·6; OMe, 31·9. $C_{13}H_{20}O_6N_2$ requires C, 52·0; H, 6·7; N, 9·3; OMe, 31·0%). In subsequent experiments the crude methylated product crystallised without previous distillation.

Methylated Nucleic Acid.—The crude methylated syrup was rubbed with acetone and the pale straw-coloured amorphous solid was collected by centrifugation, washed with ether, and dried in a vacuum-desiccator over phosphoric oxide [Found, in two preparations: C, 39·0, 39·2; H, 5·9, 6·0; N, 12·6, 12·2; OMe, 16·3, 16·4. Calc. for $C_{53}H_{77}O_{28}N_{15}P_4$ (a polymerised tetranucleotide containing 8OMe, and 7NMe groups per tetranucleotide): C, 42·5; H, 5·2; N, 14·1; OMe, 16·6%]. A portion of the product (5·4 mg.) was heated at 100° with N/10-sodium hydroxide (0·4 c.c.) for 4 hours. After neutralisation with N/10-hydrochloric acid, the solution was evaporated to dryness in a vacuum-desiccator over phosphoric oxide, and the methoxyl content of the whole of the residue was determined (Found: OMe, 7·2%).

Measurement of Diffusion Coefficients.—The diffusion coefficients of purified yeast ribonucleic acid and of the methylated nucleic acid were compared either by the method of Fletcher, Gulland, Jordan, and Dibben (*loc. cit.*) or with Gage's apparatus (*Trans. Faraday Soc.*, 1948, **44**, 253). In the latter case, the material under test (approx. 50 mg.) was dissolved in N-sodium sulphate (50 c.c.) and allowed to diffuse through two layers of Whatman No. 1 filter paper into N-sodium sulphate (400 c.c.) for 2½ hours at 37°. The percentage of diffusion was calculated from measurements of optical density of the diffusate and the original diffusing solution. The ratio of the diffusion coefficients of the nucleic acid before and after methylation was found to be 1·14; cf. 1·06 (Fletcher, Gulland, Jordan, and Dibben, *loc. cit.*), 1·01 (Gage, *loc. cit.*).

2 : 3 : 5-*Trimethyl Ribose.*—(a) *From methylated guanosine and methylated adenosine.* The methylated nucleoside (5 g.), dried at room temperature in a vacuum over phosphoric oxide, was dissolved in methyl alcohol (25 c.c.) containing 10% of hydrogen chloride and set aside at room temperature for 72 hours. The solution (A, see below) was then poured into 4% aqueous sodium hydroxide (100 c.c.) with stirring, the temperature being kept below 20°, and was repeatedly extracted with chloroform leaving an aqueous residue B (see below). After drying of the combined extracts ($MgSO_4$), the solvent was removed under reduced pressure. The residue was distilled under reduced pressure, yielding trimethyl methyl-D-ribofuranoside, b. p. 130—136° (bath-temp.)/15 mm., n_D^{25} 1·4354 (Found: OMe, 59·9. Calc. for $C_9H_{18}O_5$: OMe, 60·2%). The trimethyl methyl-D-ribofuranoside was hydrolysed as described by Barker (*J.*, 1948, 2035), yielding 2 : 3 : 5-trimethyl D-ribose, b. p. 93—100°/0·01 mm., n_D^{25} 1·4522 (Found: OMe, 47·5. Calc. for $C_8H_{16}O_5$: OMe, 48·5%). The anilide had m. p. 55·5° alone or mixed with synthetic material (Found: N, 5·1; OMe, 34·6. Calc. for $C_{14}H_{21}O_4N$: N, 5·2; OMe, 34·9%).

(b) *From methylated uridine.* 1-Methyl-3-(2 : 3 : 5-trimethyl D-ribosyl)uracil (20 mg.) in water (1·5 c.c.) was hydrogenated in presence of Adams's platinum oxide catalyst at room temperature and pressure until the uptake of hydrogen corresponded to the reduction of one double bond. The solution, after removal of the catalyst, was made 0·2N with respect to sulphuric acid and boiled under reflux for 1½ hours. Sulphate ions were removed from the cooled solution by addition of barium carbonate and filtration. The filtrate was concentrated under reduced pressure and a sample was developed on the paper chromatogram with *n*-butyl alcohol–1% aqueous ammonia (Partridge, *Biochem. J.*, 1948, **42**, 238). A single component was observed having R_F 0·73. Synthetic 2 : 3 : 5-trimethyl ribose had R_F 0·72 on the same chromatogram. The product also had the same R_F values as the synthetic material in the following solvent systems: *n*-butyl alcohol–ethyl alcohol–1% aqueous ammonia (Partridge *loc. cit.*); *n*-butyl alcohol–acetic acid–water (Partridge, *loc. cit.*); ethyl acetate–pyridine–water (Jermyn and Isherwood, *ibid.*, 1949, **44**, 402).

Chromatographic examination of hydrolysates of methylated guanosine, methylated adenosine, and hydrogenated crude methylated uridine was carried out similarly.

Isolation of Methylated Adenine.—Solution A (above) obtained from methylated adenosine was cooled to 0° and the dimethyladenine hydrochloride which was deposited was collected by filtration. It separated from aqueous ethyl alcohol (85%) in colourless needles, m. p.

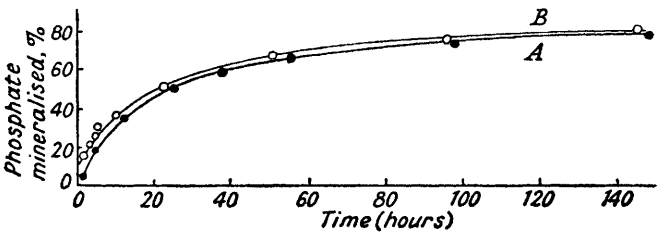
218—224° (decomp.) (Found : N, 35·3. Calc. for $C_7H_9N_5$,HCl : N, 35·1%). It was converted into the picrate, m. p. 242° (Found : N, 28·6. Calc. for $C_{13}H_{12}O_7N_8$: N, 28·6%).

Isolation of Methylated Guanine.—Solution B (above), obtained from methylated guanosine, was neutralised with acetic acid and then made alkaline with ammonia solution. The precipitated methylated guanine was collected by filtration and suspended in aqueous ethyl alcohol (85%), and hydrogen chloride was passed through the solution at 0° until crystallisation had taken place. The dimethylguanine hydrochloride was collected by filtration; it separated from ethyl alcohol in colourless needles, m. p. 282° (Found : C, 38·5; H, 5·5; N, 32·1. Calc. for $C_7H_9ON_5$,HCl : C, 39·0; H, 4·6; N, 32·5%).

4 : 5-*Dihydro*-1-*methyluracil.*—1-Methyl-3-(2 : 3 : 5-trimethyl D-ribosyl)uracil (0·55 g.) was hydrogenated and hydrolysed by sulphuric acid as described above, and, after removal of sulphate ions by barium carbonate and filtration, the solution was concentrated under reduced pressure to a small volume and percolated through a column of powdered cellulose. Elution of the column with light petroleum (b. p. 100—120°)–*n*-butyl alcohol (7 : 3) gave a fraction which after removal of the solvent yielded 4 : 5-dihydro-1-methyluracil which separated from ethyl alcohol in needles, m. p. 129° (cf. Hotchkiss and Johnson, *J. Amer. Chem. Soc.*, 1936, **58**, 525) (Found : C, 46·5; H, 6·5; N, 21·6. Calc. for $C_5H_8O_2N_2$: C, 46·9; H, 6·3; N, 21·9%).

Hydrolysis of Methylated Nucleic Acid.—The rate of hydrolysis of methylated nucleic acid by acids was determined under various conditions. A solution of the material (approx. 1 mg.

Hydolysis of methylated yeast ribonucleic acid.



A, in N/100-HCl; *B*, in N/10-HCl.

per c.c.) was added to an appropriate volume of either hydrochloric or sulphuric acid and heated in a boiling-water bath. At intervals aliquots were withdrawn and free phosphoric acid was determined by Fiske and Subbarow's method (*J. Biol. Chem.*, 1925, **66**, 375). With concentrations of acid between N/100 and N/5 and with both hydrochloric and sulphuric acids, hydrolysis proceeded at closely similar rates. Representative results are illustrated in the figure. A neutral solution of the methylated nucleic acid, hydrogenated and hydrolysed as described below, was examined by partition chromatography. The R_F values of the hydrolysis products are recorded in the annexed table.

Solvent system	R_F A	B	C	Solvent system		R_F A	B	C
Ribose	0·17	0·15	0·52	Hydrolysis products	(i)	0·16	0·16	0·52
5-Methyl ribose	0·34	0·33	0·62	,, ,,	(ii)	0·34	0·32	0·64
2 : 3-Dimethyl ribose ...	0·58	0·60	0·72	,, ,,	(iii)	0·57	0·60	0·78

A, *n*-Butanol–water; [1] B, *n*-butanol–water–1% aqueous ammonia; [1] C, ethyl acetate–pyridine–water.[2]

[1] Partridge, *Biochem. J.*, 1948, **42**, 238; [2] Jermyn and Isherwood, *loc. cit.*

Methylated yeast ribonucleic acid (100 mg.) was dissolved in water (30 c.c.) and hydrogenated at room temperature and pressure in presence of Adams's platinum oxide catalyst added portionwise (5 times 50 mg.) at intervals during 5 days. After the final addition of catalyst further hydrogenation produced no change in the absorption of the solution in the region of 260 mμ. After removal of the catalyst by filtration, the solution was made 0·5N with respect to sulphuric acid and was heated in a boiling water-bath. After varying periods of time the solution was cooled, neutralised with N-sodium hydroxide, and concentrated to dryness under reduced pressure. The residue was repeatedly extracted with boiling methyl alcohol (50 c.c.), and the combined extracts were concentrated under reduced pressure to small bulk and transferred to the paper chromatogram in a series of spots along the starting line. After development with ethyl acetate–pyridine–water (Jermyn and Isherwood, *loc. cit.*) and drying, one lane of the paper was removed and sprayed with aniline hydrogen phthalate (Partridge, *Nature*,

1949, **164**, 443), revealing the positions of the three sugar zones. Transverse strips carrying the three sugar fractions were cut from the remainder of the paper and were separately eluted with water (5 c.c.) applied dropwise to the paper from a burette. Test experiments indicated that this procedure removed all carbohydrates from the paper. Each eluate was then concentrated to small bulk and transferred to a second chromatogram, which was irrigated with the same mixture of solvents. The carbohydrate fraction on each of the three resultant chromatograms was estimated as previously described, by dividing the chromatogram into transverse strips, eluting the strips with water (5 c.c.), and subjecting the eluates to the hypoiodite titration procedure of Hirst, Hough and Jones (*loc. cit.*). The results were:

Time of hydrolysis, hours	Carbohydrate on chromatogram expressed as c.c. of N/100-sodium thiosulphate		
	Ribose	Monomethyl ribose	Dimethyl ribose
3·5	0·800	0·515	0·845
60	1·330	1·630	1·205

The presence of combined phosphoric acid in the eluates from chromatograms was detected by Fiske and Subbarow's method (*loc. cit.*). The presence of purines in the eluates from chromatograms was detected by means of the Beckman DU spectrometer.

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THE UNIVERSITY, NOTTINGHAM.
THE UNIVERSITY, MANCHESTER.

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