17. *The Constitution of Yeast Ribonucleic Acid. Part VIII. Electrometric Titration of the Acid Groups.*

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The titration of the acidic groups of samples of yeast ribonucleic acid has been performed electrometrically, hydrogen and glass electrodes being used. Yeast ribonucleic acid is found to possess four acid dissociations per tetranucleotide when existing as a polytetranucleotide, three of which are primary dissociations, and one a secondary dissociation of phosphoric acid. The deaminated acid is found to be similarly constituted. Mild hydrolysis reduces the molecular weight of the polytetranucleotide, and the titration results suggest that a further secondary dissociation of phosphoric acid is liberated. The formulæ which have been proposed previously for yeast ribonucleic acid are not in agreement with these data, and a modified formula is proposed; this is discussed in relation to the existing molecular weight and enzyme data.

The titration of the acid groups of yeast ribonucleic acid has been carried out previously by two methods: electrometrically, by Levene and Simms (*J. Biol. Chem.*, 1926, **70**, 327), Jorpes (*Biochem. J.*, 1934, **28**, 2102), and Allen and Eiler (*J. Biol. Chem.*, 1941, **137**, 757); and by titrating with alkali to the phenolphthalein end-point, thus titrating both the primary and the secondary phosphoric acid dissociation, by Makino (*Z. physiol. Chem.*, 1935, **232**, 229), Bredereck and Köthnig (*Ber.*, 1939, **72**, 121), and Bredereck and Jochman (*ibid.*, 1942, **75**, 395).

Levene and Simms titrated two different samples of yeast ribonucleic acid, and found 4·5 and 3·0 acid dissociations per tetranucleotide respectively at pH 8 (at this point the titration of primary and secondary dissociating groups of phosphoric acid is complete). The considerable discrepancy between their experimental

results and the theoretical curve constructed on the supposition that four primary and one secondary acid dissociations were present was accounted for by suggesting the formation of anhydrides, but this explanation was not further elaborated. The results obtained by Jorpes cannot be fully interpreted, since, for the purpose of comparing the titration curves of various mixtures of nucleic acid and nucleotides, a water-soluble salt of ribonucleic acid was titrated to pH 2 with an unknown quantity of acid prior to the titration with alkali. Allen and Eiler, using an Eastman preparation of yeast ribonucleic acid, found four acid dissociations per tetranucleotide at pH 8 after correcting for the presence of a small quantity of sodium salt present in the nucleic acid preparation.

Makino, using Merck's yeast ribonucleic acid, found that 2·86 acid dissociations were titrated at the phenolphthalein end-point (pH 8); this low figure was attributed to the presence of sodium nucleate, and on correcting for that part of the acid already neutralised, Makino obtained 3·73 acid dissociations per tetranucleotide. The sample on analysis showed a low phosphorus content, and Makino concluded that the acid was tetrabasic. On alkaline hydrolysis an additional 3·93 acid dissociations per tetranucleotide were obtained. Similar results were obtained for Boehringer and Takida preparations of yeast ribonucleic acid. Bredereck and his collaborators, who had found that consistent results could be obtained by titrating the desoxyribonucleic acid of thymus, observed that inconsistent results were obtained with yeast ribonucleic acid; but they concluded that most probably there were four acidic dissociations per tetranucleotide.

The titration of the acid groups of deaminated yeast ribonucleic acid to the phenolphthalein end-point has been attempted by Bredereck, Köthnig, and Lehmann (*Ber.*, 1938, **71**, 2613), who considered the impure product to be tetrabasic, but suggested that further purification might cause an increase in the number of acid dissociations. Bredereck and Hoepfner (*Ber.*, 1942, **75**, 1086) have recently prepared a material believed by them to be a deaminated tetranucleotide, which by titrating to the phenolphthalein end-point they found to be pentabasic, with the liberation of a further three acid dissociations on alkaline hydrolysis.

Results of the Present Investigation.—It will be convenient to follow other workers and calculate the results in terms of acid dissociations per tetranucleotide, assuming that this contains one of each of the nucleotides guanylic, adenylic, cytidylic, and uridylic acids.

The titration curve of yeast ribonucleic acid is given in Fig. 1, curves I and II. Curve I has been obtained for four different samples of purified B.D.H. yeast ribonucleic acid (Y1, Y2, Y21, Y8). Several separate titrations have been carried out on the sample Y1, and the results found to be accurately reproducible. A point of inflection is obtained at pH 8 which corresponds to the neutralisation of 3·4 acid dissociations per tetranucleotide. A similar curve (Fig. 1, curve II) was obtained for a purified sample of Boehringer yeast ribonucleic acid (BY1), a point of inflection occurring at pH 8 after the neutralisation of 3·6 acid dissociations per tetranucleotide. A second point of inflection has been obtained for all these preparations at pH 11·5 after the addition of a further 2·2 equivs. of alkali. The dissociating groups titrated in this pH range are most probably the hydroxyl groups of uracil and guanine in the 6-position, which are titrated over this range (Levene and Simms, *J. Biol. Chem.*, 1925, **65**, 519; 1926, **70**, 327; confirmed by us). The additional 0·2 equiv. in excess of the theoretical is to be attributed to a small amount of hydrolysis occurring in the alkaline solution. This suggestion is confirmed by the data obtained on back-titrating with acid from pH 12 immediately after titrating with alkali; an increase of 0·4 equiv. was obtained at pH 8. Very similar titration curves have been obtained for Merck (Y10, curve III) and Eastman (Y9, curve IV) preparations of yeast ribonucleic acid.

Samples of B.D.H. yeast ribonucleic acid (Y1) were hydrolysed at room temperature by standing in excess alkali (pH 12·5) and back-titrating with acid. The results of the back-titration are given in curve V, a similar curve being obtained after 22 days' and 38 days' hydrolysis. A point of inflection occurs at pH 8 corresponding to the neutralisation of 7·4 acid dissociations per tetranucleotide, an increase of 4 over the unhydrolysed substance. A second point of inflection may be discerned at pH 5, corresponding to the neutralisation of 3·6 acid dissociations. Similar results were obtained with the sample of Boehringer yeast ribonucleic acid (curve VI), which was hydrolysed by standing for 13 days at pH 11·8 at room temperature. Less alkali was used in this hydrolysis to increase the accuracy of the back titration. The cold alkaline hydrolysis (Levene, *J. Biol. Chem.*, 1923, **55**, 9; Jones and Perkins, *ibid.*, p. 567; 1924, **62**, 557; Calvery and Jones, *ibid.*, 1927, **73**, 73; Buell and Perkins, *ibid.*, 1927, **72**, 21; Calvery and Remson, *ibid.*, 1927, **73**, 593) transforms the yeast ribonucleic acid into nucleotides; provided that this hydrolysis were complete, four acid dissociations would be liberated per tetranucleotide if one acid group per nucleotide took part in the internucleotide linkage in the polynucleotide. This explanation is in agreement with the above data.

Bredereck and Hoepfner (*loc. cit.*) have described the preparation, by the mild alkaline hydrolysis of Boehringer yeast ribonucleic acid, of a substance which they consider to be a tetranucleotide. Their preparation has been repeated upon purified Boehringer yeast ribonucleic acid (BY2), and a sample of lower molecular weight isolated (BY21) (see Part VII, preceding paper). The titration of the original acid used for this preparation is given in curve VII, and that of the product of the mild hydrolysis in curve VIII; at pH 8, 4·5 acid dissociations have been titrated, an increase of 0·6 over the original acid. After hydrolysis of the Boehringer acid (BY2) in strong alkali at pH 12, as described above, curve IX was obtained on back titration with acid : it is in close agreement with curve VI, obtained with another sample of Boehringer yeast nucleic acid (BY1).

The results of the titration of deaminated yeast ribonucleic acid are given in Fig. 2, curve X, which has been obtained on several occasions (DY1, DY33, DY5). The titration curve exhibits three well-defined points of inflection : at pH 5, 8, and 12 after the neutralisation of 2·75, 4·5, and 7·6 acid dissociations, respectively. On

alkaline hydrolysis by standing at pH 12 for several days, 3·7 acid dissociations were titrated at pH 5, and 8·6 at pH 8 (curve XIII).

The deaminated yeast ribonucleic acid undergoes hydrolysis when its aqueous solution is heated. After 10 minutes' hydrolysis at 70° curve XI was obtained, and after 10 minutes or longer at 100° curve XII was obtained; no further liberation of acid groups occurred however long the time of hydrolysis. Curve XII indicates that one acid dissociation per tetranucleotide, titrated over the range pH 5—8, has been liberated by this hydrolysis.

Discussion.—The titration curve of yeast ribonucleic acid is not in agreement with the theoretical curve constructed from the known dissociation constants of the four primary phosphoric acid dissociations of the

FIG. 1.

The titration curves of various samples of yeast ribonucleic acid.

FIG. 2.

The titration curves of deaminated yeast ribonucleic acid.

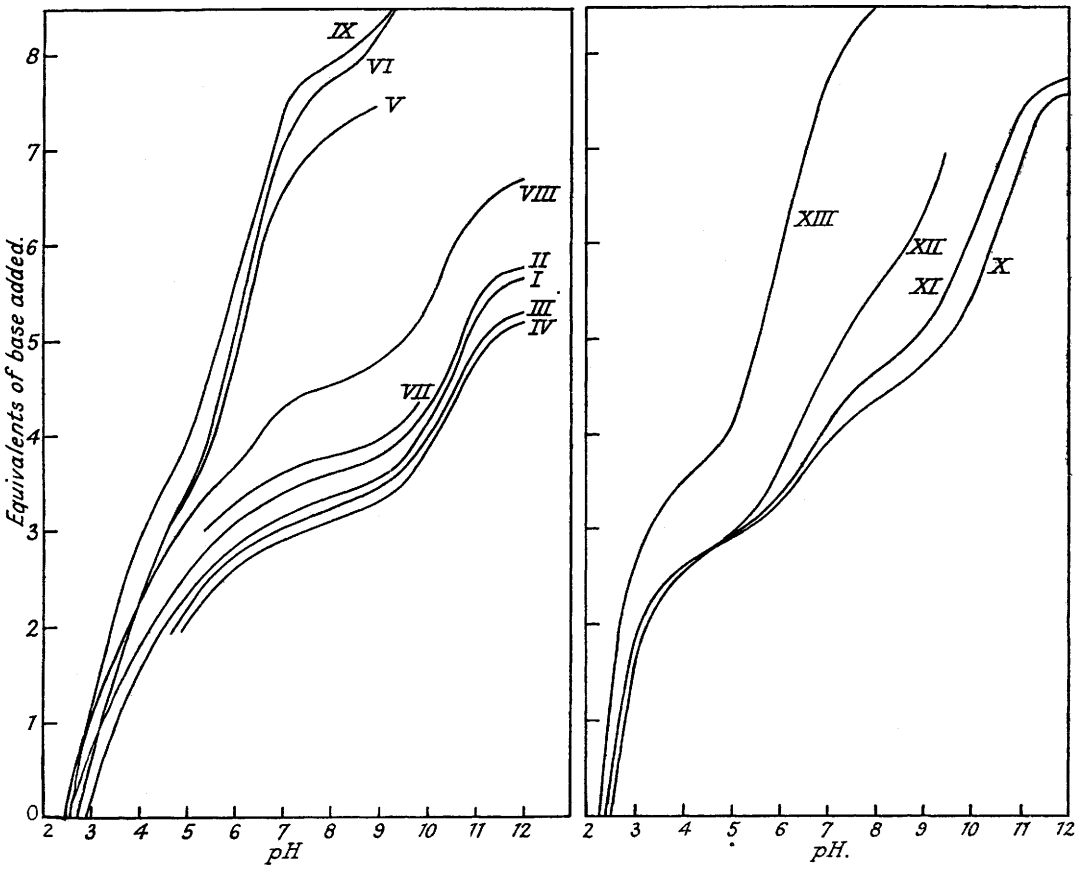


FIG. 1.—I, B.D.H.; II and VII, Boehringer; III, Merck; IV, Eastman. Back titration of the products of alkaline hydrolysis, V, B.D.H.; VI and IX, Boehringer; VIII, product isolated after the milk alkaline hydrolysis of Boehringer acid.

FIG. 2.—X, Deaminated yeast ribonucleic acid; XI, after hydrolysis at 70° for 10 minutes; XII, after hydrolysis at 100° for 10 minutes; XIII, back titration of the products of alkaline hydrolysis.

nucleotides guanylic, adenylic, uridylic, and cytidylic acid [pK: 2·3, 3·7, 1·0, and 4·2, respectively, as found by Levene and Simms (*J. Biol. Chem.*, 1925, **65**, 519) and confirmed by us], as shown by comparing curves XIV and XVII in Fig. 3, where curve XIV has been constructed from 0·4 equiv. of uridylic acid and 1 equiv. each of the three nucleotides guanylic, adenylic, and cytidylic acids, and curve XVII is the experimental curve obtained for B.D.H. yeast ribonucleic acid. This disagreement is always found however the 3·4 equivs. are chosen from the four nucleotides. This leads to the conclusion that one at least of the acidic groups of yeast ribonucleic acid must be a secondary phosphoric acid dissociation, and when curves are constructed on this basis, much closer agreement with the experimental results is obtained. This conclusion necessarily infers that one of the four atoms of phosphorus present in the tetranucleotide possesses no free acidic group, all taking part in internucleotide bonds. Furthermore, one phosphorus atom will have two free acidic groups and will be singly linked as in a nucleotide. Curves XV and XVI were constructed on this basis. The secondary dissociation constant of the phosphoric acid group was taken as having pK 6·0 for all the nucleo-

tides (Levene and Simms, *loc. cit.*; confirmed by us). For curve XV the presence of 0·7 equiv. of uridylic acid, 1·0 equiv. of guanylic and cytidylic acids, and 0·7 equiv. of a secondary group was assumed. It will be seen that a point of inflection occurs in curve XV at pH 4·5: this is always found in the theoretical curves when the primary group of uridylic acid is included. Curve XVI is a much more faithful reproduction of the experimental curve (XVII), and has been constructed on the assumption of the presence of 1 primary acidic dissociation of guanylic and adenylic acids and 0·7 equiv. each of the primary and the secondary dissociation of cytidylic acid; other distributions of the 2·7 equivs. among the nucleotides adenylic, guanylic, and cytidylic acids lead to curves closely similar to curve XVI. It would thus appear probable that the triply bound phosphorus atom is that of uridylic acid. Allen and Eiler (*loc. cit.*) realised that, in order to explain the titration curve obtained by them for yeast ribonucleic acid, one of the primary acid groups would have to have a lower dissociation constant than that normally accepted for the primary phosphoric acid group of a nucleotide, and they suggested that the ionisation of the fourth group was depressed by the ionisation of the other three phosphoric acid groups.

FIG. 3.

Comparison of the experimental and theoretical titration curves of yeast ribonucleic acid.

FIG. 4.

Comparison of the experimental and the theoretical titration curve of deaminated yeast ribonucleic acid.

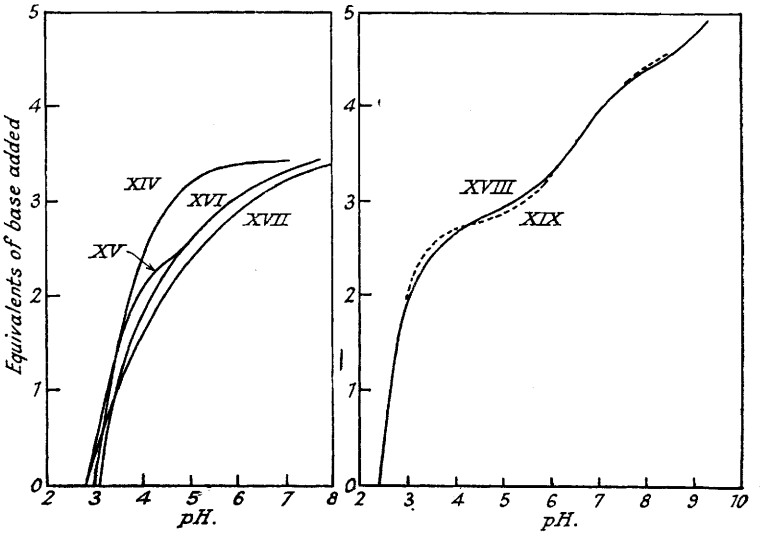


FIG. 3.—XIV. Theoretical curve constructed from 0·4 equiv. of the primary dissociation of uridylic acid, and 1·0 equiv. of the primary dissociations of guanylic, adenylic, and cytidylic acids.

XV. Theoretical curve constructed from 0·7 equiv. of the primary dissociation of uridylic acid, 0·7 equiv. of a secondary dissociation and 1·0 equiv. of the primary dissociations of guanylic and cytidylic acids.

XVI. Theoretical curve constructed from 0·7 equiv. of the primary dissociation of cytidylic acid, 0·7 equiv. of a secondary dissociation and 1·0 equiv. of the primary dissociations of guanylic and adenylic acids.

XVII. Experimental curve.

FIG. 4.—XVIII. Experimental curve (continuous line).

XIX. Theoretical curve (broken line).

Very strong evidence for the structure of yeast ribonucleic acid in which there are three primary and one secondary acid dissociations per tetranucleotide is given by the titration curve of the deaminated yeast nucleic acid. The removal of the amino-groups increases the dissociation of the primary acidic groups, and further, the hydroxyl group of xanthylic acid in the 2-position of the purine ring is titrated in the pH range 6—8. Ogston (J., 1935, 1376) gives for the dissociation constant of the purine hydroxyl group of xanthosine, the value of pK 7·0, which has been confirmed by us. These two facts lead to the formation of a marked point of inflection at pH 5 after the titration of 2·75 primary phosphoric acid dissociations (Fig. 2, curve X). At pH 8 there is a second point of inflection after the titration of 0·75 secondary dissociation and the purine hydroxyl of xanthylic acid. The titration of the hydrolysis products of the deaminated acid (curve XIII) confirms the presence of 3·8 primary phosphoric acid dissociations per tetranucleotide before hydrolysis. Furthermore, the titration curve may be very accurately reproduced theoretically, as shown in Fig. 4, on the assumption of the presence of three primary and one secondary phosphoric acid groups and one xanthine hydroxyl group. The dissociation constants of the deaminated purine nucleotides have not so far been determined. These acids are unstable in aqueous solution, undergoing hydrolysis to xanthine or hypoxanthine and a ribose phosphoric acid (Levene and Dmochowski, *J. Biol. Chem.*, 1931, **93**, 563; Levene and Harris, *ibid.*, 1932, **95**, 755; 1933, **101**, 419). The graph has therefore been constructed by assuming a dissociation constant of pK 1·0 for the primary acid dissociations, this being the value for uridylic acid. Slight changes in value of the dissociation constant in this range make insignificant differences in the position of the calculated curve.

There is a general tendency throughout the literature for the values of the phosphorus analyses of nucleic acids to be low, and this is accompanied by low titration data. An explanation of these facts now becomes possible, and is confirmed by the results obtained by introducing a correction for the missing phosphorus. The above titration data indicate the presence of a singly linked phosphorus atom, which thus possesses two free acidic groups. This atom could be removed without affecting the structure of the polynucleotide. This state of affairs could exist in the native acid, or removal could occur during some stage in the extraction and the subsequent purification process by either an enzymic or a hydrolytic fission. From the comparison of the experimental and theoretical curves, it is quite clearly shown that it is the secondary phosphoric acid group which has a low equivalent value. The theoretical phosphorus analysis per tetranucleotide is 9·5%, or per phosphorus atom 2·38%. The percentage of phosphorus linked by a single bond is therefore (for sample Y1) $8{\cdot}8 - 3 \times 2{\cdot}38 = 1{\cdot}66\%$. Thus the total number of acid groups present per tetranucleotide will be $2 + 1{\cdot}4 \times 2{\cdot}38/1{\cdot}66 = 4{\cdot}01$, in agreement with the theoretical number. By means of this method of correction, the number of acid groups per tetranucleotide has been calculated for the various samples examined. These values are given in the table, together with the uncorrected values for comparison. It will be noticed that the corrected values are very nearly whole numbers, except in the case of Merck and Eastman samples of yeast ribonucleic acid, which give anomalous results.

		Groups titrated to			
		pH 5.		pH 8.	
Sample of yeast ribonucleic acid.	P, %.	uncorr.	corr.	uncorr.	corr.
B.D.H. Y1	8·8	—	—	3·4	4·01
,, Y2	8·6	—	—	3·4	4·28
,, Y21	8·8	—	—	3·4	4·01
,, Y8	8·9	—	—	3·4	3·90
Boehringer BY1	9·1	—	—	3·6	3·95
Eastman Y9	8·9	—	—	3·2	3·62
Merck Y10	9·3	—	—	3·3	3·43
Deaminated B.D.H. DY33	8·9	2·75	3·01	4·5	5·03
,, DY1	9·0	2·75	2·96	4·5	4·92
,, DY5	8·9	2·75	3·01	4·5	5·03

It has been assumed in the above discussion that the four nucleotides, adenylic, guanylic, cytidylic, and uridylic acids, are present in yeast ribonucleic acid in equal proportion. The titration data give some evidence in support of this view. It is shown that yeast ribonucleic acid contains two acid dissociations which are titrated over the pH range 10—12; these are considered to be the nuclear hydroxyl groups of guanylic and uridylic acids, or the two groups present in two molecules of one of these acids. The titration of the deaminated acid indicates that an additional group per tetranucleotide is titrated over the pH range 5—8, which is considered to be the xanthosine hydroxyl group. These data indicate that there is one molecule of guanylic acid and one of uridylic acid present per tetranucleotide. No information can be obtained concerning the relative proportions of adenylic acid and cytidylic acid unless the ratio of purine to pyrimidine is taken to be unity. The results of Jones (" Nucleic Acids," 1920, p. 40, Longmans Green and Co., London), Hoffman (*J. Biol Chem.*, 1927, **73**, 15), Steudel (*Z. physiol. Chem.*, 1936, **242**, 100), Kobayashi (*J. Biochem. Japan*, 1932, **15**, 261), and of Ellinghaus (*Z. physiol. Chem.*, 1927, **164**, 261) tend to indicate that this ratio is unity. If this be so, then it follows that there are equal proportions of the four nucleotides present in the polytetranucleotide.

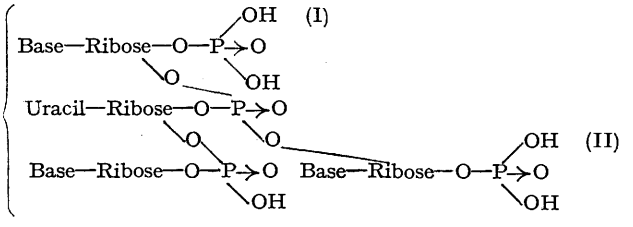
In Part VII it has been shown that the molecular weights of ribonucleic acid and of deaminated ribonucleic acid are approximately equal, and correspond to polynucleotides built up from 8—18 tetranucleotides. This is in agreement with the suggestion of a tetrabasic tetranucleotide, containing one triply linked phosphorus atom; of the four acid groups taking part in the formation of linkages, three will form inter-nucleotide links and the fourth an inter-tetranucleotide link. Further information concerning the groups taking part in the internucleotide links is forthcoming from a study of the products of mild hydrolysis. By carrying out the process of mild alkaline hydrolysis of yeast ribonucleic acid as described by Bredereck and Hoepfner (*loc. cit.*), a product having an approximate molecular weight of 5810 was isolated. The titration curve of this substance is given in Fig. 1, curve VIII, which indicates that the hydrolysis has broken linkages incorporating secondary phosphoric acid groups, since there is an increase in the number of groups titrated over the pH range 5—8. This suggests that if it were possible to isolate a tetranucleotide as claimed by Bredereck and Hoepfner, the pentabasic acid obtained would consist of three primary acid dissociations and two secondary acid dissociations. This view is substantiated by the mild hydrolysis of the deaminated acid, which on being heated to 100° liberates a secondary phosphoric acid dissociation as shown in curve XII, Fig. 2, the molecular weight of the hydrolysis product being of the order of 1800, although the isolation of the deaminated tetranucleotide free from purine has so far proved impracticable.

Bredereck and Hoepfner (*loc. cit.*) have prepared a deaminated tetranucleotide which, on titration to the phenolphthalein end-point, they found to be only pentabasic, and on back-titrating the products of alkaline hydrolysis (as distinct from the mild alkaline hydrolysis, described above), they have found only 8 acid groups. As is shown above, the xanthine hydroxyl group of deaminated guanylic acid is titrated over the pH range 5—8, and the acid, if it were a deaminated tetranucleotide should have been hexabasic. We have confirmed that

this hydroxyl group is titrated when titrating to the phenolphthalein end-point by titrating our sample of deaminated acid and finding 4·5 equivs., in agreement with the electrometric data. The results of Bredereck and Hoepfner can be explained by assuming that their supposed tetranucleotide was in fact a polytetranucleotide or that deamination was not complete, although their analysis does not suggest the latter explanation.

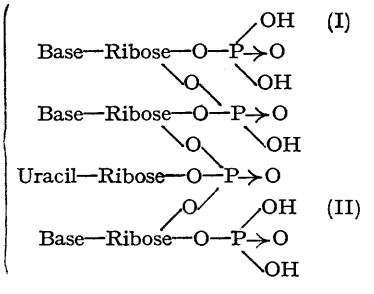
The experimental data given above suggest that yeast ribonucleic acid is a polynucleotide probably built up of tetranucleotide units, although the existence of the tetranucleotide as a chemical entity cannot be considered as proved until such a substance has been satisfactorily isolated. Each tetranucleotide present in the polytetranucleotide possesses three primary and one secondary acid dissociations, a second secondary acid dissociation being liberated on mild hydrolysis accompanied by a decrease in the molecular weight. These observations are not in agreement with the previously suggested formulæ for yeast ribonucleic acid. Feulgen's formula (*Z. physiol. Chem.*, 1918, **101**, 288) requires four primary dissociations per tetranucleotide, that of Levene and Simms (*J. Biol. Chem.*, 1926, **70**, 327) four primary and one secondary dissociation per tetranucleotide, and the cyclic formula of Takahashi (*J. Biochem. Japan*, 1932, **16**, 463) requires four primary dissociations per tetranucleotide. These formulæ have been fully described and discussed by Gulland (J., 1938, 1722). Polymerisation on the basis of Feulgen's or Takahashi's formula would produce polytetranucleotides having a maximum of three primary dissociations per tetranucleotide. On the other hand, the polymerisation of Levene and Simms' tetranucleotide would yield a polymer possessing either four primary dissociations, if linkage occurred through the secondary acidic group, or three primary and one secondary dissociation, if linkage occurred through a primary group. Although the latter result is in agreement with our titration data of yeast ribonucleic acid and deaminated yeast ribonucleic acid, it is not in agreement with our observations of the nature of the group liberated on mild hydrolysis. Possible formulæ which are in agreement with the observed data are given in (A), (B), and (C) below. Polymerisation would occur either through the group (I) or the group (II) to form the polytetranucleotide.

(A.)

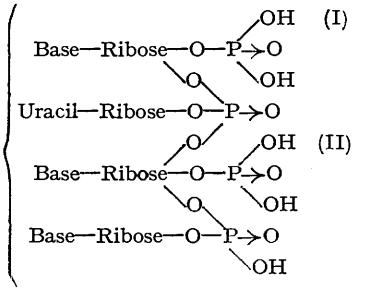


or

(B).



(C.)



It is important that such a formula should be compared with the existing enzyme data. Allen and Eiler (*loc. cit.*) observed that by the action of ribonuclease a secondary phosphoric acid group was liberated from yeast ribonucleic acid. They were uncertain whether the link was involved in ring formation, as in the formula of Takahashi, or in an inter-tetranucleotide link as in a polymer. It appears probable that the link broken by the enzyme ribonuclease may be the link broken on mild hydrolysis of ribonucleic or deaminated ribonucleic acid, which would then indicate that ribonuclease was a depolymerase. This could be proved by isolating the product after the action of ribonuclease and determining the molecular weight. Gulland and Jackson (J., 1938, 1492) found that the action of purified enzyme preparations containing a phosphomonoesterase and a diesterase produced 75% dephosphorylation of yeast ribonucleic acid, and further (unpublished result) that the mixed mono- and di-esterase produced only 75% dephosphorylation after the treatment of the yeast ribonucleic acid with an extract of boiled pancreatin which acts as a depolymerase. This indicates that one phosphorus atom is neither a di- nor a mono-ester, in agreement with the suggestion given above for the presence of one triply linked phosphorus atom per tetranucleotide. The action of bone phosphomonoesterase alone only liberated a maximum of 7% of the total phosphorus, from which Gulland and Jackson (*loc. cit.*) inferred the absence of a phosphomonoester group. This is not in agreement with the possible formulæ, and an explanation may lie in steric inaccessibility or enzyme specificity.

It is proposed to extend this investigation to other nucleic acids.

Experimental.

The titration vessel consisted of a 100-c.c. Hysil bolt-head flask fitted with a rubber stopper suitably bored to accommodate two hydrogen electrodes, or a glass electrode, two hydrogen inlet tubes, an agar–saturated potassium chloride bridge,

and a stirrer. Of the two hydrogen inlet tubes, one passed hydrogen through the solution and the second maintained an atmosphere of hydrogen in the flask above the liquid, thus preventing contamination with atmospheric carbon dioxide. Mechanical stirring was generally found to be unnecessary, the steady stream of hydrogen efficiently stirring the solution. When the glass electrode was used, the hydrogen was replaced by carbon dioxide-free air.

The platinum wire electrodes were plated by a similar method to that described by Popoff, Kunz, and Snow (*J. Physical Chem.*, 1928, **32**, 1056). The electrodes were gold plated from a 1% gold chloride solution by means of a current of 8 milliamps., the gold surface was then polished with a smooth glass rod, and platinum black deposited from a 2% platinic chloride solution containing 0·02% of lead acetate by passing a current of 30—40 milliamps. for one minute. The electrodes were finally made the cathode in an electrolytic cell, containing dilute sulphuric acid, for 15 minutes, and then thoroughly washed with distilled water. Two such electrodes were used throughout all titrations, and if disagreement was greater than 1 mv. they were replaced. Freshly plated electrodes were used for each titration. The hydrogen, obtained from a cylinder, was purified by passage over red-hot copper (freshly reduced copper oxide), then over soda-lime, and it was finally washed, first with dilute sodium hydroxide solution and then with distilled water, both wash-bottles being contained in the thermostat.

The glass electrodes were of the bulb type described by Morton (*J. Sci. Instr.*, 1930, **7**, 187) and were calibrated in 0·05M-potassium hydrogen phthalate buffer (pH 3·97) before and after each titration.

The normal calomel half cell was connected with the titration vessel by an agar–saturated potassium chloride bridge. The calomel cell was periodically standardised with 0·1N-hydrochloric acid and 0·05M-potassium hydrogen phthalate. The value of the activity coefficient of the hydrogen ion in 0·1N-hydrochloric acid was taken as 0·841 (Scatchard, *J. Amer. Chem. Soc.*, 1925, **47**, 696). The e.m.f. of the calomel half cell so determined was 0·2820 ± 0·0005 v. The titration vessel and calomel half cell were immersed in an oil thermostat maintained at 25° ± 0·05°.

The measurement of e.m.f. was made by using a Cambridge valve potentiometer. A calibrated 5-c.c. Pyrex micro-burette, reading to 0·02 c.c., was used throughout.

The samples of yeast ribonucleic acid and deaminated yeast ribonucleic acid were prepared as described in Part VII (*loc. cit.*). These samples contained only negligible amounts of sodium and potassium as determined polarographically. Titrations of these acids were carried out by suspending in 50 c.c. of conductivity water approximately 0·25 g. of the solid, accurately weighed after being dried in a vacuum at 100° over phosphoric oxide to constant weight. Solution was brought about by the gradual addition of standard alkali with mechanical stirring. The addition of alkali was so controlled that the suspension did not become more alkaline than pH 6 at any time during the process of solution. From this point alkaline titrations to pH 12 or acid titrations to pH 2 were made with approximately 0·5N-alkali or -hydrochloric acid solution respectively. Sodium hydroxide was used when titrating using hydrogen electrodes, but potassium hydroxide was used when employing glass electrodes, as the alkaline errors of the glass electrode are very much lower in the presence of potassium ions (Jordan, *Trans. Faraday Soc.*, 1938, **34**, 1305). The hydrochloric acid was standardised gravimetrically by precipitation as silver chloride, and the alkali standardised by titration with the standard acid to the phenolphthalein and the methyl-orange end-point, and electrometrically. The two methods gave identical results.

The titration curves have been corrected in the strongly alkaline pH range for the amount of alkali required to produce the same pH in conductivity water alone (see Harris, *Proc. Roy. Soc.*, 1924, **95**, *B*, 440; Kirk and Schmidt, *J. Biol Chem.*, 1929, **81**, 237).

The addition of alkali or acid during a titration was so controlled that the determination of the pH of the solution was made at intervals of approximately 0·2 pH unit.

Analyses.—All samples were dried at 110° in a vacuum over phosphoric oxide.

Found in :

	Y1.	Y2.	Y21.	Y8.	Y9.	Y10.	BY1.	BY2.	BY21.	DY1.	DY33.	DY5.
N, %	16·2	16·0	16·0	16·3	15·7	16·1	15·8	15·7	15·6	13·6	13·3	13·1
P, %	8·8	8·6	8·8	8·9	8·9	9·3	9·1	9·1	8·8	9·0	8·9	8·9

Calc. for a tetranucleotide containing 1 mol. each of the nucleotides, adenylic, guanylic, uridylic, and cytidylic acids, *i.e.*, $C_{38}H_{47}O_{28}N_{15}P_4$: N, 16·3; P, 9·5. Calc. for a deaminated tetranucleotide containing 1 mol. of the nucleotides inosinic and xanthylic acids, and 2 mols. of the nucleotide uridylic acid, *i.e.*, $C_{38}H_{44}O_{31}N_{12}P_4$: N, 12·9; P, 9·5%.

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