[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTY, UNIVERSITY OF ROCHESTER, SCHOOL OF MEDICINE AND DENTISTRY]

Nucleotide–Amino Acid Complexes in Alkaline Digests of Ribonucleic Acid*

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The presence of alkali-stable polynucleotide fractions has been detected in Schmidt–Thannhauser digests of ribonucleic acids prepared from calf pancreas, rabbit liver and yeast. These fractions are rich in purine nucleotides and contain bound amino acids or short peptides. The ratio of nucleotide to amino acid appears to be close to unity. Since these fractions are ninhydrin-negative, a phosphoamide bond is proposed which could theoretically account for the alkaline stability of the fractions by blocking the formation of cyclic phosphates. The possible relationship of these amino acid–nucleotide complexes to protein synthesis is discussed.

In a further study of the phosphorylation of RNA which was earlier attempted by Dounce and Kay,[1] rather consistent increases in the phosphorus content of RNA prepared from calf pancreas and thymus were obtained, but a phosphorus content higher than the theoretical value for RNA was not attained. It remained possible therefore that the observed increases might be due merely to a further purification of the RNA during the reisolation procedure, and hence it seemed desirable to determine, if possible, the reason for the consistently low phosphorus analyses obtained with RNA prepared by almost any of the methods that can be found in the literature.

Of the various reasonably mild methods for isolating RNA that have been reported, only that of Volkin and Carter[2] yields RNA with a phosphorus content approaching the theoretical value, as far as we have been able to determine. It should be noted, however, that in this case the RNA was isolated in phosphate buffer, and furthermore that RNA isolated by a subsequent modification of the same method[3] has a phosphorus content of considerably less than 8%. Even RNA isolated by the very recent method of Crestfield, *et al.*,[4] from yeast, involving the use of hot detergent, has a phosphorus content of only 8.2%, whereas the theoretical value is about 9.0%.

We have found that the principal "contaminants" in RNA samples prepared from calf pancreas or rabbit liver are combined amino acids, present singly or as very short peptides. The same "contaminants" are found in yeast nucleic acid isolated by the method of Johnson and Harkins.[5] Treatment of these ribonucleic acids with 1 *N* NaOH at 37° for 20 hours[6] does not result in the quantitative formation of mononucleotides, as has been rather commonly assumed in the past. In the case of one preparation of RNA from pancreas, it was found that only 62.3% of the hydrolyzed material was recoverable as mononucleotides from a Dowex-1 anion-exchange column.

Quantitative recovery of the material on the column could always be achieved however by elution with stronger acid than is required for the mononucleotides. Three additional peaks were generally obtained, after elution of the mononucleotides, through the use of 0.01, 0.1 and 1.0 *N* HCl solutions, respectively. The material corresponding to these peaks is biuret negative, ninhydrin negative and gives no precipitate with sulfosalicylic acid.

If the material corresponding to any one of these peaks is hydrolyzed with 6 *N* HCl in a sealed tube at 105°, it is then possible to reveal the presence of amino acids by two-dimensional paper chromatography. By means of hydrolysis with formic or perchloric acid, it is also possible to show that the material corresponding to all of the peaks in question is rich in purine nucleotides. The pyrimidine nucleotides, if they are present, must amount to less than 10% of the total nucleotides.

It should be noted that Smith and Allen[7] have reported that they find six spots not due to mononucleotides in the two-dimensional paper chromatography of an alkaline digest of yeast RNA, and that more recently Crestfield, *et al.*,[4] have estimated that about 3% of the RNA of yeast, isolated by their new method with hot detergent, is not degraded to the mononucleotide level by the procedure of Schmidt and Thannhauser.[6] Furthermore, de Lamirande, *et al.*,[8] have found that after treatment of rat liver RNA with 0.3 *N* KOH at 37° for 24 hours, they were able to account for only 70% of the optical density of the hydrolysate as mononucleotides using ion-exchange technique.

In the work reported in this paper, we have attempted to characterize the amino acid-containing, alkali-stable fractions obtained from the RNA of pancreas, rabbit liver and yeast after Schmidt–Thannhauser hydrolysis, and in the discussion a general type of structure is proposed for them. In addition, the possible relationship of the amino acids combined with RNA to protein synthesis is considered.

Experimental

A. Methods.—Phosphorus was determined according to the method of Fiske and SubbaRow[9] after digestion of the sample with sulfuric acid-selenium mixture. Quantitative estimations of amino acids were carried out by the method of

* We gratefully acknowledge the support of the National Cancer Institute. U. S. Public Health Service. Grant No. C994-C6 which made this work possible.

(1) A. L. Dounce and E. R. M. Kay, *Proc. Soc. Exp. Biol. Med.*, **83**, 321 (1953).

(2) E. Volkin and C. E. Carter, THIS JOURNAL, **73**, 1516 (1951).

(3) E. L. Grinnan and W. A. Mosher, *J. Biol. Chem.*, **191**, 719 (1951).

(4) A. M. Crestfield, K. C. Smith and F. W. Allen, *ibid.*, **216**, 185 (1955).

(5) T. B. Johnson and H. Harkins, THIS JOURNAL, **51**, 1779 (1929).

(6) G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.*, **161**, 83 (1945).

(7) K. C. Smith and F. W. Allen, THIS JOURNAL, **75**, 2131 (1953).

(8) G. de Lamirande, C. Allard and A. Cantero, *J. Biol. Chem.*, **214**, 519 (1955).

(9) C. H. Fiske and Y. SubbaRow, *ibid.*, **66**, 375 (1925).

Troll and Cannan.[10] When hydrolysates were required, samples were incubated in sealed tubes with 6 *N* HCl at 105° for 24 hours. Paper chromatography, utilizing 65% isopropyl alcohol in 2.0 *N* HCl as the solvent system, served for the separation of purine and pyrimidine components.[11] Paper chromatography of the amino acids was carried out with phenol–water in one direction, and collidine–lutidine–water in the other direction. Hydrolysis of samples was effected with concentrated formic acid at 175° for two hours,[12] or with 70% perchloric acid at 100° for one hour.[13] After the spots were revealed by ultraviolet illumination of the chromatogram, they were cut from the paper and eluted overnight in distilled water. The molar proportions of bases were calculated from extinction values measured in the Beckman spectrophotometer, using the coefficients of Cohn.

Other solvent systems used in this work were 5% dibasic sodium phosphate–isoamyl alcohol,[14] and butanol–acetic acid–water mixed in the proportions 40 to 10 to 10 by volume. Separations on columns of ion-exchange resins followed the method of Cohn.[15] Dowex-1 resin in the chloride form, of mesh size 200–400 and medium porosity, was used in the columns. The dimensions of the columns were 15 × 0.9 cm. for the work with RNA from pancreas and rabbit liver, and 15 × 2 cm. for the work with yeast RNA.

RNA was prepared from pancreas and rabbit liver according to the method of Kay and Dounce,[16] except that a third treatment with detergent was usually carried out. Yeast RNA was isolated by the method of Johnson and Harkins.[5,17]

B. **Experiments with RNA Isolated from Calf Pancreas.**—Thirty-six and one-tenth mg. of calf pancreas RNA was incubated with 10.0 ml. of 1 *N* NaOH at 37° for 20 hours in a stoppered tube. The solution was allowed to cool to room temperature and, after dilution to 100 ml., the *p*H was adjusted to 9.2 with HCl. A Dowex-1 chloride column was charged with the alkaline digest at a flow rate of about 1 ml. per minute. A small amount of ultraviolet-absorbing material was not held by the column. After charging the column, distilled water was run through until no more of this material could be removed. The column was then set up on an automatic fraction collector. Elution of the material remaining on the column was carried out with 0.003 *N* HCl. Twenty-ml. fractions were collected, and the optical density of each fraction was measured at 260 mμ in the Beckman spectrophotometer. In agreement with Cohn,[15] the order of elution of the mononucleotides was found to be cytidylic acid, adenylic acid a, adenylic acid b, uridylic acid and finally guanylic acid.

Reduction of the volume of eluate corresponding to the area under each peak was carried out in a modified Claisen flask *in vacuo* at temperatures between 40 and 50°. The ultraviolet spectrum of an aliquot of material corresponding to each peak was plotted and in each case the spectrum was found to be essentially identical with that of the spectrum of the corresponding pure nucleotide. A small amount of material corresponding to each peak was also chromatographed on paper, using disodium phosphate–isoamyl alcohol as the solvent system. In every case, the correct R_f value was obtained and this furnished further identification of the mononucleotides.

An inspection of Table I shows, however, that only 62.3% of the total optical density of the material initially put on the column could be accounted for as mononucleotides. As is shown in Table I, three additional peaks were obtained with 0.01, 0.1 and 1.0 *N* HCl, respectively. These are designated acid fractions I, II and III. It was possible to account completely for the material initially put on the column, in terms of optical density, by adding the extinction values for the three acid fractions to the sum of the values for the mononucleotides.

(10) W. Troll and R. K. Cannan, *J. Biol. Chem.*, **200**, 803 (1953).
(11) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).
(12) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 715 (1948).
(13) A. Marshak and H. J. Vogel, *ibid.*, **189**, 597 (1951).
(14) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).
(15) W. E. Cohn, "The Nucleic Acids," Vol. I, Academic Press, Inc., New York City, N. Y., 1955. p. 211.
(16) E. R. M. Kay and A. L. Dounce, THIS JOURNAL, **75**, 4041 (1953).
(17) We wish to express our appreciation to Dr. John Lambooy of the Department of Physiology of this University for a generous gift of RNA which was the material studied in this work.

TABLE I

RESULTS OF ANALYSIS OF CALF PANCREAS RNA BY ION-EXCHANGE CHROMATOGRAPHY AFTER ALKALINE HYDROLYSIS

Fraction	Units[a]	Fraction	Units[a]
Not held	14.5	Acid fraction I (0.01 *N* HCl)	174.0
Cytidylic acid	123.3	Acid fraction II (0.1 *N* HCl)	10.5
Adenylic acid a	38.1	Acid fraction III (1.0 *N* HCl)	74.7
Adenylic acid b	39.0		
Uridylic acid	69.2		
Guanylic acid	144.3		
Total	428.4	Total	259.2

Total units eluted = 687.6

% mononucleotide fraction 428.4/687.6 × 100 = 62.3%
% acid fractions 259.2/687.6 × 100 = 37.7%

[a] Optical density × volume = spectrophotometric units.

A detailed study of the acid fractions was next carried out. Ultraviolet absorption spectra of one of the typical acid fractions is shown in Fig. 1. Paper chromatography of

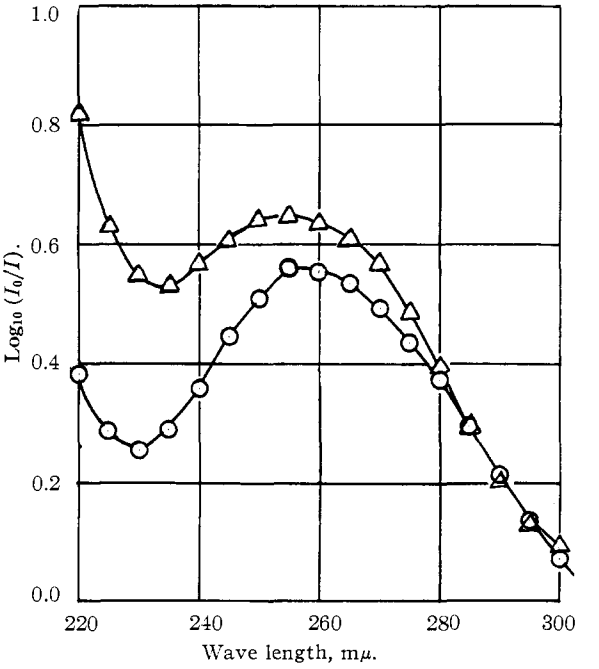


Fig. 1.—Ultraviolet spectrum of first acid fraction from pancreas RNA.

these fractions showed that they were not homogeneous. In Table II are listed the solvent systems used and the R_f values found.

Although the acid fractions are ninhydrin negative, the presence of amino acids may be revealed by paper chromatography in fractions I and III after hydrolysis with 6 *N* HCl at 105° for 24 hours. Sufficient material was unfortunately not available in fraction II to permit analysis for amino acids. Hydrolysates from fractions I and III were subjected to two-dimensional chromatography on Whatman paper No. 1, using phenol saturated with water for the first direction and collidine–lutidine–water for the second direction. The papers were dried in air and sprayed with ninhydrin reagent to reveal the spots. Both fractions yielded essentially identical chromatograms. The amino acids present in both fraction I and fraction III were glutamic acid, valine, alanine, glycine, leucine and/or isoleucine. Thus it appears that predominantly neutral amino acids are associated with the polynucleotide fractions. It should be pointed out that a certain amount of material associated with the resin is eluted together with these fractions. Since it appears that this material may adversely affect the resolution of spots on the chromatogram, the possibility that still other amino acids may be associated with these fractions is not yet excluded.

TABLE II

RESULTS OF ANALYSIS BY PAPER CHROMATOGRAPHY OF THE "ACID FRACTIONS" OBTAINED FROM CALF PANCREAS RNA

Acid fraction	Solvent system	R_f values
I	Disodium phosphate–isoamyl alc.	0.48, 0.83 (stronger)
	Butanol–acetic acid–water	0.00, 0.09, 0.18, 0.60
II	Disodium phosphate–isoamyl alc.	Not run
	Butanol–acetic acid–water	0.00, 0.11, 0.19 streak, from 0.11 to 0.52 light fluorescent spot between R_f 0.0 and 0.11
III	Disodium phosphate–isoamyl alc.	0.36; heavy band at 0.84
	Butanol–acetic acid–water	0.00, 0.11, 0.15, 0.19 then streak to 0.40

Formic acid hydrolysates of small aliquots of the acid fractions were chromatographed on paper, using 65% isopropyl alcohol in 2.0 *N* HCl as the solvent system. A formic acid hydrolysate of yeast nucleic acid was used as a source of standard adenine, guanine, cytosine and uracil. It was found that the R_f values of the standards could vary, but the relative order of mobility was always the same, namely, guanine, adenine, cytosine and uracil in order of increasing mobility.

Acid fraction I was found to give rise to two main spots with R_f values of 0.19 and 0.28 corresponding to standard guanine and adenine. There was a suggestion of a very faint spot just ahead of adenine. Guanine was present to the extent of 19 μmoles, and 11 μmoles of adenine was found. Acid fraction III gave rise to only two spots on the chromatogram, corresponding to standard guanine and adenine with R_f values of 0.26 and 0.35, respectively. The amounts of the bases were 12 μmoles of guanine and 11 μmoles of adenine.

C. Experiments with RNA Isolated from Rabbit Liver.—The presence of alkali-stable fragments in a Schmidt–Thannhauser digest of rabbit liver RNA has also been established. Table III illustrates the results of the ion-exchange separation of the digest.

TABLE III

RESULTS OF ANALYSIS OF RABBIT LIVER RNA BY ION-EXCHANGE CHROMATOGRAPHY AFTER ALKALINE HYDROLYSIS

Fraction	Units[a]
Not held	247.1
Total mononucleotides (0.003 *N* HCl)	1950.5
Acid fraction I (0.003 *N* HCl; post guanylic acid)	195.4
Acid fraction II (*p*H 1.95)	17.8
Acid fraction III (*p*H 1.45)	56.4
Acid fraction IV (*p*H 0.40)	136.1
Acid fraction V (2 *N* HCl)	46.0
Total units	2649.3

$$\%\ \text{mononucleotide fraction} \quad \frac{2197.6}{2649.3} \times 100 = 82.1\%$$

$$\%\ \text{acid fractions} \quad \frac{451.7}{2649.3} \times 100 = 17.9\%$$

[a] Optical density × volume = spectrophotometric units.

The cytidylic acid, adenylic acid and uridylic acid fractions were found to be pure mononucleotides as judged by their ultraviolet spectra and mobility upon chromatography in 5% disodium phosphate–isoamyl alcohol. However, two additional ultraviolet-absorbing spots were found in the guanine nucleotide fraction. Paper chromatography of acid fraction I showed the same three spots that were obtained with the guanylic acid fraction. The guanine nucleotide is thus present in two discrete fractions and is accompanied by at least two other components. The value for the total mononucleotide fraction shown in Table III has been corrected for this by measuring the absorption due to the two additional ultraviolet absorbing fragments in the guanylic acid fraction, and subtracting this value from the total absorption of the fraction.

TABLE IV

RESULTS OF ANALYSIS BY PAPER CHROMATOGRAPHY OF THE "ACID FRACTIONS" OBTAINED FROM RABBIT LIVER RNA

Solvent system	Fraction	R_f values
Disodium phosphate–isoamyl alc.	III	0.0, 0.41, 0.81, 0.90
	IV	0.0, 0.14, 0.41, 0.86

Acid fractions III and IV were reduced to small volume by evaporating *in vacuo* as in the experiments with pancreas RNA. The ultraviolet spectra of these fractions was similar to that shown in Fig. 1. The heterogeneity of these fractions is illustrated by the data in Table IV as it is evident that each fraction is composed of at least four components.

The nucleotide composition of these fractions is again predominantly purine in nature. The molar quantities of the purine bases in fraction III were found to be 24 μmoles of guanine, and 11 μmoles of adenine. In fraction IV there were found 11 μmoles of guanine, and 11 μmoles of adenine. The molar ratio of amino acid to phosphorus after hydrolysis in 6 *N* HCl was found to tend toward unity in both fractions III and IV. After correction for the calculated contribution of ninhydrin-positive material by the breakdown of the purines, the ratio becomes 0.71 for fraction III, and 0.6 for fraction IV.

The presence of leucine and/or isoleucine, valine, threonine, serine and glycine was detected in fraction III, and the same amino acids also in fraction IV.

D. Experiments with Yeast Ribonucleic Acid.—One gram of yeast RNA was dissolved in 40 ml. of 1 *N* NaOH, and incubated at 37° for 24 hours in a stoppered flask. The clear yellowish solution that was obtained was stored at 5° for 24 hours. The *p*H of this solution was next adjusted to 3.2 with HCl and a certain amount of material which then precipitated, causing a slight opalescence, was removed by centrifugation at 13,000 r.p.m. for one hour in a Servall centrifuge. The supernatant fluid at this time was clear and colorless. The *p*H of the solution was then adjusted to 9.0 with NaOH, and the volume made up to 200 ml. with distilled water. A Dowex-1 chloride column was charged with this solution. The results of the ion-exchange separation of the products of the alkaline hydrolysis of yeast RNA are summarized in Table V.

TABLE V

RESULTS OF ANALYSIS OF YEAST RNA BY ION-EXCHANGE CHROMATOGRAPHY AFTER ALKALINE HYDROLYSIS

Fraction	Units	Fraction	Units
Not held	940.0	Acid fraction I (HCl *p*H 1.9)	264.6
Wash (distilled water)	513.9	Acid fraction II (HCl *p*H 1.15)	674.8
Elution (0.003 *N* HCl) mononucleotides	17,128.0	Acid fraction III (HCl *p*H 0.3)	96.0
		HCl 2 *N*	19.5
Total	18,581.9	Total	1054.9

Total units eluted = 19,636.8

% not degraded to mononucleotide = 1054.9/19,636.8 × 100 = 5.4

The acid fractions, with the exception of the material eluted with 2 *N* HCl, were pooled and reduced to a volume of 10.0 ml. *in vacuo*. The ultraviolet absorption spectra of the pooled fractions are presented in Fig. 2. Shifts in the spectra of the acid fractions caused by changing the *p*H either to acid or alkaline values are clearly evident, and are suggestive of a high proportion of the guanine nucleotide in the polynucleotide fragments in question.

Paper chromatography of the acid fractions again indicated heterogeneity. In Table VI are listed the solvent systems used and R_f values observed.

Small aliquots of the pooled fractions were hydrolyzed with perchloric acid for one hour at 100°. Chromatography of these hydrolysates revealed only two spots. The R_f values of these spots in isopropyl alcohol–HCl, were 0.27 and 0.38, corresponding closely to the values observed for

TABLE VI

RESULTS OF ANALYSIS BY PAPER CHROMATOGRAPHY OF THE "ACID FRACTIONS" OBTAINED FROM YEAST RNA

Solvent system	R_f values
Disodium phosphate–isoamyl alc.	0.40, 0.93
Butanol–acetic acid–water	0.00, 0.07, 0.13, 0.19

standard guanine and adenine. The guanine content of the pooled fractions was 16 μmoles, while the adenine content was 8.1 μmoles. The predominance of guanine was also indicated from the ultraviolet absorption spectra of the fractions.

Phosphorus analysis and photometric ninhydrin determinations have demonstrated that the molar ratio of phosphorus to amino acid in the pooled acid fraction is close to unity. In this calculation, a correction again must be made for the amount of ninhydrin-positive material formed by the breakdown of the purine bases under the conditions of acid hydrolysis used. The small amount of ninhydrin-positive material found before acid hydrolysis of this fraction is presumably due to a slow release of the amino acids during the ion-exchange separation at low *p*H. Table VII shows the results of the analyses for phosphorus and amino acids. The amino acid pattern showed that leucine and/or isoleucine, valine, alanine, serine and glycine were present.

TABLE VII

RESULTS OF ANALYSIS OF THE "ACID FRACTION" OF YEAST RNA FOR PHOSPHORUS AND AMINO ACID

Phosphorus, μatom	6.6
Amino acid before hydrolysis, μmoles	0.87
Amino acid after hydrolysis, μmoles	8.89
Amino acid due to breakdown of purine bases, μmoles	1.34
Amino acid after cor. for breakdown of purine bases, μmoles	7.55
Ratio of μmoles amino acid to μatoms phosphorus	1.14

Discussion

The presence of amino acid-containing, alkali-stable fractions in the Schmidt–Thannhauser digests of ribonucleic acid samples from three sources has been described. The amino acids found in these fractions must be firmly bound to the polynucleotides, since we have found that free amino acids and all proteins tried are not retained by the ion-exchange columns under the conditions of separation of the mononucleotide fractions. Moreover, we have been unable to detect peptides or amino acids in portions of the elutriate which come off from the column between the oligonucleotide fractions that contain the bound amino acids, or in the mononucleotide fractions. Likewise, portions of the elutriate coming off between the mononucleotides did not contain peptides or amino acids.

Since the alkali-stable fractions are ninhydrin negative, it seems possible that the amino acids may be bound to phosphate groups through their amino groups, as phosphoamides. However, attempts to effect a positive identification of free terminal carboxyl groups, by means of direct reduction with lithium aluminum hydride, failed, possibly owing to the two-phase nature of the reaction. The hydrazine reaction also has thus far proved to be unsuitable for this purpose, owing to interferences caused by reaction of the hydrazine with the nucleotide fragments. It was found that carboxypeptidase would not liberate free amino acids from intact pancreas RNA in solution, but this might mean only that the amino acids are attached singly rather than as short peptides. However, since

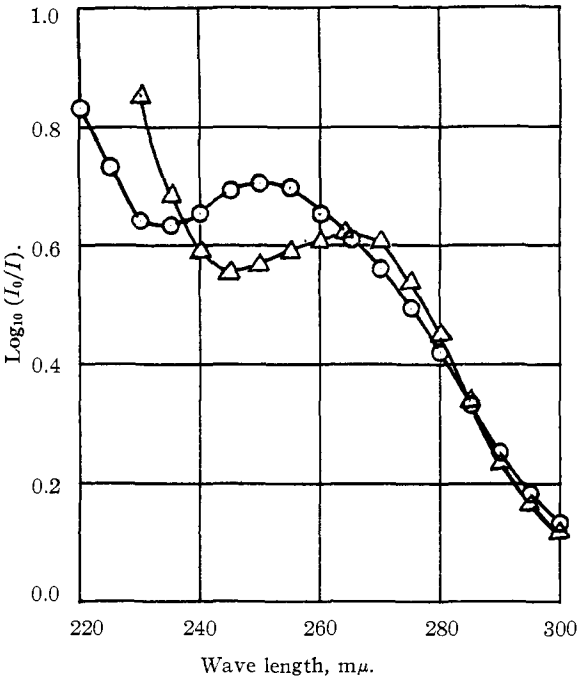


Fig. 2.—Ultraviolet spectrum of pooled acid fractions from yeast RNA.

peptide linkages stable to carboxypeptidase are known, we do not have absolute proof of the absence of short peptide chains.

We have searched without success for evidence that there is protein in our best samples of RNA from pancreas. The negative biuret reaction and the fact that the addition of trichloroacetic acid to an alkali-digest causes no visible precipitate to form, have led us to believe that no protein is present. The major "contaminant" of pancreas RNA appears to consist of the combined amino acids, since we have calculated that there are sufficient bound amino acids in our best samples to cause a lowering of the phosphorus content to within 6% of the experimentally determined value.

Our samples of RNA from rabbit liver and yeast contained less of the alkali-stable fractions than were found in pancreas RNA and did contain small amounts of protein. Small amounts of protein also were found in certain of the RNA samples from pancreas, but this protein could be removed by the use of hot detergent without materially changing the phosphorus content. When protein is present in the RNA samples, it appears in the effluent liquid collected during the initial charging of the column and may also come off in the subsequent washing and separation of the mononucleotide fractions. It is all removed however before the alkali-stable fractions containing the bound amino acids appear.

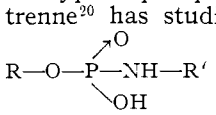
We next wish to consider possible explanations for the stability to alkali of the fractions from the Schmidt–Thannhauser digest which contain the bound amino acids.

Since Brown and Todd[18] and Lipkin, *et al.*,[19] have shown that the most likely explanation for the

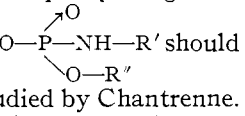
(18) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 52 (1952).

(19) D. Lipkin, P. T. Talbert and M. Cohn, THIS JOURNAL, **76**, 2871 (1954).

lability of RNA to alkali is the ability of the RNA to form a cyclic intermediate involving a phosphorus atom and the oxygens on carbons number 2 and 3 of the ribose, it is reasonable to assume that if formation of the cyclic intermediate were prevented, the internucleotide phosphate ester linkages would be stable, as they are in desoxyribonucleic acid. Formation of the cyclic intermediates might be prevented by blocking of the 2′-hydroxyl groups of the sugars or of the free hydroxyls of the phosphate groups or of both. If as already suggested the bound amino acids in the alkali-stable fractions are held in phosphoamide linkages, the formation of the cyclic intermediates could be prevented by blocking of the hydroxyls on the phosphate groups in question, provided that the phosphoamide linkages themselves were sufficiently stable to alkali. Although we have not been able to find data on the stability toward alkali of the exact type of phosphoamide bond in question, Chantrenne[20] has studied phosphoamides of the type



R—O—P(=O)(OH)—NH—R′ and finds that these are more stable than the simple phosphoamide bonds that occur in phosphocreatine and phosphoarginine. Phosphoamides of the type



R—O—P(=O)(O—R″)—NH—R′ should be at least as stable as those studied by Chantrenne.

Other possible but less probable explanations for the stability to alkali of the amino acid-containing fractions are that branching of the nucleotide chain might occur through the oxygen on carbon number 2 of the sugar, or that there might be alternate linking of nucleotides and amino acids, although the latter type of compound is not known to occur in nature.

We have some preliminary data indicating that amino acids are slowly liberated by acid from the alkali-stable fractions, and this no doubt could partly account for certain of the ratios of amino acid to nucleotide being considerably less than one. However, if the amino acids are really bound by phosphoamide linkages, the ratio of amino acid to nucleotide cannot be as high as one in any of the amino acid-containing fractions obtained after digestion of RNA by alkali, for if it were, these fractions would not be bound to the ion-exchange columns as firmly as they are. It must be assumed from the firmness of binding of the fractions that at least one strongly ionizing phosphate group is present per fragment. One is forced to draw practically the same conclusion starting from the premise that cyclic intermediates are formed during hydrolysis of RNA by alkali. According to this premise hydrolysis could take place only at the sites of occurrence of phosphate groups not bearing phosphoamide linkages, and the only hydrolytic fragments free from primary phosphate groups that could be obtained would be those fragments which included terminal nucleotides of the original RNA molecules. The value of 1.14 obtained for the ratio of amino acid to nucleotide in the case of the combined acid fractions of yeast RNA can be explained on the basis of the above arguments only as an indication of the presence of peptides or as experimental error.

The final point that we should like to consider is the possible role in protein synthesis of the amino acids bound to RNA. The attachment of amino acids to RNA through phosphoamide linkages has already been proposed recently[21] to represent possible intermediate stages in the synthesis of peptide chains. If RNA does act as a template in protein synthesis, the long-sought intermediates in this process must consist of amino acids bound in some way to the RNA. The occurrence of RNA molecules bearing partially synthesized peptide chains has also been suggested.[22] It will however be necessary to extend the work reported in this paper by the use of isotopic tracers before being able to decide whether or not the bound amino acids represent intermediates in protein synthesis. The fact that RNA samples from different tissues contain variable amounts of the bound amino acids suggests to us that we may be dealing with such intermediates.

ROCHESTER, N. Y.

(20) H. Chantrenne, *Publ. Staz. Zool. Napoli*, **23**, suppl., p. 70.

(21) A. L. Dounce, *Enzymologia* [XV] *fasc.*, **5**, 251 (1952).

(22) C. E. Dalgliesh, *Nature*, **171**, 1027 (1953).