ON THE STRUCTURE OF RIBONUCLEIC ACIDS

I. DEGRADATION WITH SNAKE VENOM DIESTERASE AND
THE ISOLATION OF PYRIMIDINE DIPHOSPHATES*

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Early concepts of the structure of ribonucleic acid (RNA) were based largely upon the isolation of the nucleotides of adenine, guanine, uracil, and cytosine from alkali-hydrolyzed RNA (1). Since these were regarded as being single compounds phosphorylated in the 3' position, and since the acid-stable ribose-5-phosphate (and its pyrimidine derivatives) could not be found in acid-hydrolyzed RNA, a 2',3'-phosphodiester linkage was proposed (2).

The introduction of a quantitative isolation technique, ion exchange chromatography, by Cohn (3) led promptly to the discovery that each of the nucleotides produced by the chemical hydrolysis of RNA exists in two isomeric forms (3-5) which are interconvertible in acid but not in alkali. These pairs of isomers were termed a and b in the order of their appearance in the elution sequence, and it soon became apparent that the four b isomers were identical with the four nucleotides isolated earlier and considered, on the basis of the optical inactivity of the derived ribitol phosphates, to be the 3' esters. Although it is very probable that the a and b isomers are indeed the 2'- and 3'-phosphonucleosides of the four known bases of RNA, it is pointed out by Brown and Todd (6) that the possibilities of intramolecular migration of phosphate are such as to make it impossible, without further evidence, to decide which is which in intact RNA.

This uncertainty did not become apparent until we attempted to find the new isomers (the a forms), considered at that time to be the missing 2' isomers (3, 4), by enzymic hydrolysis of RNA. We adapted the desoxyribonucleic acid (DNA) degradation procedure of Klein and Thannhauser (7, 8), which utilizes the diesterase activity of crude intestinal phosphatase on a nuclease digest, by substituting ribonuclease for desoxyribonuclease, thereby enabling us to isolate four nucleotides which could be shown, as Carter had already done for the desoxynucleotides (9), to be the 5'-phosphoesters of the usual four nucleosides (10). Although the b forms of uridylic and cytidylic acids were also present, it is known (10-12) that

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these arise from ribonuclease action alone. Hence, the principal products of the intestinal phosphatase action were 5'-nucleotides and not a or b nucleotides.

The finding of 5'-nucleotides in RNA digests at once recalled the early work of Gulland and Jackson (13) who had observed that snake venoms, which contain both diesterase and 5'-monoesterase activity, give rise to large amounts of inorganic phosphate from RNA. From this, they concluded that 5' esters were intermediates in the digestion, being released from RNA by the diesterase and hydrolyzed by the 5'-monoesterase. Without the means of separating these esterase activities, no further evidence was obtainable and the hypothesis of a 5' linkage in RNA was subsequently withdrawn (14).

With the analytical techniques at our disposal, we have been able recently to show (15) that the hydrolysis of Gulland and Jackson does indeed yield inorganic phosphate and nucleosides primarily, but that, in addition, about 30 per cent of the pyrimidines are left as pyrimidine nucleoside a,5'- and b,5'-diphosphates. When the crude venom is freed of 5'-monoesterase activity (16), the primary products are, as Gulland and Jackson had correctly deduced, 5'-nucleotides; the pyrimidine nucleoside diphosphates, however, remain, as does an equimolar amount of purine nucleosides (15).

It thus appears that RNA can be degraded to a mixture of the 2'- and 3'-nucleotides by alkali and to the 5'-nucleotides by diesterase. Taking account of these facts and the earlier work on intramolecular phosphomigration in glycerophosphates (Chargaff (17), Baer and Kates (18), and Bailly and Gaume (19)), Brown and Todd (6) proposed a 2',5'- or 3',5'-phosphodiester sequence for RNA. The necessary postulate of a cyclic phosphate intermediate in the course of hydrolysis is further supported by the recent observations of Markham and Smith (20) and by the synthesis of the cyclic ribophosphates themselves by Brown, Magrath, and Todd (21).

In this communication, we show that 5'-nucleotides are quantitatively the major product of the action of snake venom diesterase on intact RNA's isolated from different sources, and that there is a simultaneous production of pyrimidine nucleoside diphosphates and of purine nucleosides in equimolar quantities.

EXPERIMENTAL

 Ion Exchange Chromatography—The analysis of the enzyme digests followed essentially the procedures developed by Cohn (3) as subsequently modified (10, 15). Concentrations were determined by spectrophotometry and by phosphate analysis.
Synthetic Ribonucleoside-5'-phosphates—These compounds, with which our isolated substances were compared, were most kindly furnished by Dr. Brown and Dr. Todd.

Snake Venom Enzymes—The crude venom (Crotalus adamanteus) was obtained from Ross Allen's Reptile Farm, Silver Springs, Florida, and used as received. The removal of the 5'-esterase activity was accomplished by the method of Hurst and Butler (16). The activity of the preparation was followed by the splitting of di(dinitrophenyl) phosphate, which was kindly supplied by Dr. R. Sinsheimer. A test for residual 5'-nucleotidase activity was made against all four synthetic ribonucleoside-5'-phosphates.

Potato 5'-Phosphatase—The preparation of this enzyme was carried out as described by Kornberg and Pricer (22). In our hands, however, the preparation still contained significant hydrolytic activity toward β nucleotides and slight activity toward the α isomers. The relative rates of dephosphorylation of cytidylic acids 5', β, and α were in the ratio of 100:25:1.

Barley β Phosphatase—A preparation given to us by Dr. Shuster and Dr. Kaplan (23) and another prepared according to their direction were employed. Both enzymes, while quite specific for the β purine nucleotides, contained slight activity toward the α pyrimidine nucleotides, the β pyrimidine nucleotides being split much more rapidly. Both preparations, however, had a slow but significant diesterase activity toward the di(dinitrophenyl) phosphate substrate.

Ribonucleic Acids—The mammalian ribonucleic acids were prepared directly from the respective tissues by the method of Volkin and Carter (24). Ribonucleic acid from yeast could not be obtained by this method; the procedure used will be described.

Dried, viable yeast from Standard Brands, Inc., was ground in a ball mill for 48 hours at 5°, with stainless steel balls and 10 per cent by weight of Linde A polishing alumina. Yeast lipides were removed by successive extractions with cold acetone, alcohol, and ethyl ether. The dried powder was then suspended and stirred rapidly in 0.1 M NaCl and 0.025 M sodium citrate, pH 6.7 (6 volumes per gm. of residue). Insoluble material was centrifuged, NaCl added to give a final concentration of 0.25 M, and then 0.5 volume of 2 per cent Aerosol OT added. After incubation at 37° for 4 hours and chilling, precipitated protein was removed by centrifugation. 2.5 volumes of cold ethanol were added to the supernatant solution, any desoxynucleoprotein present being wound around the stirring rod and physically removed. The flocculent precipitate was collected, resuspended in 2 M guanidine hydrochloride, and warmed to 40°. The chloroform-octyl alcohol procedure of Sevag et al. (25) was repeated five or six times at this temperature to remove protein. RNA was again precipitated by the addition of 2.5 volumes of ethanol. After re-solution in water, larger amounts
of complex polysaccharides were removed by adding, with rapid stirring, 5 per cent trichloroacetic acid to a final concentration of 0.05 per cent. The soluble polysaccharides were removed by centrifugation and the RNA precipitate again extracted with 0.05 per cent trichloroacetic acid. Finally, the precipitate was resuspended in water, carefully adjusted to pH 6.8, and exhaustively dialyzed against water.

This material contained 9.7 per cent P and 17.0 per cent N and appeared to be about 85 per cent ribonucleic acid, small amounts of desoxynucleic acid and metaphosphate being among the contaminants.

In our opinion, the method of preparation of RNA is of critical importance in any studies of structure. It seems likely that many of the conflicting results which appear in the literature, e.g. the number of end-groups from titration studies and reduction of periodate before or after ribonuclease action, are due to partial degradation of some of the samples during isolation or preparation. The preparations made in this laboratory have on the order of 10 per cent end-groups as determined by purified bone phosphatase (13) and less than 2 per cent periodate-oxidizable residues, either before or after ribonuclease action. We do not feel that the precipitation of commercially available material, much of which has been subjected to relatively drastic chemical treatment, can undo the structural damage previously wrought or completely remove the degraded parts.

Results

The repetition of the experiment of Gulland and Jackson (13) on calf liver RNA, with whole venom and proceeding to the end-point of the reaction, resulted in a mixture which was analyzed by ion exchange chromatography (see Fig. 1). The analysis shown is that in which the two pyrimidine diphosphates were discovered. The nucleosides were separated subsequently by the method of Cohn (3) and were determined spectrophotometrically.

Table I summarizes the quantitative aspects of this analysis. From this it is evident that the inorganic phosphate was derived principally from nucleotides and, since the venom will only hydrolyze those nucleotides phosphorylated in the 5' position, they must have been nucleoside-5'-phosphates. The diphosphates comprise about one-third of the total pyrimidine content of the digest. 9 per cent of the cytidine is found as the β isomer of cytidylic acid.

The diphosphates have been characterized by the ratios of their constituent parts and by their enzymic susceptibilities. The ratio of pyrimidine (by spectrophotometric assay, extinction coefficients of 6800 and 9900 for cytidylic and uridylic acids, respectively, at 260 ma at pH 2) to ribose (by the bromination method of Massart and Hoste (26)) to phosphorus is
1:1:2. Only 15 per cent of the total phosphate is released by heating the substance at 100° for 1 hour in 1 M H$_2$SO$_4$ (compared to 3 to 5 per cent

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**TABLE I**

*Products of Digestion of Calf Liver RNA with Whole Snake Venom*

<table>
<thead>
<tr>
<th>Nucleosides</th>
<th>Diphosphates</th>
<th>Inorganic P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moles per 100 moles P</td>
<td>Cyt*</td>
<td>Ur</td>
</tr>
<tr>
<td>-------------</td>
<td>------</td>
<td>----</td>
</tr>
<tr>
<td>19</td>
<td>10.3</td>
<td>15.6</td>
</tr>
</tbody>
</table>

* 9 per cent of the total cytidine was recovered as cytidylic acid b.

from the corresponding a or b acids, thus eliminating the possibility of pyrophosphates. The potato phosphatase of Kornberg, which removes the 5'-phosphate from the diphosphoadenosine derived from triphosphopyridine nucleotide (TPN) (22), splits somewhat more than one-half of the phosphate from the cytidine diphosphate. Since this enzyme, in our hands,
splits both 5'- and b-cytidylic acids but not the a acid, this result suggests that both a, 5' - and b, 5'-cytidine diphosphates are present. The time-

![Graph showing the hydrolysis of cytidine diphosphate by barley phosphatase.](image)

**FIG. 2.** Cytidine diphosphate (4.16 mg. of P) + 3.0 ml. of potato phosphatase (20) + 5 ml. of 0.2 M NH₄Cl(NH₃) and 0.02 M MgCl₂ buffer (pH 9.0); total volume, 20 ml. incubated at 37°. Appropriate aliquots removed for inorganic phosphate and ion exchange analysis at the times indicated.

**TABLE II**

<table>
<thead>
<tr>
<th>Time</th>
<th>Cytidine</th>
<th>Cyt 5'</th>
<th>Cyt a</th>
<th>Cyt b</th>
<th>Cyt dP/O</th>
<th>Inorganic P, per cent of total P</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 min.</td>
<td>0</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>53</td>
<td>24</td>
</tr>
<tr>
<td>16 hrs.</td>
<td>9</td>
<td>77</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>55</td>
</tr>
</tbody>
</table>

1 mg. of cytidine diphosphate + 0.2 ml. of barley phosphatase + 0.2 ml. of 0.2 M tris(hydroxymethyl)aminomethane buffer (pH 7.5) incubated at 37°. 0.5 ml. samples removed for ion exchange analyses.

course of its action on cytidine diphosphate is shown in Fig. 2, the final products being cytidine, inorganic phosphate, and cytidylic acid a. We ascribe the transient appearance of cytidylic acid b and cytidine-5'-phosphate to the monodephosphorylation of cytidine-b, 5'-diphosphate; both b and 5' acids are subsequently dephosphorylated to yield cytidine.
The interpretation of these experiments as indicative of a mixture of cytidine-α,5'-diphosphate and cytidine-β,5'-diphosphate is further strengthened by the use of the phosphatase from barley (23) which hydrolyzes cytidylic acid β rapidly, cytidylic acid α slowly, and the 5' ester very slowly. The first products of the action of this enzyme on the isolated cytidine diphosphate are cytidine-5'-phosphate and cytidine diphosphate in approximately equal amounts (see Table II), the latter being presumably the α,5'- variety. The slow action upon the α and 5' phosphate groups then destroys the remaining diphosphate, and at 16 hours only the 5' and α nucleotides are found.

When snake venom was freed of 5'-monoesterase activity (16) and used with another preparation of the same type of RNA (calf liver) as above (see Fig. 1 and Table I), the ion exchange chromatogram shown in Fig. 3

Fig. 3. 16 mg. of calf liver RNA, 1.0 ml. of 0.05 M MgCl₂, 1 ml. of snake venom diesterase preparation; total volume, 5 ml. The reaction mixture was adjusted to pH 8.6 and maintained at this pH over the course of hydrolysis by the periodic addition of 0.02 N NaOH. After 7 hours digestion at 25°, the reaction was stopped by the addition of 1 M of NH₄OH and the mixture subjected to ion exchange analysis. Column, 5.8 cm. X 0.9 cm. square Dowex 1, 400 mesh; flow rate, 0.5 ml. per minute.
<table>
<thead>
<tr>
<th>Source</th>
<th>Nucleosides</th>
<th>5'-Nucleotides</th>
<th>Diphosphates</th>
<th>*Inorganic P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyt</td>
<td>Ur</td>
<td>Ad</td>
<td>Gu</td>
</tr>
<tr>
<td>Liver RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moles per 100 moles P present*</td>
<td>3.7</td>
<td>3.3</td>
<td>6.4</td>
<td>11.5</td>
</tr>
<tr>
<td>% of total recovered</td>
<td>14</td>
<td>22</td>
<td>48</td>
<td>42</td>
</tr>
<tr>
<td>Yeast RNA</td>
<td>1.8</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moles per 100 moles P present</td>
<td>0.5</td>
<td>1.3</td>
<td>7.1</td>
<td>6.0</td>
</tr>
<tr>
<td>% of total recovered</td>
<td>2</td>
<td>6</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>Thymus RNA</td>
<td>3.5</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moles per 100 moles P present</td>
<td>2.7</td>
<td>0.75</td>
<td>3.0</td>
<td>3.7</td>
</tr>
<tr>
<td>% of total recovered</td>
<td>11</td>
<td>9</td>
<td>31</td>
<td>15</td>
</tr>
</tbody>
</table>

* Spectrophotometrically determined with the following extinction coefficients (260 μm, pH 2): cytidine and cytidine-5'-phosphate, 6200; uridine and uridine-5'-phosphate, 9900; guanosine compounds, 11,800; adenosine compounds, 14,200.

† Recovered as inosine (extinction coefficient at 260 μm, pH 2, 7400).

‡ 25 per cent of this as b nucleotide (extinction coefficients at 260 μm, pH 2, 6500 and 9900 for cytidylic acid b and uridylic acid b, respectively).

§ 10 per cent as b nucleotide.

‖ Amount of b nucleotide not determined.
resulted. It will be noted that the nucleoside peaks are greatly reduced, the nucleoside-5′-phosphate peaks have correspondingly appeared, and the diphosphate peaks remain essentially unchanged. The 9 per cent of β cytidylic has now become 14 per cent and β uridylic is found to the extent of 11 per cent. The quantitative features of this analysis are listed in Table III, along with analyses of similar digests of RNA from yeast and from thymus.

It will be noted that the total nucleoside found in this experiment exceeds the inorganic phosphate present. The excess nucleoside is chiefly purine in nature and is nearly equal to the amount of pyrimidine diphosphate found. Since the diesterase preparation is always slightly contaminated with residual 5′-monoesterase, which attacks all four 5′-nucleotides indiscriminately, it is reasonable to ascribe the small amounts of pyrimidine nucleosides and an equivalent amount of purine nucleosides to this contamination, accenting the equivalence between purine nucleoside and pyrimidine diphosphate. From this equivalence, which is found in each of the three types of RNA shown in Table III and which seems to be independent of the degree of hydrolysis achieved, one is led to the conclusion that these substances arose from an asymmetrical splitting of a polynucleotide constituent of the RNA, leaving two phosphates attached to a pyrimidine riboside and none on a purine riboside in the same constituent.

The correction for 5′-phosphatase activity also raises the total of 5′-nucleotides presumably present at any time in the liver and yeast digests (the thymus RNA digest did not go to completion) to 78 and 88 per cent, respectively. The remainder is, of course, the non-5′-phosphorus in the diphosphates (a mixture of α,5′ and β,5′ as isolated) and in the β pyrimidine nucleotides. The significance of these compounds is discussed.

**DISCUSSION**

While the earlier non-quantitative data (10), derived from the use of intestinal phosphatase as a diesterase, indicated that some degree of 5′-phosphoesterification must be considered in formulating a structure for RNA, the present data indicate that about 80 per cent of the nucleotides have this characteristic and make this one of the major linkages to be considered. Thus the basic structural unit for RNA proposed by Brown and Todd (6), derived in part from our earlier data, becomes applicable essentially to the entire molecule. There remains, however, the question as to whether the chain is primarily α,5′ or β,5′ in nature, a mixture of both, or cyclic α: β,5′.

Some light is cast on the relative importance of α and β phosphoester linkages in RNA by the degree to which they appear as end-products of hydrolysis. Alkaline or acid hydrolysis liberates both in equal amounts, but, since this degradation probably proceeds through an α:β cyclic ester
STRUCTURE OF RIBONUCLEIC ACIDS. I

Ribonuclease liberates 60 per cent of the pyrimidines as \( b \) mononucleotides (10, 12, 20, 27-30), the other 40 per cent being end-groups of purine polynucleotides and having a singly linked \( b \) phosphate (12, 20, 27, 30). Thus all pyrimidine nucleotides are found in ribonuclease-treated RNA to have a \( b \)-linked phosphate. This hydrolysis has been shown to proceed through the same \( a:b \) cyclic phosphate intermediate as chemical hydrolysis; however, ribonuclease selectively breaks the \( a \) bond to leave the \( b \) (20, 31). However, since it is now known\(^1\) that this cyclic intermediate can be formed by ribonuclease only from the \( b \) phosphoesters of pyrimidine nucleosides, and not from the \( a \) esters, essentially all pyrimidine nucleotides in RNA must be regarded as not possessing \( a,5' \) linkages. Pyrimidine \( b \) nucleotides also are found in the diesterase digests; only in the diphosphonucleoside fraction of this hydrolysate are \( a \) groups found. As for the purine nucleotides, some of these are liberated as \( b \) nucleotides by the action of a nuclease from spleen (along with \( b \) pyrimidine nucleotides),\(^2\) but lack of knowledge of the mechanism of action of this enzyme makes this observation of little value. Nevertheless, the evidence so far obtained indicates a predominance of \( b \) over \( a \) linkages in RNA.

A proper interpretation of the data presented in this paper on the products of the action of venom diesterase upon RNA requires some information as to the mechanism of action of the enzyme. The diesterase prepared by us is practically inactive toward all mononucleotides, whether singly or doubly phosphorylated, but is active toward polynucleotides of all sizes, provided that they do not have a singly esterified \( b \) phosphate group. Thus the diesterase will hydrolyze the polynucleotides from the action of desoxyribonuclease on desoxyribonucleic acid (16), which end in \( 5' \)-phosphate,\(^3\) but not those from the action of ribonuclease upon RNA, which end in a \( b \) phosphate group. On the other hand, the \( a:b \) cyclic precursors of the latter (20), or the \( b \)-ending polynucleotides after dephosphorylation (12), are susceptible to rapid diesterase hydrolysis. It would thus appear that the singly phosphorylated \( b \) phosphate is inhibitory to a hydrolysis that splits the \( b \) links of doubly esterified phosphates.

If diesterase hydrolysis proceeded through cyclic intermediates, as postulated for ribonuclease action, further information as to the initiation and destruction of these intermediates would be required in order to relate the end-products to the original substrates. Cyclization involving \( 5' \) linkages is unlikely on steric grounds. Stronger evidence is afforded by the fact

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\(^1\) Todd, A. R., personal communication.

\(^2\) Volkin, E., and Cohn, W. E., unpublished data.

\(^3\) Sinsheimer, R. L., personal communication.
that diesterase will hydrolyze the polydesoxynucleotides derived from DNA by the action of desoxyribonuclease (16, 32). In these compounds, no cyclization is possible; yet the end-products are the same and are seemingly as readily produced as are those of the ribose analogues. From this analogy it may be argued that cyclization is not a necessary prerequisite to diesterase action and that the nucleotides found in diesterase digests are, therefore, a true reflection of the initial structure.

Prior to the admission of the 5' position as of significance equal to the 2' and 3' positions in the backbone of RNA, branching through triply phosphorylated ribose was not considered possible. The proposed new structure requires the consideration of such branching, e.g. through the 2' position in a 3',5' chain, which Brown and Todd have discussed (6). While some chemical evidence, based upon the isolation of ribose from methylated RNA, has been presented to indicate that triply esterified ribose molecules do exist in RNA (33), the strength of this evidence is impaired by lack of proof that all sugar hydroxyls react with the methylating reagent.

In seeking an explanation for the appearance of equal amounts of the pyrimidine diphosphonucleosides and the purine nucleosides in the diesterase digests, we have been led to the consideration of such branching through ribose. An unbranched RNA ending in b phosphate is eliminated from consideration as the source of these substances. They could so arise if the end-group were a cyclic a:b phosphate, but, since they could only originate from opposite ends of such a polynucleotide sequence, only 1 mole of each could be realized from 1 mole of RNA. This would place the maximal molecular weight of RNA in the neighborhood of 3000, i.e., six to ten nucleotides. Finally, we have found that removal of monoesterified phosphates ("end-phosphates") from our RNA by bone phosphatase (which reaches an end-point in the neighborhood of 10 per cent, consistent with titration data (34)) does not impair significantly the diesterase yield of cytidine diphosphate; thus, this cannot arise from the same end-groups that can be hydrolyzed by bone phosphate. We are led therefore to consider groups other than end-groups as sources of the diphosphonucleosides.

The type of branching proposed by Brown and Todd (6) offers an interpretation of the observations that is free of the objections raised to the unbranched chain. As shown diagrammatically in Fig. 4, the diesterase should effect cleavage of the b—O—P bonds as shown. At the branch

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4 That b nucleotides are 3'-nucleotides cannot be supported by this evidence alone without knowledge of the behavior of dinucleoside a,5'-monophosphates toward diesterase. Should these be inactive, the case for this eventuality would be greatly advanced, but, if they should prove as easily split as the b,5' compounds, the question would still be open.
point, a split at bond A would liberate nucleoside A as the α,5′ diphosphate, while the end-groups would be liberated as nucleosides. These are, therefore, labeled Py and Pu, respectively. Since the ribonuclease-produced polynucleotides are unbranched and contain b,5′-pyrimidine nucleoside and b (or a) purine nucleoside residues at either end, we consider the first member of the branch to be a pyrimidine and the rest of it to be purine.

![Diagram](image-url)

**Fig. 4. Schematic partial structure for RNA (after Brown and Todd)**

There remains to be established the significance of the b pyrimidine nucleotides found in the diesterase digests. We are inclined to regard these as originating from branches of but one nucleotide in length involving triply esterified phosphate. While such branches have not been considered in the formulations of RNA as discussed, their presence is indicated by the observation that about 10 per cent of the phosphorus in RNA is singly esterified\(^2\) (34).

**SUMMARY**

The hydrolysis of ribonucleic acids, isolated from yeast, from calf liver, and from thymus, by crude snake venom (which contains diesterase and 5′-nucleotidase activities) is shown to yield most of the substrate as nucleosides and inorganic phosphate, about a third of the cytidine and uridine...
being recovered as a mixture of \(2',5'-\) and \(3',5'-\)diphosphonucleosides. When the 5'-nucleotidase activity is removed, the same substrates are found to yield the same pyrimidine diphosphonucleosides plus purine nucleosides in nearly equimolar amounts, with the production of much less inorganic phosphate than nucleoside; the bulk of the substrate is recovered as nucleoside-5'-phosphates. Thus about 80 per cent of the nucleotide content of each ribonucleic acid is recoverable as 5'-phosphoesters. These findings confirm the earlier qualitative indications that this type of bond is a major one in ribonucleic acid.

In addition, the production of pyrimidine nucleoside diphosphates and purine nucleosides in equal amounts, taken together with the finding that the latter is a mixture of short unbranched polynucleotide chains ending in pyrimidine nucleotides and not oxidizable by periodate, indicates the possibility that these products arise from branch points and branch ends, respectively, the branch points being \(2',3',5'-\)triphosphopyrimidine ribosides and the branch ends being \(3'\) (or \(2'\)) singly phosphorylated purine ribosides.

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