STUDIES ON RIBONUCLEIC ACID

III. ON THE COMPOSITION OF THE RIBONUCLEIC ACID OF BEEF PANCREAS, WITH NOTES ON THE ACTION OF RIBONUCLEASE

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In a recent report on a procedure for the isolation of ribonucleic acid (RNA) from beef pancreas (1) it was shown that this compound is relatively labile, being partially depolymerized on incubation in solution at 25°, or when fractionated by precipitation with acetic acid (2, 3) or with a mixture of acetone and acetic acid (4). The widely varying composition reported in the literature for this compound (3, 5–10) may, consequently, be due to fragmentation of the native material during extraction and purification of the sample. A comparative study of the composition of RNA subjected to various methods of fractionation is, therefore, reported below.

Feulgen (11) was the first to report the presence of guanine in pancreas RNA in excess of that required for a tetranucleotide. Hammarsten (5), analyzing nucleoprotein prepared from pancreas with avoidance of both heat and alkalinity, found guanine and adenine in the ratio of 3:1, an amount of pentose consistent with a hexanucleotide, and purine nitrogen 75 per cent of the total. Jorpes (6), on the basis of determinations of total and acid-hydrolyzable phosphate, pentose, and total and purine nitrogen, concluded that the substance was a pentanucleotide. Isolation of the purine hydrochlorides from a suspension of the nucleic acid in methyl alcohol, however, revealed the guanine to adenine ratios in two experiments to be 3.3:1 and 4.6:1 (3). Jorpes (7) in a third attempt to solve this problem determined total purine before and after destruction of guanine, finding the guanine to adenine ratio to be 2:1, and concluded again that the pancreas ribonucleic acid was a pentanucleotide. Steudel (8), starting with Hammarsten’s (12) procedure for preparation of the β-nucleoprotein, prepared the barium nucleinate according to Jorpes (6), converted this to the copper salt, and found it to contain 4 molecules of guanine to each of adenine nucleotide. He concluded that the RNA of pancreas contained five purine and two pyrimidine nucleotides. He had isolated only the cytidylic acid, but assumed uridylic acid to be present.

More recently Plentl and Schoenheimer (9), using the procedure of Levene and Jorpes (3), isolated the purines from the mixed nucleic acids of rat and beef pancreas, which they had extracted with hot 10 per cent NaCl.
The ratio of guanine to adenine in the RNA of rat pancreas was found to be 3:1, but that for beef pancreas was 0.55:1. Vischer and Chargaff (10) applied their chromatographic procedure to the RNA of pig pancreas, isolated by the method of Jorpes (6) and purified according to Levene and Jorpes (3) but still containing 3 per cent of desoxyribonucleic acid. They accounted for 82 per cent of the nitrogen, finding the nucleotides of guanine, adenine, cytosine, and uracil in the proportion of 2.42:1:1.23:0.3.

**EXPERIMENTAL**

The analyses presented in Table I were made on pancreas RNA prepared by the procedure recently described from this laboratory (1) and fractionated in several different ways. These included (a) removal of the low polymer fraction by dialysis, (b) precipitation with a mixture of 20 parts of acetone, 40 of acetic acid, and 40 of 0.14 M NaCl, (c) precipitation with 83 per cent acetic acid, followed by (d) precipitation from the supernatant solution with an equal volume of alcohol.
The analyses were made by the spectrophotometric procedure described in the preceding paper of this series (13), the results being expressed as molar concentrations in order to permit comparison of determinations which serve as checks. For example, total purine (Table I, Column 3) was determined by dividing the optical density at wave-length 276 m\(\mu\) by the molecular extinction coefficient 7100, which at that wave-length is identical for adenine and guanine. The value thus obtained is in close agreement with the sum of the concentrations of adenine and guanine (Columns 1 and 2) as well as with the purine N determined by the micro-Kjeldahl procedure (Column 4). In the case of the pyrimidine nucleotides the sum of the concentrations given in Columns 5 and 6 does not agree so satisfactorily with total pyrimidine determined by optical density measurement at 265 m\(\mu\) (Column 7). In order to permit comparison of N determined by the Kjeldahl procedure after removal of the purines (Column 8) with the sum of N contained in cytidylic and uridylic acids, the values in Columns 5 and 6 were recalculated to represent mM of N rather than of nucleotides (Column 9). It is evident that an amount of N varying from a minimum of 0.18 to a maximum of 1.32 mM is found in the acid hydrolysate which cannot be accounted for as purine or pyrimidine.

The sum of the concentrations of adenine, guanine, cytidylic acid, and uridylic acid, each determined individually (Column 10), is in close agreement with the total phosphate (Column 11), the average difference being only 0.02 mM, or 1 per cent of the total.

The relative proportions of the four nucleotides are given in Table II, uridylic acid being taken as unity. In most of the specimens, adenine is

**Table II**

<table>
<thead>
<tr>
<th>RNA fraction</th>
<th>Guanine</th>
<th>Adenine</th>
<th>Cytidylic acid</th>
<th>Uridylic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated 36-10A</td>
<td>4.41</td>
<td>1.04</td>
<td>3.30</td>
<td>1.00</td>
</tr>
<tr>
<td>Non-dialyzable 36-10B</td>
<td>4.08</td>
<td>0.96</td>
<td>3.11</td>
<td>1.00</td>
</tr>
<tr>
<td>Acetone acetic acid ppt. 36-6</td>
<td>2.68</td>
<td>1.13</td>
<td>2.29</td>
<td>1.00</td>
</tr>
<tr>
<td>Acetone acetic acid ppt. 36-4</td>
<td>2.91</td>
<td>1.05</td>
<td>2.42</td>
<td>1.00</td>
</tr>
<tr>
<td>Acetone acetic acid ppt. 36-15</td>
<td>3.65</td>
<td>1.14</td>
<td>2.18</td>
<td>1.00</td>
</tr>
<tr>
<td>Acetone acetic acid ppt. 36-20A</td>
<td>3.31</td>
<td>1.20</td>
<td>2.43</td>
<td>1.00</td>
</tr>
<tr>
<td>Acetone acetic acid ppt. 36-20B</td>
<td>2.73</td>
<td>1.00</td>
<td>2.14</td>
<td>1.00</td>
</tr>
<tr>
<td>Acetone acetic acid ppt. 36-24</td>
<td>3.50</td>
<td>1.29</td>
<td>2.59</td>
<td>1.00</td>
</tr>
<tr>
<td>Acetone acetic acid (83%) ppt. 33-24</td>
<td>3.11</td>
<td>0.81</td>
<td>2.44</td>
<td>1.00</td>
</tr>
<tr>
<td>Alcohol ppt. in acetic supernatant 33-32</td>
<td>3.73</td>
<td>1.23</td>
<td>2.73</td>
<td>1.00</td>
</tr>
<tr>
<td>Alcohol ppt. in acetic supernatant 33-32</td>
<td>2.21</td>
<td>1.15</td>
<td>1.40</td>
<td>1.00</td>
</tr>
</tbody>
</table>
found in nearly the same concentration as uridylic acid. The molar ratio
of the purines varies from 2.36 to 4.21 of guanine (average 3.5) to 1 of
adenine. The pyrimidine nucleotides are found in ratios varying from
2.16 to 3.18 of cytidylic acid to 1 of uridylic acid.

DISCUSSION

Fletcher, Gulland, and Jordan (14) called attention to the low phos-
phorus content of some commercial preparations of yeast RNA, and sug-
gested that a singly esterified phosphate might be lost from a nucleotide
branching from the main nucleic acid structure. In our preparations, the
close agreement between the molar concentrations of phosphate and the
sum of the individual nucleotides indicates that each of the nucleosides
is fully phosphorylated, regardless of whether the preparation was precipi-
tated by alcohol, acetic acid, or the mixture of acetone, acetic acid, and
NaCl.

The analytical procedures we employed account for 88 to 105 (average
97) per cent of the total nitrogen of the nucleic acid. Purine N deter-
mined by the Kjeldahl procedure is in close agreement with the sum of
adenine and guanine N, any nitrogen unaccounted for being found in the
hydrolysate after removal of the purines. Although our preparations have
negative biuret tests, it is possible that the extra nitrogen represents the
hydrolysis products of traces of protein. A qualitative test revealed the
presence of some ammonium salts in the acid hydrolysate.

We are not satisfied that the hydrolysis of nucleic acid by 2 \( \text{N}_2\text{H}_2\text{SO}_4 \) at
100° is without influence on cytidylic acid. We find that evaporation of
cytidylic acid in \( \text{N} \) HCl solution to dryness at 100° in a stream of air re-
sults in the conversion of the major part of it to uridylic acid. A com-
parison of the analyses in Experiments 36-10A and 36-20B (Table I),
however, reveals no loss of cytidylic acid when the hydrolysis period was
increased from 30 to 60 minutes. The presence of ammonium salts in the
hydrolysate may be of significance.

The variable ratio of guanine to adenine found is not surprising in view
of the findings of others reviewed earlier in this paper. From the results
summarized in Table II, it will be noted that the highest proportion of
guanine (4.24:1) is found in the unfractionated specimens, and lower pro-
portions in the preparations subjected to dialysis or to fractionation by
means of acetic acid or acetone-acetic acid mixtures. The preparation
containing the lowest proportion of guanine is that precipitated by alco-
hol after removal of the fraction insoluble in 83 per cent acetic acid. These
variations may be attributed in part to alterations in the structure of the
RNA during the course of its preparation or fractionation. The labile
nature of the compound is indicated by its decomposition or depolymeri-
zation on incubation in aqueous solution (1).
Autolysis in the interval between death of the animal and fixation of the tissue must be considered as another factor causing variation in the ratios of guanine to adenine and cytidylic to uridylic acid. It is possible that RNA may also differ in composition in accordance with its participation in the metabolic processes of the cell. Investigation of the last two factors requires the development of quantitative procedures which may be applied to the tissue itself rather than to purified preparations of nucleic acid. A study of such procedures is in progress.

Our results are in harmony with the suggestion of Gulland (15) that the nucleotides may occur in random sequence in the polynucleotide.

Action of Ribonuclease—Since circumstances prevent the continuation of studies begun on the behavior of pancreas RNA with ribonuclease, the results of a typical experiment are presented in some detail below, together with a summarized statement of our general findings. It has already been noted (1) that, in aqueous solution, pancreas RNA is readily depolymerized at room temperature; hence an estimate of ribonuclease activity requires a careful comparison with control specimens incubated under the same conditions.

A specimen of pancreas RNA precipitated by a mixture of acetone, acetic acid, and NaCl, and analyzed for its content of purines and pyrimidines (13), was dissolved in water, adjusted to pH 5.3, and divided into two equal parts, each in a cellophane dialysis bag suspended in water at 25°. After 62 hours, the diffusion of phosphate having practically ceased, the dialysates were evaporated to a small volume in a vacuum and analyzed. To one of the bags containing the non-diffusible fraction crystalline ribonuclease (16) was added (0.8 mg. per 100 mg. of RNA) and the incubation of both control and enzyme-treated specimens was continued for another 24 hours. Only traces of phosphate were found in each dialysate. The non-diffusible enzyme-resistant fraction remaining in the bag, as well as the control, was then analyzed. In both specimens the RNA, although not diffusible, was no longer precipitable by 83 per cent acetic acid.

The results of analysis of the four fractions are given in Table III. Since no appreciable amount of phosphate diffused out after addition of the ribonuclease, it is evident that the non-diffusible fraction is resistant to the enzyme. Yeast RNA similarly treated behaved in an entirely different way. After the diffusible fraction had been removed by dialysis in the cold room, no further loss occurred when the dialysis was continued at 25°. Addition of ribonuclease at that point resulted in the diffusion of more than a third of the purine and more than half of the pyrimidine nucleotides. The hydrolytic effects of incubation of pancreas RNA thus resemble the enzymatic action of ribonuclease on yeast RNA. The addition of copper sulfate to the incubated RNA solution to a concentration of
0.02 per cent failed to inhibit this effect (17), which indicates that it cannot be attributed to the inclusion of Kunitz' enzyme in our preparation.

During incubation of pancreas RNA without added enzyme, the diffusion of the various nucleotides into the dialysate is not uniform, the loss of adenine exceeding that of guanine, with the result that the ratio of guanine to adenine becomes higher in the non-diffusible fraction. Uridylic acid also appears to diffuse to a greater extent than cytidylic acid. Other experiments, not recorded here, showed that incubation and dialysis of pancreas RNA resulted in an increase in the proportion of hydrolyzable to total phosphate in the non-diffusible fraction. This is interpreted as evidence of the loss by diffusion of pyrimidine nucleotides to a greater extent than that of the purine nucleotides.

The material which diffuses into the dialysate may be fractionated by alcohol, a fifth of the phosphate being precipitated at 70 per cent concentration. No phosphate is split from the alcohol precipitate by the monophosphoesterase prepared from hypertrophic prostate tissue (18), and is presumably a low polymer nucleic acid. The remaining four-fifths of the diffusible fraction, after removal of alcohol by distillation in a vacuum, loses from 45 to 65 (average 55) per cent of its phosphate on treatment with the prostatic enzyme, which suggests that it may be a dinucleotide or a mixture of mononucleotides with nucleic acid of low molecular size.

### Table III

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Specimen</th>
<th>Incubated and dialyzed</th>
<th>Per cent of original nucleotide (molar ratios in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hrs.</td>
<td>Guanine</td>
</tr>
<tr>
<td>36-27</td>
<td>RNA (acetone-acetic acid ppt.)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>36-27A</td>
<td>Control, diffusible</td>
<td>62</td>
<td>48.2</td>
</tr>
<tr>
<td>36-27A</td>
<td>&quot; non-diffusible</td>
<td>86</td>
<td>51.8</td>
</tr>
<tr>
<td>36-27B</td>
<td>Duplicate of Experiment 36-27A (no enzyme), diffusible</td>
<td>62</td>
<td>40.5</td>
</tr>
<tr>
<td>36-27B</td>
<td>Same + ribonuclease, non-diffusible</td>
<td>24</td>
<td>53.5</td>
</tr>
<tr>
<td>36-29A</td>
<td>Unfractionated RNA, control, non-diffusible</td>
<td>106</td>
<td>(4.58)</td>
</tr>
<tr>
<td>36-29B</td>
<td>Same + ribonuclease, non-diffusible</td>
<td>106</td>
<td>(3.84)</td>
</tr>
</tbody>
</table>
That ribonuclease may hasten the effects also produced by long incubation without enzyme is revealed by the fact that, in short periods of dialysis (5 hours) at 25°, the enzyme greatly increased the fraction of nucleic acid rendered incapable of precipitation by uranyl acetate in 10 per cent trichloroacetic acid (16, 19).

Our observations confirm those reported by Schmidt (20) concerning the existence of a fraction of pancreas RNA resistant to the depolymerizing action of Kunitz' ribonuclease (16). In a personal communication, Schmidt states, however, that the RNA disappears almost completely within 3 hours during autolysis of a pancreas suspension at 37°, and suggests that the pancreas contains some other enzyme or factor which is necessary for the hydrolysis of the nucleic acid.

Ribonuclease has been used as a tool for differentiating between ribo- and deoxyribonucleoprotein in histochemical studies (21), disappearance of basophilia after treatment of a tissue with the enzyme being considered evidence of the presence of ribonucleic acid. In view of the fact that a fraction of RNA is resistant to the action of this enzyme, caution must be used in the interpretation of histochemical findings based on its use.

SUMMARY

Analyses are reported for individual purines and the pyrimidine nucleotides in pancreas RNA. These account for all of the phosphorus and for 97 per cent (average) of the total nitrogen.

The molar ratios of the purines vary from 2.36 to 4.21 molecules of guanine to 1 of adenine. The pyrimidine ratios vary from 2.16 to 3.18 molecules of cytidylic acid to 1 of uridylic acid. Adenine and uridylic acid are found in nearly equal molar concentrations.

The composition of the RNA appears to be dependent partly on the mode of preparation. After removal of diffusible material by dialysis in the cold, further incubation at 25° causes extensive depolymerization of pancreas RNA (but not of yeast RNA), adenine and uracil nucleotides being lost by diffusion to a greater extent than the others. Treatment of the non-diffusible residue with ribonuclease results in the production of no new diffusible fragments.

BIBLIOGRAPHY

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