the RNA biosynthetic pathway, possibly at the IMP stage, is more readily accessible to MAP administered as the free base than to adenine.

Such a conclusion implies the existence of an efficient salvage pathway for MAP. Preliminary results indicate that certain of the yeast fractions active with respect to AMP and IMP pyrophosphorylases[10] can condense 5-phosphoribosylpyrophosphate with MAP. Studies directed toward the isolation of the resulting nucleotide and its possible subsequent conversion by deaminases have been undertaken.

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Separation of nucleotides, nucleosides, and bases from ribonucleic acid on Dowex-1 X8

No single method for the chromatographic separation of the bases, nucleosides, and nucleotides obtainable from RNA has been described. In previous studies incomplete separation of members of these three classes of compounds have been obtained on an anion-exchange column employing buffers ranging from pH 10.2 to 2.75 (ref. 1). During the chromatographic analysis of acid-soluble nucleotides from liver, a number of closely spaced peaks were observed very early in the run. Subsequent work suggested that these were nucleosides and bases. Extended studies have shown that excellent separation of these substances, in addition to nucleotides, may be obtained on Dowex-1 resin by adjustment of temperature, flow rate, resin particle size, column length, pH and ionic composition.

A representative chromatogram obtained with an artificial mixture is shown in Fig. 1. To maintain the relative order of all components, the entire elution was carried out at one pH, in this instance pH 4.4. Data on buffers, temperature and flow rates are given in the figure and legend. Thymine and inosine were added to indicate their position on the chromatogram. The technique allows the breakdown products obtained from RNA by either alkaline or acid hydrolysis or by irradiation to be analyzed on one column. A complete description of the automatic analytical system and opera-

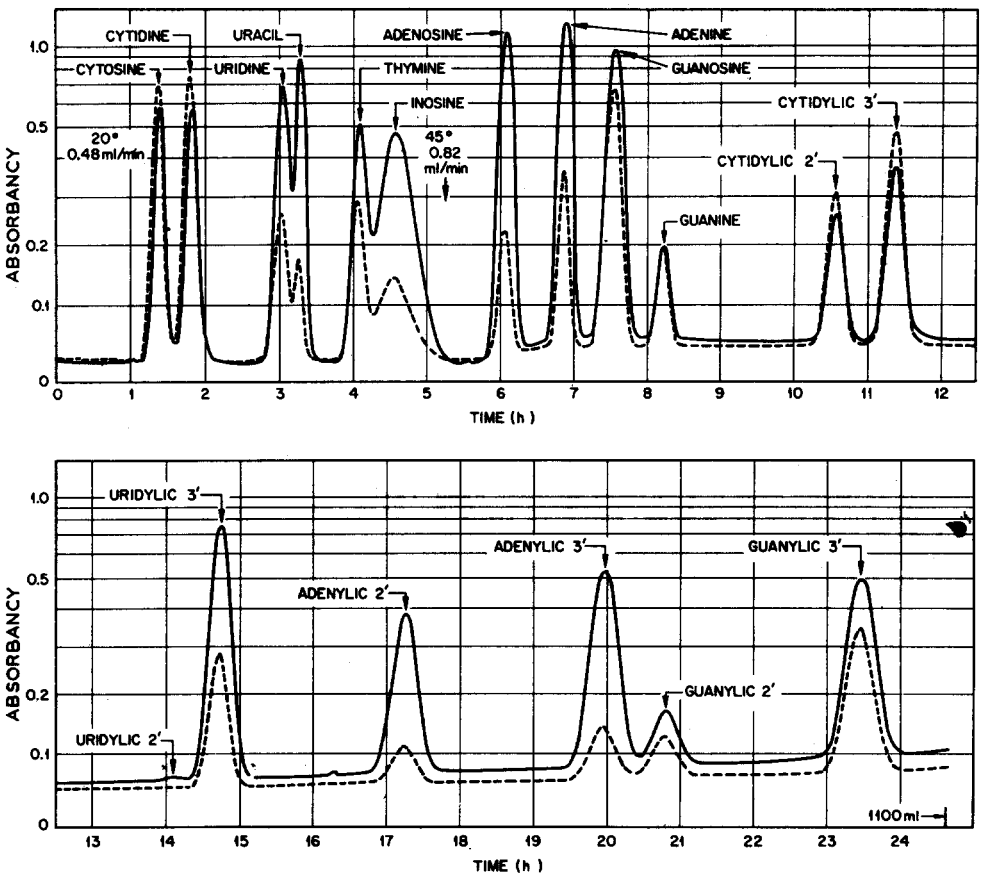

Fig. 1. Separation of a mixture of ribonucleotides, nucleosides, and bases by anion exchange. Exchanger: Dowex-1 X 8 acetate, hydraulically sized from through 400-mesh material, 0.9 × 150 cm. Eluting solution: 0.6 *M* sodium acetate grading linearly to 2 *M* sodium acetate, all at pH 4.4; flow and temperature: 0.48 ml/min at 20° changing to 0.82 ml/min at 45°; recording: absorbancy at 260 (solid line) and 280 mμ (dashed line) recorded at 5-sec intervals using 1-cm quartz flow cells and a recording system developed at ORNL (ref. 2).

tional procedures together with results obtained with DNA degradation products and tissue acid-soluble nucleotides will be published elsewhere.

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