Biosynthesis of bromegrass mosaic virus ribonucleic acid
by Dean Russell Branson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in BOTANY
Montana State University
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Abstract:
Both de novo synthesis of nucleotides and rearrangement of ribosomal breakdown products appear to
be precursor sources for bromegrass mosaic virus (BMV)-RNA. The incorporation of C^14-orotic acid
and C^14-uridine, intermediates of the de novo synthesis and breakdown pathways respectively, were
compared in BMV-infected barley during a period of virus synthesis.

Analyses of the C^14-BMV-RNA specific activities using the dilution factor method showed that the
carbon-14 from C^14-uridine was incorporated into BMV-RNA two to three times more efficiently
than the carbon-14 from C^14-orotic acid.

The essential enzymes for both pathways were demonstrated by using crude enzyme preparations.
Those enzymes considered essential were: 1) uridine 5'kinase, 2) orotidine 5'phosphate
pyrophosphorylase, and 3) orotidine 5'phosphate decarboxylase.

Ribosomal content was lower in BMV-infected tissue than in non-infected tissue suggesting that BMV
incorporated the products of host-RNA breakdown, in a manner similar to that reported for tobacco
mosaic virus infections. Continuation of ribosomal synthesis in virus infected tissue was demonstrated
by the incorporation of C^14-orotic acid into ribosomal RNA.

Pyrimidine metabolism, involved in the formation of RNA precursors in BMV-infected and
non-infected barley, appeared unchanged except for the additional function of BMV-RNA synthesis.
Evidence for this unchanged metabolism was: 1) radioactive ribosomal RNA from C^14-orotic
acid-fed barley, infected and non-infected, contained C^14-uridylic acid 3' and C^14-cytidylic acid 3'
when acid hydrolysed, and 2) ribosome breakdown was not a prerequisite for BMV-RNA precursor
formation. Direct incorporation, without delay, of C^14-orotic acid into BMV-RNA was observed.

These findings were consistent with the following relationship between host-RNA metabolism and
BMV-RNA biosynthesis: Pyrimidine Metabolism in BMV-infected Barley

*5-Phosphorylribose-1-pyrophosphate Orotic acid PRPP* Uridine Uridylic acid 5' Orotidine 5'
phosphate Uridylic acid 3' UDP UTP CTP Ribosomal - RNA BMV-RNA Breakdown De Novo
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in

BOTANY

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TABLE OF CONTENTS

LIST OF TABLES ................................................................. v
LIST OF FIGURES............................................................... vi
ABSTRACT ........................................................................ vii
INTRODUCTION ................................................................ 8
MATERIALS AND METHODS .................................................. 17
  Virus Inoculation and Purification........................................ 17
  Time Course of BMV Increase: Analytical Procedures .......... 18
  BMV Incorporation of Orotic Acid-C\textsuperscript{14} and Uridine-C\textsuperscript{14}: Analytical Procedures .......................... 19
  Pathway Enzymes: Analytic Procedures ............................... 21
  BMV and Ribosomal Incorporation of Orotic Acid-C\textsuperscript{14} ................................. 25
  Method of Testing BMV-Ribosome Separation by Ribonuclease Treatment ................................................. 27
  Method of Testing for Non-specifically Incorporated Carbon-14 ................................. 28
  Source of Chemicals and Reagents...................................... 28
RESULTS ............................................................................. 29
  Time Course of BMV Increase .......................................... 29
  Orotic acid-C\textsuperscript{14} and Uridine-C\textsuperscript{14} Assimilation into BMV-RNA ................................................. 32
  Enzymes of the Breakdown and de novo Synthesis Pathways ................................................. 37
  Orotic acid-C\textsuperscript{14} Incorporation into Ribosomal and BMV-RNA ................................................. 42
DISCUSSION ........................................................................ 56
LITERATURE CITED ............................................................. 65
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Dilution factors of assimilated orotic acid-C(^{14}) and uridine-C(^{14})</td>
<td>33</td>
</tr>
<tr>
<td>II</td>
<td>Radioactivities of BMV-RNA-C(^{14}) hydrolysates</td>
<td>36</td>
</tr>
<tr>
<td>III</td>
<td>Uridine 5'kinase and OMP pyrophosphorylase: decarboxylase in healthy barley</td>
<td>40</td>
</tr>
<tr>
<td>IV</td>
<td>Characterization of a product of orotic acid decarboxylase activity</td>
<td>41</td>
</tr>
<tr>
<td>V</td>
<td>A test of the method of separating virus from virus-ribosome mixtures with ribonuclease treatment</td>
<td>44</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1. Time course of BMV increase ........................................ 31

Figure 2. Distribution of C\textsuperscript{14} in BMV-RNA hydrolysates ........... 35
   2a. Typical C\textsuperscript{14} distribution in the BMV-RNA hydrolysates
       from uridine-C\textsuperscript{14} fed barley
   2b. Typical C\textsuperscript{14} distribution in the BMV-RNA hydrolysates
       from orotic acid-C\textsuperscript{14} fed barley

Figure 3. Uridine 5'kinase and OMP pyrophosphorylase-decarboxylase
   activity in cell-free extracts of barley .................................... 39
   3a. Uridine 5'kinase
   3b. OMP pyrophosphorylase:decarboxylase

Figure 4. Specific radioactivity of BMV and ribosomes
   isolated from barley .................................................................. 47

Figure 5. Accumulation of BMV in infected plants:
   testing the method of ribonuclease treatment
   for estimating virus content ............................................. 49

Figure 6. Orotic acid-C\textsuperscript{14} incorporation into BMV
   during virus synthesis ......................................................... 51

Figures 7 and 8. Orotic acid-C\textsuperscript{14} incorporation into ribosomes .......... 53
   7. Ribosomes from BMV-infected plants
   8. Ribosomes from non-infected plants

Figure 9. Proposed breakdown and de novo synthesis pathways:
   diagram of BMV-RNA precursor sources .................................... 62
Both de novo synthesis of nucleotides and rearrangement of ribosomal breakdown products appear to be precursor sources for bromegrass mosaic virus (BMV)-RNA. The incorporation of C\textsuperscript{14}-orotic acid and C\textsuperscript{14}-uridine, intermediates of the de novo synthesis and breakdown pathways respectively, were compared in BMV-infected barley during a period of virus synthesis. Analyses of the C\textsuperscript{14}-BMV-RNA specific activities using the dilution factor method showed that the carbon-14 from C\textsuperscript{14}-uridine was incorporated into BMV-RNA two to three times more efficiently than the carbon-14 from C\textsuperscript{14}-orotic acid.

The essential enzymes for both pathways were demonstrated by using crude enzyme preparations. Those enzymes considered essential were: 1) uridine 5'-kinase, 2) orotidine 5'-phosphate pyrophosphorylase, and 3) orotidine 5'-phosphate decarboxylase.

Ribosomal content was lower in BMV-infected tissue than in non-infected tissue suggesting that BMV incorporated the products of host-RNA breakdown, in a manner similar to that reported for tobacco mosaic virus infections. Continuation of ribosomal synthesis in virus infected tissue was demonstrated by the incorporation of C\textsuperscript{14}-orotic acid into ribosomal RNA.

Pyrimidine metabolism, involved in the formation of RNA precursors in BMV-infected and non-infected barley, appeared unchanged except for the additional function of BMV-RNA synthesis. Evidence for this unchanged metabolism was: 1) radioactive ribosomal RNA from C\textsuperscript{14}-orotic acid-fed barley, infected and non-infected, contained C\textsuperscript{14}-uridylic acid 3' and C\textsuperscript{14}-cytidylic acid 3' when acid hydrolysed, and 2) ribosome breakdown was not a prerequisite for BMV-RNA precursor formation. Direct incorporation, without delay, of C\textsuperscript{14}-orotic acid into BMV-RNA was observed.

These findings were consistent with the following relationship between host-RNA metabolism and BMV-RNA biosynthesis:

\[
\text{Orotic acid} + \text{PRPP} \rightarrow \text{PRPP} \rightarrow \text{Uridine} \rightarrow \text{Uridylic acid 5'} \rightarrow \text{UDP} \rightarrow \text{UTP} \rightarrow \text{CTP} \rightarrow \text{BMV-RNA}
\]

* 5-Phosphorylribose-1-pyrophosphate
INTRODUCTION

The general scheme of virus synthesis by converted cells is considered to be 1) the synthesis of virus protein and virus nucleic acid, and 2) the assembly of these virus components to form viral nucleoproteins (78).

Experiments reported below are concerned with the synthesis of viral RNA, origin of the precursors, and the metabolism of host-RNA during virus synthesis.

Precursors of viral RNA originate from host-RNA breakdown products and/or from de novo synthesis. The host-virus relationship governing the origin of viral RNA precursors is apparently not the same for all systems studied. Within the animal, bacterial and plant systems there are variations which prevent generalizations about the activity of a de novo synthesis pathway or a breakdown pathway during virus synthesis.

The basic questions of host-RNA metabolism during virus synthesis are: 1) continuation or cessation of host-RNA synthesis, and 2) stability or rapid decay of host-RNA content. Since a majority of the host-RNA is ribosomal and ribosomes have been demonstrated to be necessary for protein synthesis, investigations concerning the metabolism of ribosomes during virus synthesis seem important.

Precursor sources and ribosome metabolism are the subjects of this thesis. A literature review of these subjects follows.
Pyrimidine metabolism of the de novo synthesis pathway in plants has been studied only recently and it tentatively appears to be similar to that found in microbial and animal cells (16, 17, 44, 66, 67, 80, 81, 82). The proposed sequence of reactions includes a step leading to the formation of uridine-5'-phosphate (UMP5') from orotic acid (29).

**PRECURSOR SOURCE: DE NOVO SYNTHESIS PATHWAY**

![Diagram of pyrimidine metabolism](image)

Orotic acid-6-C\(^{14}\)  
Orotidine 5'PO\(_4\)  
Uridine 5'PO\(_4\)

*PRPP = 5'phosphoribosyl-1-pyrophosphate

Orotic acid formation in plants appears to be similar to other systems. The initial reaction is the formation of the pyrimidine precursor, L-ureidosuccinic acid, from carbamyl phosphate and L-aspartic acid (66).

Shigematsu (74) reported that H\(^3\)-aspartic acid introduced to tobacco mosaic virus (TMV)-infected tobacco could be recovered in the nucleic acid fraction, implying that the proposed de novo synthesis pathway is active during TMV biosynthesis.

Pyrimidine metabolism of the breakdown pathway in TMV-infected plants as proposed by Reddi and Mauser (62) directly contributes to the formation of TMV-RNA. Similarly, Gilliland et al. (38) suggest that
tobacco ringspot virus-RNA is formed by the rearrangement of host breakdown products.

**PRECURSOR SOURCE: BREAKDOWN PATHWAY**

\[
\text{Host-RNA} \rightarrow \text{ribonucleoside} \rightarrow \text{ribonucleoside 3'PO}_4 \rightarrow \text{ribonucleoside 5'PO}_4
\]

Leaf ribonucleases attack all the internucleotide links giving at first nucleoside 2'3'phosphates and later completing the hydrolysis of the pyrimidine and purine cyclic phosphates to give 3'phosphates (53).

Reddi and Mauser (62) demonstrated these nucleases in ribosome preparations from TMV-infected tobacco and called them RNase I and RNase II. Nucleotide phosphatase and nucleoside 5'phosphokinase activity which catalyze the formation of UMP5' from ribosomal breakdown products could be demonstrated in the same preparations.

Other pyrimidine metabolic pathways contributing to the formation of UMP5' that may play a significant role in viral RNA biosynthesis have been investigated in non-plant systems. Hurlbert and Kammen (43) reported the deamination of cytidine to uridine and its phosphorylation to UMP5'. Crawford et al. (28) and Reichard and Skold (63) and Reichard (64) described the direct formation of UMP5' from uracil, PRPP and UMP5' pyrophosphorylase activity. The metabolic activity of orotidine has been suggested (20, 82) but no direct evidence has been supplied. According to White et al. (79), the only known pathway for formation of a cytidine nucleotide involves amination of uridine triphosphate to yield the
corresponding cytidine triphosphate (16, 29). Uridine triphosphate formation involves consecutive transfers of phosphate from ATP to uridine 5'phosphate with intermediate formation of uridine diphosphate (79). The hypothesized viral RNA polymerase substrate "pool" consists of UTP, CTP, ATP and GTP. The formation of UTP and CTP is by the above described pathways.

The de novo synthesis and breakdown pathways leading to the formation of viral RNA precursors are the major interests of this thesis. Direct evidence is lacking for the activity of the de novo synthesis pathway during virus synthesis. Acceptable evidence is the incorporation of an intermediate, like orotic acid, into viral RNA.

Evidence for the activity of the breakdown pathway during virus synthesis is available in the animal virus, bacteriophage and plant virus systems (18, 48). This evidence is, in some cases, the incorporation of an intermediate of the breakdown pathway into viral RNA. In other cases it is an evaluation of host-RNA metabolism during virus synthesis.

Animal virus-RNA biosynthesis has been extensively investigated using poliovirus-infected cells. So sophisticated have been the experiments that one must identify the type of host-RNA and its subcellular location when discussing host-RNA metabolism in virus-infected cells. Holland and Peterson (42) have shown that synthesis of nuclear RNA is inhibited and cytoplasmic RNA synthesis is stimulated in poliovirus-infected human cells. If the synthesis of cytoplasmic RNA is
stimulated and the cytoplasm is the site of poliovirus-RNA replication (3, 4, 5) then the most likely source of viral RNA precursors would be de novo synthesis. Consistent with de novo synthesis would be reports on the stability of ribosomal RNA in poliovirus-infected cells by Holland (41), Zimmerman et. al. (84) and Fenwick (32). Evidence supporting the alternate source of viral RNA precursors (host-RNA breakdown products) as reported by Salzman et. al. (70) and Salzman and Sebring (71), is that poliovirus incorporated radioactive uridine, adenine and cytidine in infected S 3 cells. These compounds are intermediates of the proposed breakdown pathway. Diamond and Balis (30) suggests both de novo synthesis and host-RNA breakdown as sources for poliovirus-RNA. Perhaps nuclear ribosome breakdown products cross the nuclear membrane and become available for viral RNA synthesis.

Myxoviruses such as fowl plague virus and Newcastle disease virus do not induce synthesis of inhibitors of cellular RNA synthesis (68). Inhibition of nuclear RNA appears to be characteristic of picornaviruses (31, 35, 42, 54, 70, 89). In the early stages of myxovirus-RNA biosynthesis the process appears to be an additional performance of the host cells (68), meaning that the viral RNA precursors originate from both sources; de novo and breakdown products.

Bacteriophages have been used in studies which have contributed to a model for viral RNA replication (50). Recent studies on viral RNA replication are reviewed by Burke (18), Levintow (48), Ochoa et. al. (38), and Zinder (85). The aspects of viral RNA replication of much interest
are the demonstration and characterization of a viral induced DNA-independent-RNA polymerase and a double stranded replicative form of viral RNA, which is a parental viral RNA (plus strand) complementarily paired with a template strand (minus strand) (59).

Friesen (36) reported evidence suggesting that the control of phage-RNA synthesis is subject to those factors described for the control of ribosomal and transfer RNA synthesis. This indirectly means both *de novo* synthesis and breakdown pathways are available to supply phage-RNA precursors. The fact that many *in vivo* phage labeling studies use radioactive uridine (33), an intermediate in the proposed breakdown pathway, indicates that at least host-RNA breakdown products could be virus precursors. Ellis and Paranchych (31) and Cooper and Zinder (27) conclude that viral RNA and protein are synthesized from soluble precursors. This notion is compatible with precursors arising from any source including breakdown products and *de novo* synthesis. The cessation of host-RNA synthesis and/or a variation in the host-RNA content in infected cells as well as the source of viral RNA precursors appear to be aspects of bacteriophage-RNA biosynthesis that have been overlooked.

RNA biosynthesis of plant viruses has been studied primarily in TMV-infected tobacco and to a lesser degree in turnip yellow mosaic virus (TYMV)-infected cabbage. Precursor sources and ribosome metabolism, aspects of viral RNA biosynthesis, are the subjects of several recent reports (1, 25, 60, 61, 62). Reddi (60) has suggested that TMV induces the rapid breakdown of tobacco microsomal (ribosomal) RNA and that the breakdown products are
enzymically assimilated into TMV-RNA. The amount of TMV-RNA synthesized is approximately equal to the amount of ribosomal RNA degraded. The use of uracil-H³ and uridine-H³ for studies on TMV synthesis indirectly assumes a precursor source (40, 75, 76, 83).

Continuation of ribosome synthesis after TMV infection was observed by Reddi (60). He found large amounts of radioactivity in the ribosome fraction several days after inoculation and after introduction of C¹⁴-precursors of ribosomes. Babos (1) and Sanger and Knight (72) were also able to demonstrate the continuation of ribosome synthesis after TMV infection.

Disagreement with Reddi on the source of viral RNA precursors was reported by Commoner et al. (25) who indicated that TMV-RNA was synthesized largely de novo. This finding was confirmed by Babos (1) and Kubo (47) on the basis that the rate of host-RNA synthesis and host-RNA degradation was unaffected by TMV synthesis in the cells. Babos did not show, however, that de novo or breakdown products were specifically incorporated into TMV-RNA. One possible explanation suggested by Babos (1) for the disagreement was that Reddi used much younger tissue.

Stability of ribosome content in TMV-infected tissue was also supported by evidence reported by Basler and Commoner (6), Röttger (69), Commoner et al. (24), Balandin et al. (2), Fry and Matthews (37), and Babos (1). Generally, the ribosome content of TMV-infected samples taken at intervals after inoculation increases at first and then gradually decreases at a rate equal to or slightly greater than that of non-infected tissue samples.
The effects of virus infection on the distribution of the various classes of ribosomes have not been adequately studied except in the case of a turnip yellow mosaic virus infection in cabbage (55, 56). The 68 S ribosomes of Chinese cabbage leaves appear to be confined to chloroplasts of healthy leaves (23) in a concentration of 0.2 mg/g fresh weight (65). No 68 S ribosomes could be detected in symptomatic tissue of TYMV-infected cabbage. The concentration of 83 S cytoplasmic ribosomes (22) decreases during leaf expansion in both infected and non-infected tissue. This decrease in chloroplast ribosomes along with other indirect evidence suggests not only that TYMV-RNA is synthesized in the chloroplast but also that biosynthesis takes place by the ribosomal breakdown mechanism proposed by Reddi and Mauser (62). The decrease of ribosome content in beans infected with southern bean mosaic virus, as demonstrated by Meister (57), is also evidence for a ribosome breakdown mechanism. It is obvious that future studies on viral RNA biosynthesis should be at the subcellular level.

In light of the above review there appear to be conflicting views on the origin of plant virus RNA precursors as well as the synthesis of host-RNA during virus synthesis. The stability of ribosome content during virus synthesis is also questionable.

The main purpose of the following investigation was to determine whether orotic acid, an intermediate of the de novo pathway, and/or uridine, an intermediate of the breakdown pathway, could be incorporated into BMV-RNA. The relative activity of these two pathways was determined
by introducing radioactive orotic acid and uridine into similar lots of BMV-infected barley and comparing the amount of assimilated radioactivity. Parallel studies dealt with two other aspects of viral RNA biosynthesis; 1) ribosomal content, and 2) ribosomal synthesis.

Bromegrass mosaic virus infected barley is a favorable system for investigating the plant-virus relationship during biosynthesis of viral ribonucleic acid. The advantages of this system are: 1) several milligrams of virus are easily purified from a gram of infected tissue (11,21); 2) the RNA content (2%) is relatively high (10); 3) rapidly translocated radioactive viral precursors can be introduced to infected tissue with ease (7); and 4) BMV is structurally similar to other small plant, animal and bacterial RNA viruses (45, 46, 85).
MATERIALS AND METHODS

Virus Inoculation and Purification  The type strain of bromegrass mosaic virus (BMV) AC-66 American Type Culture Collection, 1958 1/ was used in this study. The virus was cultured in barley (Hordeum vulgare L. var. Blackhulless) in a greenhouse at an air temperature of 20-30°C unless otherwise stated.

Plants in the two-to three-leaf stage were inoculated by rubbing the leaves with a sterile cheesecloth pad soaked in crude sap from BMV-infected plants diluted 1:10 with tap water containing Celite.2/ This method of inoculation was used for all time-sequence studies of BMV infections, virus purifications and maintenance of the stock culture.

Infected leaves were harvested two weeks after inoculation, or, in the case of replication studies, the inoculated leaves were harvested at designated times after inoculation. Leaf samples of less than fifty grams were ground with a mortar and pestle and larger samples were ground with a meat grinder. Ground samples were diluted with equal volumes for tissue weight (w/v) of pH 7.0, 0.01M potassium phosphate buffer and then frozen. Frozen samples were thawed, heated for one hour at 40°C and clarified by centrifugation for 15 min at 20,000 g in a refrigerated centrifuge. The supernatant fluid containing the crude virus preparation

1/ BMV-infected tissue was obtained from Dr. R. I. Hamilton of the Botany and Microbiology Department, Montana State University, Bozeman.

2/ Diatomaceous silica, prepared by Johns-Manville Co.
was either analysed for virus mass by the sucrose density gradient centrifugation method or was further purified by differential centrifugation. The method of differential centrifugation was similar to that used by Chiu and Sill (21), Brakke (14) and Bockstahler and Kaesberg (11), except the low speed centrifugations were 15 min at 20,000 g, the high speed centrifugations were 3 hr at 100,000 g or 2 hr at 130,000 g, and the virus pellets were resuspended in 0.01M phosphate buffer pH 6.5.

**Time Course of BMV Increase: Analytical Procedures**

BMV replication rates were estimated by sucrose density gradient centrifugation analysis for virus mass at intervals after inoculation. Two gram samples of inoculated leaves were used to obtain crude virus preparations for sucrose density gradient centrifugation analysis. Crude virus was prepared from fresh tissue by the grind-freeze-thaw-heat method previously described. A 0.2 ml sample was overlayed with 0.1 ml buffer and floated on a gradient column prepared by layering 1.1 ml of buffered solutions (0.01M phosphate, pH 6.5) of 100, 200, 300 and 400 mg sucrose per milliliter. Gradient columns were centrifuged for 1.5 hr at 38,000 rpm in the SW39L rotor and fractionated using an ISCO Density-Gradient Fractionator. Virus zones were quantitated by their UV absorption at 254 nm as recorded on a Nesco graphic recorder. Several runs using known concentrations of highly purified BMV established a relationship between virus mass and the area under the absorption curves. This

---

\[ UV = \text{ultraviolet} \]
relationship was used to convert area into mg BMV per gram of infected leaf tissue. Similarly prepared samples from non-infected barley were tested by the same method for UV absorbing material at a position corresponding to the virus zone for samples from infected tissue.

BMV concentrations were determined using the mass to UV absorption conversion factors of $4.5 \, A_{254\text{nm}}^2$ and $4.8 \, A_{260\text{nm}}^2$ for a 1 mg/ml solution (13). Ultraviolet spectrophotometry was done using a Beckman DU Spectrophotometer and a 1 cm light path.

Relative virus mass at intervals after inoculation was also determined by a microprecipitation test as described by Hamilton (39). Anti-BMV serum furnished by Dr. R. I. Hamilton was used to determine dilution end points of crude virus preparations.

**BMV Incorporation of Orotic acid-$^{14}$C and Uridine-$^{14}$C: Analytical Procedures**

Barley plants used in these studies of the precursors of viral nucleic acid were grown in an ISCO E-2 Environment Chamber with a diurnally fluctuating temperature of 4.5 to 21°C at 60 per cent relative humidity and a photoperiod of 14 hours at 3,000 foot-candles.

Orotic acid-$^{14}$C and uridine-$^{14}$C were injected with the aid of a 27 guage needle into the leaf whorls of BMV-infected plants. Neither injection volumes nor the amount of radioactivity injected were controlled because of the irregularity of the spaces between leaf sheaths. Solutions of orotic acid-$^{14}$C for injection were 1 μmole/ml of 0.01M phosphate buffer pH 6.0, containing a stoichiometric amount of 1-phosphoribosyl-5-
pyrophosphate (PRPP, 1 μmole/ml) required for the formation of orotidine 5'phosphate (OMP) (29, 49, 79). Solutions of uridine-C\(^{14}\) for injection were 1 μmole/ml of 0.01M phosphate buffer pH 6.0. Plants were injected on post inoculation (PI) day 12, (one day before initial symptoms) again on PI, day 13 and were harvested on PI, day 14, thus allowing a 48 hr carbon-14 incubation period. The eclipse period and rate of BMV synthesis at this temperature profile were determined by the method of BMV increase described above.

Incorporated carbon-14 was estimated by the amount of radioactivity that sedimented with BMV in a sucrose density gradient column. Appropriate checks were conducted to determine the amount of non-specifically incorporated carbon-14 (see Method of testing for non-specifically incorporated carbon-14, p. 29). These experiments were conducted in a fashion that allowed a comparison of the incorporation efficiencies of orotic acid and uridine into BMV.

Preparation of a crude extract from the harvested leaves was similar to that described for the BMV increase studies. Crude extracts were then subjected to sucrose density gradient analysis and the virus zones were collected. Specific activities (sp act) of the collected virus zones were determined by converting virus absorbancy at 260 nm into mg BMV and then calculating the total C\(^{14}\) in the sample from the disintegrations per minute (dpm) of an aliquot. Quenching was determined by the channels ratio method. The dpm were determined in a Nuclear-Chicago scintillation counter using 12.5 ml of scintillation fluid composed of 4.0 g PPO (2, 5
diphenyloxazole) and 100 mg POPOP (2, 2' paraphenylene bis 5-phenyl-
oxazole) in 1 liter of toluene. To this was added 1.5 ml of absolute reagent grade methanol. Usually 10 μl aliquots were counted for 10 min.

The dilution factor method (26) of incorporation efficiency analysis was employed with the following expressions defining the dilution factors x and y for this experiment:

\[
\frac{\text{sp act (μc/μ mole) orotic acid-C}^{14}}{\text{sp act (μc/μ mole) BMV-C}^{14} \text{ from orotic acid-C}^{14} \text{-injected plants}} = x
\]

and

\[
\frac{\text{sp act (μc/μ mole) uridine-C}^{14}}{\text{sp act (μc/μ mole) BMV-C}^{14} \text{ from uridine-C}^{14} \text{-injected plants}} = y
\]

Dilution factors are inversely proportional to the incorporation efficiency of the fed compound.

Translocation of the fed compounds was determined by autoradiography. Harvested leaves from plants that had been injected 48 hr previously with orotic acid-C\(^{14}\) and uridine-C\(^{14}\) were exposed to X-ray films for six days at -10°C. Exposed X-ray film was developed in D-19 developer for 10 min, then fixed and washed. Translocation of orotic acid and uridine was evaluated by the dispersion of carbon-14.

**Pathway Enzymes: Analytic Procedures**

Demonstrations of enzymic activities were based on general requirements for the conversion of a carbon-14 substrate to a carbon-14 product. These general requirements are:

1. amount of product in the reaction mixture increases with time;
2. activity increases with greater amounts of enzyme (protein
3. activity decreases to zero in absence of the enzyme or with enzymes that have been previously heated (55°C for 5 min). Enzymes selected were uridine 5' kinase, OMP pyrophosphorylase and OMP decarboxylase because they have been reported (41) to be essential for the incorporation of orotic acid and uridine into nucleic acids (31) and to the author's knowledge, their activities have not been reported in barley.

Protein extracts were prepared from barley leaf homogenates by grinding leaf tissue in 1:10 (w/v) 0.01M phosphate buffer pH 7.0 containing sufficient (NH₄)₂SO₄ to make the final volume 20% saturated. Solubilized proteins were separated from cellular debris by centrifuging at 30,000 g for 20 min and making the supernatant fraction 60% saturated with respect to (NH₄)₂SO₄ according to the method of Branson (15). Resulting precipitates were collected by low speed centrifugation and resuspended in 1:1 (w/v) 0.01M phosphate buffer pH 7.0. Following another low speed centrifugation to remove any insoluble material, the resuspended protein extract was dialysed against 2,000 volumes of 0.01M phosphate buffer pH 7.0. All steps were carried out at 4°C unless otherwise stated. Protein concentrations were determined by the method of Lowry et al. (51) and adjusted to 0.5 mg/ml and 1.0 mg/ml with respect to the reaction mixture.

The assay method for uridine 5' kinase was similar to that used by Reddi (61). Reaction volumes of 5 ml were a mixture of 1.0 ml uridine-
C\textsuperscript{14} (50 m\textmu c/ml), 1.0 ml adenosine triphosphate (ATP, 0.01M), 0.5 ml MgCl\textsubscript{2} (0.05M) and 2.5 ml enzyme (protein extract) buffered with 0.01M phosphate. Protein concentrations of 1 mg/ml and 0.5 mg/ml were equivalent to 2X and X amounts of enzymes, respectively. The pH was controlled by the 0.01M buffer pH 7.0, in the enzyme preparation.

Assay reactions were started by the addition of the uridine-C\textsuperscript{14}, incubated for 0, 2, 10 and 20 min at 37\textdegree C, and stopped by the addition of 2.5 ml of 18\% trichloroacetic acid. To facilitate precipitation reaction vessels were placed in an ice bath for 30 min. Following the removal of resulting precipitates by centrifugation supernates were supplied with approximately 1 mg of uridine and uridylic acid (uridine 5' monophosphate or UMP 5') as carriers for the substrate and product of the reaction.

Twenty \mu l aliquots of the reaction mixtures were spotted on Whatman #1 chromatography paper and developed in butanol: ethanol: formic acid: water (5:3:2:1) for 12 hr at 25\textdegree C according to the method of Fink (34). Uridine and UMP 5' were located on chromatograms with the aid of a short wave UV mineral light before determining their radioactivities in a Packard Radiocromatoscanner. Strip scanning was done at 2 cm per min with a time constant of 30, in a range of 0 to 1000 cpm and with collimators set at 5 mm. Radioactivities of individual spots were determined by placing spot cut-outs in scintillation vials and counting by the method described above.
Orotidine-5'-phosphate pyrophosphorylase and OMP decarboxylase were assayed as a single enzyme that catalyzed the formation of UMP 5' from orotic acid.

Reaction mixtures were similar to those used by Kapoor and Waygood (44), but the assay method was analogous to that used for uridine-5'-kinase with orotic acid-\(^{14}\)C being converted to UMP5'. Reaction volumes of 3 ml were a mixture of 0.1 ml orotic acid-\(^{14}\)C (5 \(\mu\)c/ml; sp. act. 44.5 mc/mM), 0.3 ml PRPP (0.57 mg/ml), 0.3 ml Mg Cl\(_2\) (7.5 mg/ml), 1.8 ml phosphate buffer (0.01 M, pH 8.0) and 0.5 ml enzyme (3 mg/ml). Assay reactions were started by the addition of the orotic acid-\(^{14}\)C, incubated for 0, 2, 10 and 20 min at 37°C and stopped by the addition of 1.5 ml of 18% trichloracetic acid. Reaction vessels were placed in an ice bath for 30 minutes to facilitate precipitation. Following the removal of resulting precipitates by centrifugation, supernates were supplied with approximately 1 mg of orotic acid and UMP5'. Orotidine 5' phosphate, the intermediate of this reaction, was unavailable for co-chromatography with every sample but sufficient OMP was available\(^4\) for a few experiments. OMP-\(^{14}\)C was further identified by a comparison of its UV absorption properties with authentic OMP.

\(^4\)Authentic OMP was graciously supplied by Dr. Cleon Ross of the Botany and Plant Pathology Department, Colorado State University, Fort Collins.
Analyses of the reaction mixture by chromatography and radiochromatography were similar to those described above except that the chromatograms were developed in acetonitrile:water (ammoniacal to pH 10) (70:30), for 4 to 6 hr at 25°C, a system selected by Berger and Hedrick (9) for separating certain pyrimidine derivatives.

**BMV and Ribosomal Incorporation of Orotic acid-C14**

BMV-infected and non-infected barley plants growing in the greenhouse were injected with orotic acid-C14 on P.I. day 7 (one day after initial symptoms) by the method described above. Three-gram samples of leaf tissue were harvested from BMV-infected and non-infected plants at daily intervals on post carbon-14 (P.C14). On P.C14 day 0, a 3 gm leaf sample was obtained from four plants and on P.C14 day 7 from 3 plants. Fractions of plants to make up a 3 gm sample were obtained from the youngest leaves.

A BMV-ribosome mixture (80 S fraction) was prepared from infected plants by differential centrifugation since the sedimentation coefficients of BMV and plant cytoplasmic ribosomes were 86 S and 80 S, respectively (11, 12). Preparation of an 80 S fraction from both BMV-infected and non-infected samples was similar to that described by Bonner (12) for preparation of ribosomes from plant material. Three gram tissue samples were mixed with sand, pulverized in a mortar and pestle with 20 ml of ribosomal extraction buffer5/ and frozen. Frozen samples were

5/ Ribosomal extraction buffer was the same as that used by Bonner (12) except 0.1% deoxycholate was used.
thawed and clarified by centrifugation for 20 min at 20,000 g, in a refrigerated centrifuge. The supernatant fractions were submitted to high-speed centrifugation for 2 hours at 40,000 rpm in the Spinco #50 rotor. Resulting pellets, containing the 80 S fraction, were resuspended in 2 ml ribosomal extraction buffer without the deoxycholate and 0.4 M sucrose. Resuspended 80 S fractions were separated from any insoluble material by a further low-speed centrifugation. Purity of the 80 S fraction was estimated by sedimentation in sucrose density gradient columns and UV absorption.

Analysis of the orotic acid-C\textsuperscript{14} incorporation into the 80 S fraction (ribosomes and virus) was based first on counting a 10 ul aliquot for 10 min in the scintillation counter and then by determining the specific activity of the 80 S fraction. Specific activity was assigned an arbitrary definition of:

\[ \text{dpm per unit RNA} = \text{specific activity} \]

A unit of RNA was that amount of RNA with an absorbancy of 1.0 at 260 mu. The A\textsubscript{260} of the 80 S fraction was primarily due to RNA. Non-RNA material, absorbing UV at 260 mu, was neglected.

To determine the incorporation of C\textsuperscript{14} into ribosomes, ribosomes were separated from virus particles in the 80 S fraction on the basis of their sensitivity to pancreatic ribonuclease Type II. Sucrose density gradient analysis of the 80 S fraction did not resolve a BMV zone and a ribosome zone. The ribonuclease treatment method of separating ribosomes and virus is analogous to that used by Reddi (60). One ml aliquots of the 80 S fractions
were mixed with 0.25 ml ribonuclease (1 mg/ml) and incubated for 3 hrs at 30°C. Ribonuclease hydrolysis of ribosomes was stopped by the addition of 1.25 ml of 18% trichloracetic acid followed by immersion of the reaction vessel in an ice bath for 30 minutes. Resulting precipitates were removed by low speed centrifugation. After decanting the supernatant liquid the precipitates were solubilized in 2.5 ml 1N NaOH. These two solutions, supernatant liquid and solubilized precipitate, represent the enzymic hydrolysate of ribosomal RNA and the alkaline hydrolysate of BMV-RNA respectively.

Ribosomal RNA hydrolysates and BMV-RNA hydrolysates were analysed for C¹⁴ (dpm) and total A₂₆₀ and the specific activity calculated by the methods described above. Spectrophotometric references were 9% trichloroacetic acid and 1N NaOH for the ribosomal RNA and BMV-RNA hydrolysates, respectively. Hyperchromicity of the RNA hydrolysates at 260 μm was not subtracted from the total absorption.

Method of testing BMV-ribosome Separation by Ribonuclease Treatment

Separation of ribosomal RNA from BMV-RNA by their differential sensitivity to ribonuclease was tested by base ratio analysis. Samples hydrolysed by acid and ribonuclease for analysis were purified BMV, ribosomes purified from non-infected barley and mixtures of BMV and ribosomes from infected barley. Acid hydrolysis conditions of 1N HCl for 1 hour at 100°C were maintained according to the method of Markham and Smith (52). Ribonuclease hydrolysis was accomplished by the previously described method.
Hydrolysate aliquots were chromatographed on Whatman #1 paper with a solvent of tert-butanol: 0.8 N HCl (70:30) (52) for 25 hr at 25°C. After locating the bases on the chromatograms with an Ultra-violet Products, Inc. Mineralight, the bases were eluted in 4 ml 0.1 N HCl and quantitated using the absorbance values of Markham and Smith (52). Ratios of purines to pyrimidines for the respective hydrolysates were compared in determining the insensitivity of BMV to ribonuclease.

Method of Testing for Non-specifically Incorporated Carbon-14

Acid hydrolysates of RNA samples that were being checked for non-specific incorporation were chromatographed on Whatman #1 paper in a solvent of acetonitrile: water ammoniacal to pH 10 (70:30). Criteria of specific incorporation were: 1) association of radioactivity with the UMP 3' and cytidylic acid 3' (CMP3') moieties, and 2) low amounts of radioactivity in the $R_f$ zone corresponding to the fed compounds (uridine-C$^{14}$ or orotic acid-C$^{14}$) on chromatograms. Radiochromatoscanning was accomplished by the method described above.

Source of Chemicals and Reagents

Orotic acid-6C$^{14}$ and uridine-2C$^{14}$ (specific activities of 44.5 mc/mM and 42.3 mc/mM) were purchased from Nuclear-Chicago, Des Plaines, Ill. Adenosine triphosphate, uridine, uridylic acid, orotic acid, ribonuclease Type II, uridine 3' monophosphate, cytidine 3' monophosphate, adenine, and guanine were purchased from Sigma Chemical Co., St. Louis, Mo.
RESULTS

Time Course of BMV Increase

BMV accumulated in inoculated plants at rates that varied with the environment in which the plants were grown. Accumulation rates were calculated from the initial slopes of the growth curves in Fig. 1. These rates were 1.42 mg BMV/g tissue/day for 40/20°C (summer environment), 0.48 mg/g/day for the 27/13°C (fall environment), 0.82 mg/g/day for the 27/20°C (winter environment), and 0.18 mg/g/day for the 20/4°C (chamber environment). The fact that these rates were constant rather than exponential was in agreement with the general virus model reported by Bentzon et al. (8).

Growth curves, as illustrated in Fig. 1, are typical of the multiplication of plant viruses according to Matthews and Ralph (56) and Schuster (73) and provide information about the timing of certain events in virus synthesis. These events, originally described by Stent (77) for the phage model and redefined for this thesis are: 1) eclipse period - time between inoculation and the beginning of virus increase; 2) accumulation period - time during which the rate of virus increase is constant; and 3) terminal events - time during which virus increase approaches a minimum, virus accumulation reaches a maximum and the virus content decreases.

Times for feeding C¹⁴-viral precursors were arbitrarily selected at two days after the eclipse period, thus taking advantage of several days of maximum virus increase for carbon-14 incorporation. These selected
Figure 1. **Time course of BMV increase**

Crude virus preparations from BMV-infected barley were analysed for virus content by the sucrose density gradient method. BMV content in barley leaves increased at intervals post inoculation (P.I.) during summer greenhouse air temperature range (40/20°C, O—O ), winter (27/20°C, ▽—▽), fall (27/13°C, O—O ), and environment chamber (20/4°C, ▽—▽).

Significance of the figure: time selected for introduction of virus precursors before the period of rapid virus synthesis, indicated by arrows.
times for feeding C^{14}-viral precursors were indicated for each growth
curve in Fig. 1 and were used in subsequent experiments.

**Orotic acid-C^{14} and uridine-C^{14} assimilation into BMV-RNA**

Radioactive BMV from plants injected with orotic acid-C^{14} and
uridine-C^{14} was compared to evaluate the relative activities of the
breakdown and de novo synthesis pathways during virus synthesis.
Precursors assimilated into viral RNA were determined by the previously
described dilution factor method. Activity of the de novo synthesis and
breakdown pathways were indicated by the relative amounts of C^{14}
assimilated into BMV-RNA. Activities of the pathways were interpreted
as being inversely proportional to the dilution factors (Table I). By
these standards, uridine was found to be assimilated two to three times
more rapidly than orotic acid, indicating that the breakdown pathway
was more active than the de novo pathway.

The phrase "incorporated radioactivity" as used in this thesis refers
to the total amount of radioactivity associated with the particle and
is the sum of assimilated and non-specifically adsorbed radioactivities.
Incorporated radioactivity sedimented with BMV in a sucrose density
gradient column but only that radioactivity which was assimilated was
found with the uridylic acid 3' (UMP3') and cytidylic acid 3' (CMP3')
hydrolysates of BMV-RNA (Fig. 2a, 2b). Orotic acid-C^{14} and uridine-C^{14},
that was non-specifically associated with BMV, accounted for about 10
per cent of the total incorporated radioactivity (Table II). Radio-
activity within spots on chromatograms corresponding to hydrolysate
Table I. Dilution factors of assimilated orotic acid-$^{14}\text{C}$ and uridine-$^{14}\text{C}$

<table>
<thead>
<tr>
<th>Expt.</th>
<th>$^{14}\text{C}$-precursor fed</th>
<th>Specific Activity $^{1}$</th>
<th>Dilution factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Orotic Acid</td>
<td>18.8 μc/μmole BMV$^{2}$</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>33.0 μc/μmole BMV</td>
<td>1.4</td>
</tr>
<tr>
<td>1</td>
<td>Uridine</td>
<td>61.0 μc/μmole BMV</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>59.6 μc/μmole BMV</td>
<td>0.7</td>
</tr>
</tbody>
</table>

1/ Reported specific activities are the average of duplicate analyses.

2/ BMV molecular weight is $4.6 \times 10^6$ as reported by Bockstahler (11). 1 μc is equivalent to $2.2 \times 10^6$ dpm.

BMV purified from plants that had been fed radioactive viral precursors during viral RNA biosynthesis was radioactive. Orotic acid-6-$^{14}\text{C}$ (sp. act. 44.5 mc/mM) and uridine-2-$^{14}\text{C}$ (sp. act. 42.3 mc/mM) were introduced into different BMV-infected plants within the same lot. Approximately $10^5$ dpm per plant were introduced. After 48 hr incubation BMV was purified and virus specific radioactivity determined. Dilution factors were calculated using the expression already mentioned.
Figure 2. Distribution of C\textsuperscript{14} in BMV-RNA hydrolysates

Radioactive virus isolated from uridine-C\textsuperscript{14}-fed BMV-infected plants was acid hydrolysed, chromatographed and scanned.

Figure 2a. Typical C\textsuperscript{14} distribution in the BMV-RNA hydrolysates from uridine-C\textsuperscript{14}-fed barley

Chromatograms were developed in butanol: ethanol: formic acid: water (5:3:2:1) for 12 hr at 25\textdegreeC.

Figure 2b. Typical C\textsuperscript{14} distribution in the BMV-RNA hydrolysates from orotic acid-C\textsuperscript{14}-fed barley.

Chromatograms were developed in acetonitrile: water-ammoniacal to pH 10 (70:30).

Significance of these radiochromatograms: 1) illustration of the relative amount of non-specifically incorporated precursor, 2) proof of assimilation and 3) indirect evidence for the UTP-GTP "pool".
Table II. Radioactivities of BMV-RNA-C\textsuperscript{14} hydrolysates

<table>
<thead>
<tr>
<th>Hydrolysate</th>
<th>Radioactivity</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orotic acid-C\textsuperscript{14}-BMV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine</td>
<td>30</td>
<td>0.8</td>
</tr>
<tr>
<td>Adenine</td>
<td>40</td>
<td>1.0</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>1370</td>
<td>35.4</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>2030</td>
<td>52.5</td>
</tr>
<tr>
<td>Orotic acid</td>
<td>390</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Total dpm</strong></td>
<td><strong>3860</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hydrolysate</th>
<th>Radioactivity</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine-C\textsuperscript{14}-BMV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine</td>
<td>25</td>
<td>0.3</td>
</tr>
<tr>
<td>Adenine</td>
<td>30</td>
<td>0.4</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>2160</td>
<td>26.0</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>5060</td>
<td>62.0</td>
</tr>
<tr>
<td>Uridine</td>
<td>855</td>
<td>11.0</td>
</tr>
<tr>
<td><strong>Total dpm</strong></td>
<td><strong>8120</strong></td>
<td></td>
</tr>
</tbody>
</table>

BMV purified from plants that had been fed radioactive viral RNA precursors was acid hydrolysed and the resulting base derivatives analyzed for assimilated precursors. Analysis was by paper chromatography and determination of radioactivity of the compounds corresponding to the spots on chromatograms. These results were obtained when BMV-infected barley was grown in a 20/4°C controlled chamber, eclipse period was 12 days, C\textsuperscript{14}-precursors were administered on P.I. day 12, and C\textsuperscript{14}-precursors were incubated for 48 hours.
compounds was used to determine the $^{14}\text{C}$ absorbed but not assimilated into BMV-RNA. Compounds were identified by cochromatography or relative $R_F$ values and UV absorption properties.

**Enzymes of the Breakdown and de novo Synthesis Pathways**

Uridine 5' kinase and OMP 5'-pyrophosphorylase: decarboxylase activities were demonstrated in barley leaf extracts by the procedures previously described. Typical radiochromatoscans of reaction mixtures incubated for 15 minutes are represented in Fig 3a and 3b. Carbon-14 products of the enzyme reactions were isolated chromatographically and their radioactivities were determined.

The substrate to product conversion activities of OMP pyrophosphorylase: decarboxylase and uridine 5'-kinase were demonstrated (Table III) with active enzyme preparations. No conversion was detected when the enzyme preparations were heat inactivated or omitted from the reaction. Product radioactivities were obtained by averaging three assays using freshly prepared enzymes for each.

These results demonstrate that the above mentioned enzyme activities were present in barley leaf tissue. Uridylic acid 5' formation in barley leaf tissue by at least the two pathways (breakdown and de novo synthesis) was implied by these results since uridine has been reported to be a breakdown product of ribosomes (62) and orotic acid to be a precursor of UMP5' in de novo synthesis (49).

Uracil-$^{14}\text{C}$ was identified (Table IV) as one of the products in the enzyme reaction mixture when orotic acid-$^{14}\text{C}$ was incubated with extracts
Figure 3. **Uridine 5'kinase and OMP pyrophosphorylase: decarboxylase activity in cell-free extracts of barley**

*Figure 3a. Uridine 5'kinase*

Uridine-$\text{C}^{14}$, ATP and a crude enzyme preparation incubated for 10 min at 35°C was the reaction mixture that was analysed for products by paper chromatography. Chromatograms were developed in butanol: ethanol: formic acid: water (5:3:2:1) for 12 hr at 25°C. The distribution of radioactive compounds found in this reaction mixture is represented.

*Figure 3b. OMP pyrophosphorylase: decarboxylase*

Orotic acid-$\text{C}^{14}$, 5-phosphoribosyl-1-pyrophosphate and a crude enzyme preparation incubated for 10 min at 35°C was the reaction mixture that was analysed for products by paper chromatography. Chromatograms were developed in acetonitrile: water-ammoniacal to pH 10 (70:30). The distribution of radioactive compounds found in this reaction mixture is represented.

Significance of these radiochromatograms: 1) formation of UMP$'\text{5}'$ by two different enzyme activities found in non-infected barley, and 2) preliminary evidence for an orotic acid decarboxylase activity in barley.
Figure 3a

Figure 3b
Table III. Uridine 5' kinase and OMP pyrophosphorylase: decarboxylase in healthy barley

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Enzyme</th>
<th>Reaction Product Radioactivity (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>None</td>
<td>UMP5' 17 dpm</td>
</tr>
<tr>
<td>10 min</td>
<td>heated</td>
<td>29</td>
</tr>
<tr>
<td>0 min</td>
<td>0.5 mg</td>
<td>127</td>
</tr>
<tr>
<td>2 min</td>
<td>0.5 mg</td>
<td>325</td>
</tr>
<tr>
<td>5 min</td>
<td>0.5 mg</td>
<td>ND</td>
</tr>
<tr>
<td>10 min</td>
<td>0.5 mg</td>
<td>1438</td>
</tr>
<tr>
<td>15 min</td>
<td>0.5 mg</td>
<td>1558</td>
</tr>
<tr>
<td>10 min</td>
<td>1.0 mg</td>
<td>1720</td>
</tr>
</tbody>
</table>

1/ Reaction mixture for uridine 5'phosphokinase activity: uridine-2-C14 (50μμc/ml), ATP (1 ml, 0.01M), MgCl2 (0.5 ml, 0.05M) and enzyme (2.5 μl). Reaction mixture for OMP pyrophosphorylase-decarboxylase activity, orotic acid-6-C14 (0.1 ml, 6μμc/ml), 5'phosphoribosyl-1-pyrophosphate (0.3 ml, 0.57 mg/ml), MgCl2 (0.3 ml, 7.5 mg/ml) and enzyme.

2/ Chromatographed, cut out and counted

3/ Carbon-14 disintegrations per min.

4/ No Data

5/ 5 min at 55°C

The reactions were started with the addition of the radioactive substrate, incubated for various times at 35°C and stopped by the addition of trichloroacetic acid. Thirty μl of the reaction mixture were chromatographed and analyzed for product radioactivity.
Table IV. Characterization of a product of orotic acid decarboxylase activity

<table>
<thead>
<tr>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Authentic Uracil</strong></td>
</tr>
<tr>
<td>280/260⁴/</td>
</tr>
<tr>
<td>250/260</td>
</tr>
<tr>
<td>290/260</td>
</tr>
<tr>
<td>max</td>
</tr>
<tr>
<td>min</td>
</tr>
<tr>
<td>Rᶠ solvent 1⁵/</td>
</tr>
<tr>
<td>Rᶠ solvent 2⁵/</td>
</tr>
</tbody>
</table>

⁴/ Ratio of UV absorption at μμ wave lengths. Measurements were made in a Beckman DU Spectrophotometer on samples that had been eluted from chromatograms with 0.01 M PO₄ buffer, pH 7.0. The reference sample was an equivalent eluant without uracil.

⁵/ Authentic uracil was purchased from Sigma Chemical Co. and purified by paper chromatography.

⁶/ Standard spectrophotometric data, Burton (19).

⁷/ Rᶠ values were determined on uracil that separated from the reaction mixture.

⁸/ Solvent 1. Acetonitrile: water-ammoniacal to pH 10 (70:30); 4 to 6 hr at 25°C.

⁹/ Solvent 2. Butanol: ethanol: formic acid: water (5:3:2:1); 12 hr at 25°C.
from leaf tissue. Uracil-\(^{14}\text{C}\) was singled out of the other products of enzyme reactions for identification because it was an unexpected product according to Wolcott and Ross (82). Uracil-\(^{14}\text{C}\) was the first product of the enzyme reaction since it appeared approximately two minutes before UMP\(^{5}\text{P}\). Cochromatography of authentic uracil with the radioactive product showed that it was uracil. The enzymic decarboxylation of orotic acid-\(^{14}\text{C}\) was demonstrated but the description and characteristics of this enzyme were not within the scope of this report. To the author's knowledge this activity has not previously been reported in plants.

The area under peaks corresponding to UMP\(^{5}\text{P}-\^{14}\text{C}\) formed from orotic acid-\(^{14}\text{C}\) where incompletely dialysed enzyme preparations were used was about twice the area when completely dialysed enzyme preparations were used. Incompletely dialysis of an enzyme preparation presumably leaving considerable \((\text{NH}_4)_2\text{SO}_4\), resulted in more UMP\(^{5}\text{P}\), less uracil-\(^{14}\text{C}\) and virtually no OMP\(^{5}\text{P}-\^{14}\text{C}\) being enzymically formed from orotic acid-\(^{14}\text{C}\). Additional accumulation of UMP\(^{5}\text{P}-\^{14}\text{C}\) in the presence of \((\text{NH}_4)_2\text{SO}_4\) might be explained by inhibition of UMP\(^{5}\text{P}\) kinase activity that would increase the amount of UMP\(^{5}\text{P}\) in a reaction mixture.

Orotic acid-\(^{14}\text{C}\) Incorporation into Ribosomal and BMV-RNA

BMV-infected and non-infected barley plants were fed orotic acid-\(^{14}\text{C}\) and at daily intervals an 80 S fraction was prepared from the leaves by differential centrifugation. The radioactivity incorporated into BMV was distinguished from that associated with the ribosomes by
treating the 80 S fraction with ribonuclease. Ribosomal RNA was hydrolysed and rendered acid-soluble by ribonuclease whereas BMV-RNA was protected (Table V) from ribonuclease by the protein coat of the virus and remained acid-insoluble.

Testing the ribonuclease treatment method of distinguishing ribosomes and virus from an 80 S fraction was described in Materials and Methods. Ribosomal sensitivity to ribonuclease was evidenced by the similarity of the purine:pyrimidine ratio for both acid and ribonuclease hydrolysed samples of ribosomes from non-infected leaves. BMV insensitivity to ribonuclease was shown by the purine:pyrimidine ratio of ribonuclease hydrolysed 80 S fraction from BMV-infected leaves. The ratio was similar to samples not containing BMV, indicating that BMV-RNA does not contribute to the purine:pyrimidine ratio when hydrolysed by ribonuclease.

Ratios for acid-hydrolysed BMV are sufficiently dissimilar to the ribosomal ratio that a significant contribution by BMV could be detected, i.e., the ratio for acid-hydrolysed-BMV was found to be 1.11 and for ribonuclease hydrolysed ribosomes the ratio was 1.37. If BMV-RNA were contributing to the acid soluble material from the 80 S fraction after ribonuclease treatment, the ratio would drop as it did when the BMV-ribosome mixture was acid-hydrolysed; from 1.54 to 0.83. The ratio was 1.38, similar to that for ribosomes for non-infected leaves, indicating that BMV was not contributing acid-soluble material.
Table V. A test of the method of separating virus from virus-ribosome mixtures with ribonuclease treatment

<table>
<thead>
<tr>
<th>Sample</th>
<th>C+G/U+C</th>
<th>A</th>
<th>G</th>
<th>C</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-H+</td>
<td>1.54</td>
<td>30.0</td>
<td>29.6</td>
<td>23.0</td>
<td>17.3</td>
</tr>
<tr>
<td>H-RNase</td>
<td>1.37</td>
<td>26.0</td>
<td>31.8</td>
<td>24.0</td>
<td>18.2</td>
</tr>
<tr>
<td>B-H+</td>
<td>0.83</td>
<td>27.7</td>
<td>17.7</td>
<td>26.0</td>
<td>28.6</td>
</tr>
<tr>
<td>B-RNase</td>
<td>1.38</td>
<td>24.7</td>
<td>33.8</td>
<td>24.1</td>
<td>18.4</td>
</tr>
<tr>
<td>BMV purified</td>
<td>1.11</td>
<td>26.2±2.0</td>
<td>26.4±2.0</td>
<td>18.7±2.0</td>
<td>18.6±2.0</td>
</tr>
</tbody>
</table>

H = ribosomes from non-infected tissue
B = ribosomes plus BMV from BMV-infected tissue
H+ = sample was acid hydrolysed
RNase = sample was hydrolysed with ribonuclease

An 80 S fraction prepared from BMV-infected barley by differential centrifugation contained a BMV:ribosome mixture. Ribonuclease (250 µg/ml) treatment of this mixture for 3 hr at 37°C followed by TCA precipitation resulted in ribosomal RNA hydrolysates in the supernate and precipitated BMV in the pellet. Base ratio analyses of these respective fractions by paper chromatography demonstrate that BMV was insensitive and ribosomal RNA was sensitive to ribonuclease.
Specific radioactivities associated with BMV-RNA and ribosomal-RNA in the 80 S fraction were determined by the previously described method and the results summarized (Fig. 4). The similarity of specific radioactivities of ribosomes from infected and non-infected tissue indicates an unchanged ribosome metabolism in BMV-infected tissue. The nearly linear increase of BMV specific radioactivity indicated that the ratio of assimilated carbon-14 to virus was approaching unity by a constant rate mechanism. Virus synthesis occurs at one rate and assimilation of orotic acid-C\textsuperscript{14} at another (Fig. 6) and the nearly linear increase of BMV specific radioactivity indicated that the combined rates of carbon-14 assimilation and virus synthesis were nearly constant for this time period.

The rate of virus synthesis as determined by the ribonuclease treatment method was shown (Fig. 5) to be consistent with the rate determined by the sucrose density gradient centrifugation method already reported (Fig. 1).

The rate of carbon-14 incorporation into BMV-RNA (Fig. 6) is initially constant and similar to the constant rate of virus synthesis. These constant rates start decreasing about P.I. day 12 when virus accumulation approaches its maximum.

Orotic acid-C\textsuperscript{14} incorporation into ribosomes from infected and non-infected tissue was determined by measuring radioactivity associated with those fractions (Fig. 7 and 8), further supporting the continuation of ribosome synthesis during BMV synthesis. The 20 per cent decrease in
Figure 4. **Specific radioactivity of BMV and ribosomes isolated from barley**

A BMV-ribosome mixture was prepared by differential centrifugation from plants that had been fed orotic acid-C\(^{14}\). That amount of radioactivity associated with BMV and associated with ribosomes was estimated by rendering ribosomal RNA as acid soluble with a ribonuclease treatment. RNA content of each fraction was determined by total \(A_{260}\) (absorbancy x dilution x volume) and incorporated radioactivity (dpm) by direct count.

Barley plants were grown in a greenhouse at an air temperature range of approximately 27/20°C. In this environment BMV had an eclipse period of six days. Orotic acid-C\(^{14}\) (1 \(\mu\)mole/ml: 10\(^5\) dpm per plant) was introduced into BMV-infected and non-infected plants on P.I. day 7.

Ribosomes from non-infected barley \(\bigcirc - \bigcirc\), ribosomes from BMV-infected barley \(\times - \times\), and BMV \(\triangleleft - \triangleright\), indicate BMV and ribosome metabolism measured by incorporation and turnover of orotic acid-C\(^{14}\).

Significance of the figure: 1) the similarity of ribosome metabolism in BMV-infected and non-infected barley, and 2) the \(C^{14}\) per unit BMV-RNA increase appears linear.
Figure 4

Specific Radioactivity vs. Days P.I.

- dpm / RNA

Graph showing specific radioactivity over days post infection (P.I.) with various symbols representing different conditions or samples.
Figure 5. Accumulation of BMV in infected plants: testing the method of ribonuclease treatment for estimating virus content

Virus increase from Fig. 1 (mg BMV/g/day) for a winter environment was plotted using an ordinate described by an average of the non-viral material in the 80 S fraction from non-infected plants. Estimations of non-viral material from non-infected plants and viral material from BMV-infected plants were by total $A_{260}$ (absorbancy x dilution x volume) after ribonuclease treatment.

Two hundred-fifty µg of ribonuclease was incubated with an 80 S fraction (ribosome-virus and/or non-viral material mixture) for 3 hr at 30°C. Virus and/or non-viral material remained acid insoluble after this treatment. The recovered insoluble material was alkaline hydrolysed with 1N NaOH and $A_{260}$ determined for the resulting solubilized material.

BMV remaining after ribonuclease treatment of BMV-ribosome mixtures (80 S fraction) was indicated by O—O, BMV content by sucrose density gradient analysis, winter environment 27/20°C ▼▼, and non-viral material remaining after ribonuclease treatment of 80 S fraction from non-infected tissue O——O.

Significance of the figure: the standardization of ribonuclease method of estimating virus in tissue extracts by checking the method against a more acceptable method; the sucrose density gradient method described for Figure 1.
Figure 5
Figure 6. Orotic acid-$^{14}C$ incorporation into BMV during virus synthesis

The 80 S fraction containing a ribosome-virus mixture prepared by differential centrifugation from orotic acid-$^{14}C$-fed, BMV-infected tissue was separated into a ribosome and virus fraction by the ribonuclease treatment method. The virus fraction was analysed for RNA content by total $A_{260}$ (absorbancy x dilution x volume) and incorporated radioactivity (dpm) by direct count.

Significance of the figure: rates of RNA synthesis and orotic acid-$^{14}C$ assimilation appear to follow similar constant rate mechanisms. The parallel rates of increase indicate: 1) orotic acid incorporation and virus synthesis follow similar kinetics, 2) orotic acid did not pass through ribosomal material as a prerequisite of incorporation, and 3) the method of total $A_{260}$ of BMV-RNA hydrolysates agrees with other methods for the linear increase of BMV-RNA.
Figure 6
Figures 7 and 8. Orotic acid-C\textsuperscript{14} incorporation into ribosomes

BMV-infected and non-infected plants were fed orotic acid-C\textsuperscript{14} and an 80 S fraction containing a ribosome-virus and/or non-viral material mixture was prepared by differential centrifugation. The ribosomes from the 80 S fraction were rendered acid soluble by ribonuclease treatment and analysed for RNA by total $A_{260}$ (absorbancy x dilution x volume) as well as incorporated radioactivity (dpm) by direct count.

**Figure 7. Ribosomes from BMV-infected plants**

Ribosomes analysed from BMV-infected plants incorporated 20 per cent less orotic acid-C\textsuperscript{14} at a time of maximum radioactivity than did ribosomes from non-infected barley.

**Figure 8. Ribosomes from non-infected plants**

The fact that incorporated radioactivity reached a maximum indicates an average life expectancy of ribosomes, which means it was three days after synthesis until ribosomes were degraded or turned over. The rate of degradation seemed to exceed the rate of synthesis causing the gradual negative slope after three days.

Significance of these figures: continuation of ribosomal synthesis in virus infected tissue.
Oroic acid - C14 incorporation into ribosomes (dpm X10^3)

Days P.I.

Figure 7

Days P.I.

Figure 8
radioactivity in ribosomes from BMV-infected tissue probably reflects ribosome content but could also indicate a lower rate of synthesis due to competition for the UTP-CPT "pool".

Ribosome content in BMV-infected tissue was approximately 16 per cent lower than that in non-infected tissue ribosomes 14 days after inoculation. The gradual ribosome decrease indicated in both cases was based on fresh weight and probably was caused by leaf expansion. Ribosome content was determined during infections from P.I. 7 to P.I. 14 and was lower in BMV-infected tissue at all intervals. This lower ribosome content was indirect evidence for activity of the breakdown pathway.

Ribosome turnover time was indirectly determined for infected and non-infected tissue (Fig. 7 and 8). Turnover was defined as "life expectancy" and was that time required to accumulate a maximum amount of radioactivity. The average ribosome turnover was about three days, which means that a period of three days elapsed between synthesis of a ribosome and its degradation.

The difference between the rate of synthesis and the rate of degradation was observed as a net loss of radioactivity. If this loss of radioactivity could be interpreted as a greater rate of degradation than synthesis then the available orotic acid-C\(^{14}\) could not have been limiting. Evidence that orotic acid-C\(^{14}\) was not limiting was provided by radiochromatograms of acid-soluble extracts of fed barley leaves.

The area under the orotic acid-C\(^{14}\) peak after a seven day incubation was 50-80 per cent that of a 10 minute incubation. Sufficient precursor
was thus available to justify a constant ribosomal synthesis rate. Several other radioactive compounds appeared on the radiochromatogram of the samples incubated for seven days but identification of the compounds awaits further investigation.

Orotic acid-$^{14}\text{C}$ administered to plants was quickly translocated and uniformly dispersed throughout the leaf tissue as evidenced by autoradiography of the leaf tissue fed the radioactive compound. Subcellular availability of the fed orotic acid-$^{14}\text{C}$ was not investigated.

Orotic acid-$^{14}\text{C}$ that was non-specifically absorbed to ribosomes from non-infected tissue, to ribosomes from BMV-infected tissue and to BMV, was estimated by the previously described method. Radiochromatograms (Fig. 2b) were also typical of the results found for the above mentioned samples. Approximately 10 per cent of the incorporated carbon-14 in the 80 S fractions from orotic acid-$^{14}\text{C}$-injected plants was non-specifically absorbed. When separating the 80 S fraction into ribosomal-RNA and BMV-RNA this 10 per cent of the total carbon-14 becomes included in the ribosomal-RNA fraction since orotic acid was acid-soluble. Therefore 10 per cent of the radioactivity associated with the 80 S fraction was subtracted from the ribosomal RNA counts before any calculations were made.
DISCUSSION

The purpose of this investigation was to determine the relationship between de novo synthesis of RNA precursors and of host-RNA breakdown products for BMV-RNA precursor sources. This relationship was reported by Reddi (60) to be inherit in the limiting supply of one base from cytoplasmic ribosomes. Reddi concluded that for his TMV system, the base uracil from ribosomes was about 34 per cent below the requirement for TMV synthesis and that the deficit was supplied by de novo synthesis.

Evidence presented in this report demonstrated the relative activities of the de novo synthesis and breakdown pathways during BMV synthesis. Precursor-uridine, as intermediate of the breakdown pathway, was 2-3 times more efficiently assimilated than precursor-orotic acid, an intermediate of the de novo synthesis pathway. The above fact supports Reddi's model since the higher efficiency of uridine assimilation (Table I) compared to that of orotic acid could be interpreted as 67-75 per cent of the BMV-RNA uridylic acid being derived from ribosomal breakdown.

The uracil requirement for BMV-RNA synthesis was 24-28 moles/100 moles RNA (Table V). The uracil that was potentially supplied by ribosomal breakdown was 18 moles/100 moles RNA (Table V) or a calculated average difference of 67 per cent. The activity observed for the breakdown pathway was 67-75 per cent and therefore consistent with the calculated 67 per cent. The remaining 33-25 per cent uridylic acid incorporated into BMV was derived from de novo synthesis, as indicated
by the assimilation of orotic acid-C\textsuperscript{14} into BMV-RNA.

The apparent similarity in the amounts of RNA precursor furnished by ribosomal degradation in TMV and BMV systems were possibly fortuitous. For example, the difference in the amount of uracil potentially supplied by ribosomal breakdown for the formation of viral RNA was 33 per cent in both systems.

The total mass difference between ribosomal breakdown and viral RNA formed represented a negligible amount in TMV reported by Reddi (60). This fact represented a major dissimilarity between the TMV and BMV systems. Virus content in the TMV system reported by Reddi (60) was approximately 300 \( \mu \)g TMV-RNA per g of leaf. Tobacco ribosomal-RNA breakdown for the same period of time was approximately 300 \( \mu \)g RNA per g of leaf which was nearly 75 per cent of the pre-infection ribosome content. The difference was therefore negligible which means the demand on the de novo pathway was only slight.

In the BMV system, however, a decrease of approximately 16 per cent of the ribosomal RNA occurred in the BMV-infected barley. The amount of RNA represented by a decrease of 16 per cent per g of leaf was not determined. If applied to the TMV system, the 16 per cent represents 64 \( \mu \)g RNA (16 per cent of 400 \( \mu \)g RNA per g leaf). Hypothetically then, 64 \( \mu \)g RNA was supplied by the ribosomal breakdown and at the same time the BMV-RNA synthesis requirement was 1,250 \( \mu \)g RNA (Fig. 1; 21 per cent of 6 mg BMV/g tissue). Even though the figures are hypothetical and one \( \mu \)gram of ribosomal RNA may not necessarily be equivalent to one \( \mu \)gram...
of viral RNA, the argument remains that the amount of viral RNA precursors supplied by ribosomal RNA breakdown probably was less than the requirement of 1,250 μgram. This deficit in the requirement for viral RNA synthesis could then have been met by de novo synthesis.

Factors that might change the relative amounts of assimilated precursors are the temperature at which the infected plants were grown and the time after inoculation at which radioactivity was introduced. The temperature range during the assimilation experiments was abnormally low, 20/4°C, and might have differentially reduced the enzymic activities of both pathways of the virus biosynthetic process. The pathway activities might have also been different had the introduction of the C\textsuperscript{14}-precursors been sometime other than two days after the eclipse period.

Differences in pathway activity might even be explained by the differences in precursor penetration or availability. Autoradiographic evidence of leaf material suggested a uniform dispersal of the C\textsuperscript{14}-precursors only hours after its introduction but movement at the cellular level is open to question. Preliminary incorporation experiments using precursor-adenosine triphosphate-C\textsuperscript{14} show only small amounts of incorporated radioactivity suggesting either adenosine triphosphate is not a BMV-RNA precursor or, more likely, that cellular transport of the charged adenosine triphosphate is restricted. Adenosine triphosphate-C\textsuperscript{14} would, therefore, be unavailable for incorporation at the site of BMV-RNA synthesis.
It might be argued that if uridine-$^{14}$C or orotic acid-$^{14}$C were differentially converted to charged intermediates before movement to the site of BMV-RNA synthesis, then the availabilities might have been different for each precursor. Pathway activity would then have been a measure of precursor availability. Autoradiograms of thin sections reported by Smith and Schlegel (75, 76), Hirai and Hirai (40), and Yasuda and Hirai (83) indicate the rapid translocation of radioactivity of uridine-$^3$H and uracil-$^3$H into TMV-infected tobacco cells. The actual compound moving into the nuclei would have been difficult to determine.

Equal molar amounts of each precursor were introduced in the experiments with orotic acid-$^{14}$C and uridine-$^{14}$C to minimize the differences due to reaction rates that vary with substrate concentrations. Even equal molar amount would not necessarily equalize incorporation rates by two different pathways because of possible differences in equilibrium constants, enzyme Michaelis-Menten constants, and the effect of the precursor "pool".

The steady state equilibrium could be argued to have been significantly altered by feeding only one precursor per sample and thus the equilibrium conditions used in the comparison of two precursors might have been different. The effect could have been controlled by mixing equal amounts of orotic acid-$^{12}$C and uridine-$^{14}$C in the assimilation experiments.

Uridine and orotic acid metabolism was only partly understood by demonstrating uridine 5' kinase, OMP 5' pyrophosphorylase, and OMP 5' decarboxylase activities. These activities were considered essential
for the in vivo conversion of uridine and orotic acid to uridylic acid 5', but other activities might have contributed to viral nucleic acid synthesis. Suggested activities for future RNA biosynthesis investigations were:

\[
\begin{align*}
\text{Uridine} & \xrightarrow{\text{PRPP}} \text{Uracil} \xrightarrow{\text{PRPP}} \text{UMP}^1 \text{P}^1 \\
\text{Orotic acid} & \xrightarrow{\text{CO}_2, \text{PRPP}} \text{Uracil} \xrightarrow{\text{PRPP}} \text{UMP}^1 \\
\text{Orotic acid} & \xrightarrow{\text{OMP}^1, \text{PRPP}} \text{Orotidine} \\
\text{Orotidine} & \xrightarrow{\text{CO}_2} \text{Uridine} \xrightarrow{\text{PRPP}} \text{UMP}^1
\end{align*}
\]

Feeding intermediate precursors other than uridine and orotic acid (eg. uracil, orotidine, etc.) would have predictably resulted in assimilation into RNA as long as they were a precursor to UMP^1 and were transported to the cellular sites of synthesis. Yasuda and Hirai (83) and Hirai and Hirai (40) indicated that uracil was a TMV-RNA precursor in studies on the site of viral synthesis.

The fact that uracil-C^{14} was rapidly formed from orotic acid-C^{14} and uridine-C^{14}, when incubated with barley leaf protein extracts (Fig. 3a, 3b) implicates other pathways. Products of these other pathways were referred to in this thesis when chromatograms of acid-soluble extracts from plants fed radioactive precursors showed several radioactive compounds. Identification of these orotic acid and uridine metabolites would have contributed to the understanding of the other pathways. Studies on the effect of BMV synthesis on uridine and orotic acid metabolism would have shown further, the relationship between de novo
and breakdown pathways during BMV biosynthesis.

Ribosomal synthesis was shown to compete with BMV-RNA synthesis for precursor-orotic acid by the fact that the assimilation of orotic acid-$^{14}$C into BMV-RNA was concurrent with the assimilation into ribosomal RNA (Fig. 4). If ribosomal turnover were a prerequisite for BMV-RNA synthesis, then a lag in the increase in radioactivity would have been observed. Such a lag was not observed (Fig. 6). Such a competition for the precursor pool probably explains the reduction in the ribosomal biosynthetic process that was observed (Fig. 7).

Observed phenomena reported in this thesis were consistent with the pathway model (Fig. 9). Results considered important in suggesting this model were: ribosomal metabolism continues during BMV-RNA biosynthesis (Fig. 4) but at a competitive rate (Fig. 7 and 8); ribosome content was somewhat less in BMV-infected tissue than in non-infected tissue (Fig. 7); maturation of BMV and assimilation of orotic acid-$^{14}$C occurred concurrently and without a lag (Fig. 6); both the de novo synthesis pathway (orotic acid-$^{14}$C) and the breakdown pathway (uridine-$^{14}$C) were active during virus synthesis (Table 2); and CMP $3'$-$^{14}$C and UMP $3'$-$^{14}$C were hydrolysis products of BMV and ribosomes isolated from orotic acid-$^{14}$C and uridine-$^{14}$C fed plants infected with BMV (Fig. 2a, 2b).

Interpretation of these phenomena suggested that the breakdown and de novo synthesis pathways were precursor "supply-pathways". Other "supply-pathways" have already been discussed. For example, the formation
Figure 9. Proposed breakdown and de novo synthesis pathways: diagram of BMV-RNA precursor sources

Compounds
1. Orotic acid
2. 5-phosphoribosyl-1-pyrophosphate
3. Orotidine 5'-phosphate (OMP)
4. Uridine 5'-monophosphate (UMP5')
5. Uridine 3'-monophosphate (UMP3')
6. Uridine

Enzymes
a) OMP pyrophosphorylase  b) OMP decarboxylase  c) Uridine 5' kinase
of UMP 5' from uracil, reported by Cawford et al. (28), Reichard and Sköld (63) was implicated in this thesis. Wolcott and Ross (82) suggested that crude enzyme preparations from bean seedlings contained a phosphatase that converted OMP 5' to orotidine. Orotidine metabolism studies in barley might have then indicated still another "supply-pathway". The contributions of these other "supply-pathways" to BMV-RNA biosynthesis would have been speculative.

Demands on the precursor "pool" (UMP 5', UDP, UTP and CTP) might also be other than those in the model; for example, carbohydrate metabolism and synthesis of other types of host-RNA (messenger RNA and transfer RNA). Turnover of this host-RNA probably contributes to the precursor pool in a cyclic fashion similar to ribosomal RNA.

Ribosomes purified from non-infected barley sedimented like an 80 S particle with smaller amounts of UV absorbing material sedimenting slower, probably at 60 S and 40 S suggested by Bonner (12) to be ribosome subunits. Reducing the concentration of Mg ++ caused the ribosomal preparation to sediment almost exclusively as 60 S and 40 S particles, thus behaving like typical ribosomes. The method of purification, however, suggests that the ribosomal preparations were probably a mixture of several ribosomal types; nuclear, chloroplastic, mitochondrial and cytoplasmic.

Base ratios reported in Table 5 were determined from crude 80 S preparations and were considered only to show the sensitivity of BMV to ribonuclease. An unidentified fifth hydrolysis product (A2),
different from the usual products of hydrolysis, was present only when the 
80 S fraction was ribonuclease hydrolysed. UV absorption properties of A₂ 
at pH 7.0 were 280/260 = 0.67, 250/260 = 0.83, max = 260 nm and min=230 nm. 
Base ratios used in Table 5 excluded A₂ because of the agreement with base 
ratios determined by acid-hydrolysis. The significance of A₂ remains un­
determined until it is identified but it was found that A₂ was not a con­
taminant of the ribonuclease.

The base ratio reported (Table 5) for BMV-RNA was repeated eight 
times on purified BMV and was considered statistically significant. 
Bockstahler and Kaesberg (11) also reported a base ratio for BMV-RNA that 
was in some disagreement with the ratio herein reported. At least two 
possible explanations were suggested for this discrepancy, 1) the range of 
experimental findings included the results reported by Bockstahler, which 
meant the same virus was investigated in both laboratories, or 
2) Bockstahler used one strain of BMV, reported by Chiu and Sill (21) and 
another strain was used in this study.

The UTP-CTP pool concentration could have been sufficiently lowered 
by BMV-RNA synthesis to reduce the reaction rate of ribosomal RNA synthe­
tase; thus accounting for the loss of ribosomal content.

Rott and Scholtiscek (68) have suggested a relationship between the 
de novo synthesis and breakdown pathways during myxovirus RNA synthesis. 
The relationship was that viral RNA biosynthesis seemed to be an addition­
al function of the host cell. Similarly, BMV-RNA synthesis seemed to be 
an additional function of the host tissue because, 1) both the de novo syn­
thesis and breakdown pathway were active and 2) ribosomal synthesis 
continued during virus synthesis.
LITERATURE CITED


