One of the remarkable properties of a leaf infected with tobacco mosaic virus is the unusually large amount of virus protein which appears as a consequence of the infection. Some weeks after inoculation, 25 per cent or more of the total protein in tobacco plants can be directly isolated as crystalline virus protein (1, 2, 7, 10). This massive synthesis of virus protein is, however, accomplished without a significant change in either the total or the soluble nitrogen content of tobacco plants (8). In fact, virus multiplication occurs even in detached and darkened leaves (13), conditions under which protein hydrolysis normally predominates (5). Virus multiplication must occur therefore at the expense of preexisting and normal nitrogenous constituents of tobacco leaf protoplasm.

What, then, are the leaf constituents which are utilized in virus synthesis? Recent work on the protein constituents of the leaf (14, 15) makes it possible to attempt to obtain an experimental answer to this question. The cell-free protoplasm of leaves can be divided into three distinctly different types of nitrogenous materials: (1) The soluble, low molecular weight, nitrogenous constituents such as amino acids, amides, etc. (2) The particulate matter, consisting of the nucleus, chloroplasts, and any other as yet unknown materials of a similar particulate nature. It is estimated that 90 to 95 per cent of the particulate matter in spinach leaf protoplasm consists of chloroplasts. Because of the large particle sizes involved, the particulate matter is separated readily from other leaf proteins by moderate centrifugal fields. (3) The soluble proteins of the leaf cytoplasm; the cytoplasmic proteins. These proteins are not sedimented even by centrifugal fields up to 43,000 \( \times g \) for 2 hours. It is thus possible simply and quantitatively to extract the protoplasm of leaf tissue and to separate the protoplasm into particulate and soluble cytoplasmic...
protein. It should therefore be feasible to determine whether either the particulate matter or the soluble proteins of the cytoplasm bear a relation to the formation of virus protein.

The cytoplasm of spinach leaves, which has been studied in detail (14, 15), consists of two protein constituents, one of which, Fraction I protein, makes up approximately 75 per cent of the total cytoplasmic proteins. This protein contains bound purine, pentose, and phosphorus (15) and is hence a nucleoprotein. Purified Fraction I protein is electrophoretically homogeneous and has a molecular weight, as indicated by osmotic measurements, in excess of 200,000.

Frampton and Takahashi (6) have shown by electrophoretic analysis that a new cytoplasmic protein appears in tobacco leaves after the leaves are infected with tobacco mosaic virus. This new protein component appears to be the virus itself. In the present communication, it will be shown that tobacco leaves contain a main protein component in the cytoplasm analogous to that already described from spinach leaves. Quantitative chemical and electrophoretic analyses of the cytoplasmic proteins of healthy and virus-infected leaves strongly suggest that virus protein is synthesized at the direct expense of this normal protein.

Methods and Materials

Plants—Young Nicotiana tabacum plants, variety Havana 38 or Turkish Samsun as indicated, were used in these experiments. Seeds were supplied through the courtesy of Dr. E. E. Clayton of the United States Department of Agriculture, Beltsville, Maryland. The plants were individually grown in pots containing washed sterilized sand, and were supplied with Hoagland's complete nutrient solution including micro elements. They were protected from contamination in an insect-free greenhouse with the usual aseptic precautions. In comparative experiments, plants as uniform as possible were chosen.

Virus—Common tobacco mosaic virus 1, obtained through the courtesy of Professor James Johnson of the University of Wisconsin, was used to infect tobacco plants by the carborundum technique.

Fractionation of Leaf Protoplast—The methods (14) found useful for the fractionation of spinach leaf protoplasm were only slightly modified for use with tobacco leaves. At all times during the fractionation of the leaf protoplasm, an effort was made to keep the temperature as close to 0° as possible and to work rapidly. The following method was used throughout this study both for healthy and virus-infected leaves.

All leaves were clipped from the stems except for the top clusters which were omitted from the analysis, since they had been used to infect some of the plants with virus. The petioles and midribs were removed and
discarded so that essentially only the parenchymatous cells of the leaves were analyzed. The leaves were weighed, stacked on top of each other, and two leaf disks removed from each leaf with a cork borer. This representative aliquot was weighed, frozen, dried by lyophil, again weighed, and used to estimate the total dry matter contained in the original leaf sample.

The remainder of the leaves was rapidly sliced into approximately 1 sq. cm. pieces, and then ground in an Eppenbach colloid mill. The mill was started circulating with 150 ml. of ice-cold potassium maleate buffer, pH 6.9. It is essential to use a solution buffered near the neutral point in order to prevent precipitation of the cytoplasmic proteins. In the absence of buffer, a pH of about 5 is rapidly attained upon grinding tobacco leaves in water, which is sufficient to cause a considerable isoelectric precipitation of the cytoplasmic proteins. Enough leaves were added to the buffer to produce a thick slurry, which was then freed of cell walls and unbroken cells in a basket centrifuge lined with sharkskin filter paper.

The cell-free protoplasmic juice which passed through the paper was returned to the mill and used in grinding more leaves. This process was repeated until all of the leaves were ground and the protoplasmic contents of the cells dispersed into solution. The concentrated juice was finally passed through two thicknesses of sharkskin filter paper to insure complete removal of all cell walls. The final pH of the juice was 6.7. The residues remaining on the paper were dried and weighed. Since the total amount of dry matter was ascertained from the aliquot, it was possible to calculate the total amount of dry matter dispersed as a cell-free juice. In all instances, 60 to 65 per cent of the total dry matter was found in the cell-free juice. Since about 10 per cent of the original dry matter consisted of insoluble cellulose, it is evident that more than 65 per cent of the leaf cells was ruptured by the colloid mill treatment, with consequent extraction of their protoplasmic contents as a cell-free juice.

The filtered and concentrated protoplasmic juice was immediately frozen with the aid of a dry ice-methyl cellosolve bath. This freezing treatment results in aggregation of the particulate matter so that when the suspension is thawed, the particulate matter can be completely sedimented by centrifuging for 1 hour at 5000 \( \times g \). The sediment was dried and the dry weight taken to represent the total particulate matter of the protoplasm. The clear brown supernatant was distributed into 1 inch Visking casings which were placed before a fan until the contents were reduced to about one-third of their original volume, usually about 25 ml. of concentrated juice per 100 gm. of fresh leaf tissue. The content of total cytoplasmic proteins was about 3 per cent. After tying off the sacs
to prevent excessive dilution, the concentrated protein solution was dialyzed with mechanical stirring for 48 hours against two separate 2 liter portions of buffer. The total amount of cytoplasmic proteins in the sample was ascertained from an aliquot, and the protein after dialysis was rapidly frozen and stored at -16° until it was used for electrophoresis analysis. Freezing produced no apparent deleterious effect on the proteins since, when thawed, the protein solution remained perfectly clear. Protein determinations were made either by drying or by trichloroacetic acid precipitation as described previously (14).

**Fig. 1.** Comparison of the electrophoretic behavior of Havana tobacco leaf cytoplasmic proteins in phosphate and cacodylate buffer. 1.0 per cent solutions of total proteins in buffer of 0.1 ionic strength, pH 6.9. Migration time, 180 minutes at 15 ma. Arrows indicate positions of starting boundaries. Right, ascending; left, descending.

**Electrophoresis**—Through the courtesy of Dr. S. Swingle of the Chemistry Department of this Institute, cytoplasmic proteins were examined in a modified Tiselius electrophoresis, moving boundary, apparatus with the Longsworth scanning device (12). Conventional, double length analytical cells were used. Before analysis, the proteins were previously dialyzed against a 0.0233 M cacodylic acid buffer which was brought to pH 6.9 and a total ionic strength of 0.1 by the addition of 0.02 M NaOH and 0.08 M NaCl. This univalent ionic buffer gave much more satisfactory resolution of cytoplasmic protein mixtures than phosphate or a buffer such as potassium maleate. With phosphate buffer for example, anomalous δ boundaries were encountered such as are illustrated by Fig. 1, where it can be observed that a pronounced boundary at the original protein buffer interface is still present even after the cytoplasmic protein mixture
had been allowed to migrate for 3 hours. However, in the presence of
cacodylate at the same pH and ionic strength as phosphate, a normal
spreading of the starting boundary was found after the protein mixture
had migrated for 3 hours. Cacodylate buffer was therefore used in all
subsequent electrophoresis experiments.

In comparing the cytoplasmic proteins obtained from healthy and
virus-infected leaves, analysis was performed in paired electrophoresis
cells at identical total protein concentrations. Both samples therefore
received the same treatment as far as current, migration time, etc., were
concerned. The proteins were usually allowed to migrate for 2 or 3 hours
with a current of 15 ma. At pH 6.9, this time of migration was adequate
for the separation of virus protein from the normal protein of tobacco
leaf cytoplasm.

EXPERIMENTAL

Electrophoretic Analysis of Cytoplasmic Proteins of Healthy Tobacco
Leaves—Cytoplasmic proteins were prepared, as described above, from
two varieties of Nicotiana tabacum, Havana 38 and Turkish Samsun, and
from Nicotiana glutinosa. After removal of the particulate matter, the
cytoplasmic proteins were examined in a Tiselius electrophoresis appa-
tratus, and tracings made from the scanning patterns are presented in
Fig. 2. The three preparations are strikingly similar in that each appears
to contain a protein component comprising 75 per cent or more of the
total cytoplasmic proteins. Even after 3 hours migration at 15 ma. in
cacodylate buffer, pH 6.9, there is no evidence of electrophoretical in-
homogeneity in the principal component in any preparation. The simi-
larity of these cytoplasmic protein spectra to spinach cytoplasm is further
augmented by the presence of minor protein components which, in the case
of Havana and Turkish tobacco, appear as faster moving components
than the main protein, while with N. glutinosa, the minor component
moves more slowly. Therefore, it can be concluded that tobacco leaves
are closely similar to spinach leaves as far as the general protein spectrum
of the normal cytoplasm is concerned.

An indication of the experimental error involved in this type of quanti-
tative protein analysis is shown by the data in Table I. Two sets of
fourteen Havana tobacco plants were separately analyzed for total par-
ticulate proteins and total cytoplasmic proteins by the methods described
above. The data show that, on a dry weight basis, the amount of par-
ticulate protein isolated from the two sets of leaves agreed within 6 per
cent, while the amounts of total cytoplasmic proteins agreed within 5
per cent. In order to exaggerate any differences in the ratio of total
particulate proteins to total cytoplasmic proteins which might result from
insufficient grinding of the leaf cells, the second sample was ground to a lesser extent in the colloid mill than the first. However, on a dry weight basis, the yield of the two constituents was essentially the same and the

![Electrophoretic patterns of the cytoplasmic proteins of different tobacco leaves. Cacodylate buffer of 0.1 ionic strength, pH 6.9. Current, 15 ma. 1.0 per cent solutions of Havana tobacco and N. glutinosa proteins; 1.3 per cent solutions of Turkish tobacco proteins. Right, ascending; left, descending.](image)

**Fig. 2.** Electrophoretic patterns of the cytoplasmic proteins of different tobacco leaves. Cacodylate buffer of 0.1 ionic strength, pH 6.9. Current, 15 ma. 1.0 per cent solutions of Havana tobacco and N. glutinosa proteins; 1.3 per cent solutions of Turkish tobacco proteins. Right, ascending; left, descending.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Fresh weight analyzed</th>
<th>Per cent dry weight</th>
<th>Per cent dry weight extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>323.3</td>
<td>10.05</td>
<td>63.4</td>
</tr>
<tr>
<td>2</td>
<td>283.3</td>
<td>10.02</td>
<td>54.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mg. per gm. dry weight of leaves</th>
<th>Total particulate proteins</th>
<th>Total cytoplasmic proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>148</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>158</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

ratios were unchanged. Evidently, these methods can be used here quantitatively to detect changes in either the particulate or the cytoplasmic constituents of the leaf as a result of virus multiplication.
Electrophoretical Character of Cytoplasmic Proteins Obtained from Healthy and Virus-Infected Tobacco Leaves—In December, 1948, two sets of thirty-three Havana tobacco plants were chosen for uniformity and arranged for experimental use. One set of plants was inoculated with tobacco mosaic virus; the other set was kept as a virus-free control. The top two leaves only of each plant were inoculated with a crude preparation of virus by means of the carborundum technique. The plants were approximately 1.5 months old, averaged about 12 inches in height, and had eight to ten well expanded leaves. 13 days after inoculation, all but the top cluster of leaves from each set of plants were removed and analyzed for particulate protein and soluble cytoplasmic protein. The only evidence of virus infection in the inoculated leaves at this time was the appearance of a slight amount of vein clearing in the leaves which had been used for inoculation, but which were not included in the protein analysis. Other than these very mild symptoms, the control and infected leaves gave every appearance of being alike.

The results of the protein analyses are given in Table II together with the electrophoretic scanning patterns of Fig. 3. From Table II it is evident that the weights of the two lots of leaves were closely similar and, more important, that the same proportion of dry matter, 65 per cent, was dispersed as a cell-free protoplasmic juice. Similarly, the amount of particulate matter contained in the leaf protoplasm of virus-infected leaves was closely similar to the amount contained in the healthy leaves. The total amount of cytoplasmic proteins in the two instances was also nearly the same. Therefore, it can be concluded that there had been no great change in either the total amount of protein of the leaf protoplasm or the distribution of particulate protein compared to the cytoplasmic protein as the result of 13 days infection with virus.

However, electrophoretic analysis of the soluble cytoplasmic protein contained in the healthy and virus-infected leaves showed that a profound

<table>
<thead>
<tr>
<th>Condition of leaves</th>
<th>Fresh weight analyzed</th>
<th>Dry weight</th>
<th>Dry weight extracted as cell-free juice</th>
<th>Mg. per gm. dry weight of leaves</th>
<th>Amount of virus protein in cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>477</td>
<td>31.3</td>
<td>64.8</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>Virus-infected</td>
<td>463</td>
<td>35.5</td>
<td>64.5</td>
<td>150</td>
<td>113</td>
</tr>
</tbody>
</table>

TABLE II
Effect of Tobacco Mosaic Virus Infection on Total Protein Composition of Havana 38 Tobacco Leaves

December, 1948; leaves analyzed 13 days after inoculation.
change had occurred in the physical properties of the proteins as a result of infection. As shown in Fig. 3, a new and distinctly different protein had appeared. Since the concentration of total protein used for electrophoresis was in both instances the same, it is possible to compare amounts by planimeter measurements (4), and this comparison shows that 37 per cent of the total cytoplasmic protein of the virus-infected leaf consists of the new component. Correspondingly, the virus-infected leaf pos-

![Diagram of electrophoresis results, showing two peaks labeled 'Virus' and 'Normal'.](http://www.jbc.org/)

**Fig. 3.** Comparison of the electrophoretic behavior of the total cytoplasmic proteins obtained from normal and virus-infected Havana tobacco leaves. 1.0 per cent solutions of total proteins. Conditions the same as in Fig. 2. Migration time, 180 minutes. Right, ascending; left, descending.

sessed 37 per cent less normal protein than the non-infected leaf. It can also be observed that the mobility of the normal protein in both kinds of leaves was the same, but that the new component in the virus-infected leaves migrates more rapidly than the main protein.

Two facts suggest that the new component is indeed virus protein similar in properties to the virus previously isolated in crystalline condition (11). In the first place, the narrowness of the faster moving peak suggests a protein of very high molecular weight, which consequently diffuses
during the time of electrophoresis at a much slower rate than the normal protein. Secondly, if the protein mixture is allowed to migrate for 6 hours, sufficient separation of the virus protein from the normal protein occurs so that it is possible to remove the faster moving virus component with little or no contamination by the normal protein. When tested on *N. glutinosa*, the new component produced local lesions at high dilutions in a manner characteristic of crystalline tobacco mosaic virus preparations.

![Virus and Normal Proteins](image)

**Fig. 4.** Comparison of the electrophoretic behavior of the total cytoplasmic proteins obtained from normal and virus-infected Turkish tobacco leaves. 0.8 percent solutions of total proteins. Migration time, 180 minutes. Other conditions the same as in Fig. 2. Right, ascending; left, descending.

The data in Table II show the total amount of cytoplasmic proteins found in a kilo of fresh leaves. Since in the virus-infected leaves 37 percent of the total cytoplasmic proteins is the new virus component, calculation reveals that 3.2 gm. of virus protein per kilo of fresh leaves are present 13 days after inoculation. This yield compares favorably with those previously reported by others (3, 7).

Experiments similar to that of Table II and Fig. 3 have been carried out on five separate occasions, and the results in every instance suggest that the appearance of tobacco mosaic virus protein is related to the
disappearance of a corresponding amount of the normal protein of tobacco leaf cytoplasm. For example, the experiment was repeated in January, 1949, with another set of Havana tobacco plants, except that the leaves were harvested 5 days after inoculation. Even with this short time of incubation after inoculation and in complete absence of symptoms suggestive of virus infection, electrophoretic analysis indicated that about 20 per cent of the normal protein had disappeared and a corresponding quantity of the new virus component had appeared. The rapidity of virus protein formation will be brought out shortly in connection with another experiment. When the experiment was repeated with Turkish tobacco leaves, approximately 35 per cent of the main protein component contained in these leaves had disappeared in favor of virus protein 14 days after infection, as shown in Fig. 4.

![Fig. 5. Comparison of the electrophoretic behavior of the minor protein components of Havana tobacco leaf cytoplasm obtained from normal and virus-infected leaves. 1.5 per cent solutions of total proteins. Migration time, 60 minutes. Other conditions the same as in Fig. 2. Ascending boundaries.](image)

The scanning pattern illustrated by Fig. 5 shows that virus formation does not seem to occur at the expense of the cytoplasmic proteins other than the main protein. 1.5 per cent solutions of normal and virus-infected Havana tobacco cytoplasm were allowed to migrate for 1 hour. At this increased protein concentration and shorter period of migration, some of the proteins comprising Fraction II (14) of cytoplasm can be observed as fast moving components. However, they are present to about the same extent in both samples, even though 37 per cent of the total cytoplasmic protein in the virus-infected leaves represents virus protein. Evidently, there is not only insufficient protein in this fraction to account for the observed synthesis of virus protein, but there is also
no evidence that the quantity of the protein mixture has been significantly decreased as a consequence of virus formation.

Formation of Virus Protein As Function of Time after Inoculation—Turkish tobacco plants, approximately 1.5 months old, were divided into seven groups of fifteen plants each. The experiment was performed in January, 1949. A terminal leaf of each plant was inoculated with a crude preparation of tobacco mosaic virus. With the exception of the top cluster of leaves, all of the lower leaves of fifteen plants were removed 2, 3, 4, 8, 12, 16, and 20 days after inoculation. The soluble cytoplasmic proteins of each sample were isolated and examined for the presence of virus protein by the Tiselius electrophoresis method. For comparative purposes, 1.0 per cent solutions of total cytoplasmic proteins were allowed to migrate for 120 minutes at 15 ma. in cacodylate buffer, pH 6.9. The results of electrophoresis are shown in Fig. 6.

![Fig. 6. Time course of virus protein formation in the cytoplasm of Turkish tobacco leaves. 1.0 per cent solutions of total proteins. Migration time, 120 minutes. Other conditions the same as in Fig. 2. Descending boundaries.](image-url)
No new component was detected in the leaves removed 48 hours after inoculation, but the unmistakable appearance of a new component in the cytoplasm of leaves removed 3 days after inoculation is evident. There was a progressive increase in the quantity of the new component as the length of time after inoculation increased up to the 12th day, when about 40 per cent of the total cytoplasmic proteins consisted of virus protein. Virus protein formation then appeared to level off and there was no further increase in the amount of the new component when the leaves were analyzed 16 and 20 days after inoculation. Simultaneously with the appearance of the new component, there was an equivalent decrease in the amount of the normal protein of the cytoplasm, and this reciprocal relationship obtains during the entire course of the experiment.

<table>
<thead>
<tr>
<th>Condition of leaves</th>
<th>Days after inoculation</th>
<th>Dry weight of leaves</th>
<th>Dry weight extracted as cell-free juice</th>
<th>M g. per gm. dry weight of leaves</th>
<th>Virus protein in total cytoplasmic protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>11.0</td>
<td>64.8</td>
<td>146</td>
<td>87</td>
</tr>
<tr>
<td>Virus-infected</td>
<td>10.8</td>
<td>10.9</td>
<td>65.7</td>
<td>144</td>
<td>81</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
<td>11.4</td>
<td>63.7</td>
<td>136</td>
<td>84</td>
</tr>
<tr>
<td>Virus-infected</td>
<td>17.2</td>
<td>12.4</td>
<td>64.4</td>
<td>146</td>
<td>78</td>
</tr>
</tbody>
</table>

It should be noted again that maximum virus protein formation occurred in leaves which gave no outward signs of virus infection except slight symptoms indicative of virus in the inoculated leaf 12 days after infection, but it will be recalled that this leaf and closely adjacent leaves were not included in the analysis.

The time experiment was repeated in April, 1949, with Havana tobacco plants approximately 12 inches high, having about twelve leaves per plant. Growth at this season of the year was rapid, as evidenced by the rapid expansion of the young leaves compared to the slow growing conditions encountered in the December experiments. As before, a single terminal leaf was inoculated with a crude virus preparation, but this leaf was not included in the subsequent protein analyses.

96 plants were divided into three sets of thirty-two plants each. One-
half of the plants was inoculated with virus; the remainder was kept as virus-free controls. 5, 12, and 17 days after infection, the lower leaves were removed from one set of virus-infected and one set of control plants and the leaves analyzed for total particulate and total cytoplasmic proteins. The cytoplasmic proteins were investigated for the presence of virus protein by electrophoretic analysis. No characteristic symptoms of virus infection were detected until 14 days after inoculation. On the 17th day, symptoms were apparent on the younger leaves of all of the infected plants. The results of the protein analysis are presented in Table III and in the scanning pattern tracings in Fig. 7.
Virus protein formation was not as rapid in this experiment as in the previous experiment with Turkish tobacco, but by the 12th day after infection, it is evident that virus protein constitutes about 20 per cent of the total cytoplasmic proteins, while by the 17th day, 40 per cent of the total cytoplasmic proteins consists of virus protein. The data of Table III show that no significant change in total amount of cytoplasmic proteins occurred during the time of this experiment irrespective of whether the leaves were infected with virus or not, even though it is evident from electrophoresis that 12 and 17 days after inoculation the cytoplasm contained substantial amounts of the new virus protein which was absent from the control leaves. There is a small and probably insignificant change in the total particulate proteins as between virus-infected and healthy leaves, but the fact that the virus-infected leaves still contain the same amount of particulate matter on the 17th day after infection as on the 5th day strongly suggests that virus protein formation does not occur at the expense of the chloroplasts or other particulate materials. The data in Tables II and III could be interpreted as showing a slight reduction in particulate matter during virus protein formation. However, the reduction is no greater than that anticipated by the data in Table I, where the amounts of particulate matter from two sets of healthy leaves are compared, but the experimental errors inherent in this type of analysis are still large enough to make a positive statement impossible with the present data.

Evidence has already been presented that the minor protein components of cytoplasm do not change markedly during virus protein synthesis. It seems reasonable to draw the conclusion that virus protein is formed principally at the expense of the main protein component of tobacco cytoplasm.

**DISCUSSION**

The electrophoresis experiments described above seem to establish two facts concerning the formation of tobacco mosaic virus protein in Turkish and Havana tobacco leaves. (1) Virus protein formation is a process which appears to be completed in the parenchymatous cells of leaves about 2 weeks after inoculation of a terminal leaf. (2) Virus protein appears to be formed at the expense of a normal protein already present in the cytoplasm of tobacco leaves.

The formation of tobacco mosaic virus protein as a function of time after inoculation was investigated by Stanley (10) shortly after he succeeded in isolating the virus in crystalline condition. Virus was isolated from expressed leaf juice at weekly intervals after inoculation, and it was found that the amount of virus protein which could be isolated reached
a maximum about 6 weeks after infection. At first sight, these results seem to be at variance with the conclusions reached above. A proper evaluation of the discrepancy can be achieved, however, by a consideration of the different extraction methods and different tissues used for analysis. In Stanley's experiments, the entire aerial portion of the plant was frozen and passed through a meat grinder, and the juice expressed from the ground pulp by pressure. His data show that less than 25 per cent of the total nitrogen contained in the tissue was actually recovered in the juice in the sample analyzed 1 week after inoculation. Such a low recovery doubtless indicates that many cells were left unruptured by the grinding process. This method is to be contrasted with the grinding procedure used here in which it has been shown that extraction of about 75 per cent of the total leaf nitrogen is achieved (14). Chibnall (5) has shown that intact cell walls act as ultrafilters, and although soluble small molecular constituents can be expressed from tissues whose semipermeability has been destroyed by freezing, etc., the proteins are nevertheless largely retained within the cell. The fact that the first samples of sap analyzed by Stanley were essentially free of trichloroacetic acid-precipitable protein, and were not heavily charged with green color, suggests again that only relatively small amounts of leaf cell protoplasm were extracted as cell-free juice. With the methods used here, the cell-free extract is not only intensely green in color, but it also gives voluminous precipitates when trichloroacetic acid is added to the extract. It is therefore possible that the delayed appearance and relatively slow increase in virus protein in juice expressed in Stanley's experiments may be attributed to incomplete grinding of the leaf tissue. Secondary factors operative in the diseased plants over long periods apparently increase the case with which the virus may be recovered, although secondary increases in virus content perhaps in tissues other than the parenchyma of the leaves may also be involved.

The fact that formation of virus protein in leaf cells under our conditions seems to reach a maximum about 12 to 14 days after inoculation of apical leaves makes much of the previous work on the mechanism of virus formation of doubtful significance, because most studies have generally utilized plants which were inoculated some 2 to 3 weeks before the investigation (9). The same criticism can be made of experiments in which virus formation is explained on the basis of transformation of the chlorophyll protein (17). Since maximum virus synthesis can be achieved before changes in chlorophyll protein can be detected, it is probable that any subsequent disappearance of chlorophyll is a secondary result of derangement of the cytoplasm by virus. The main protein component in spinach cytoplasm is an enzyme having to do with phosphorus metabo-
lism, and there is evidence that the normal protein of tobacco cytoplasm performs this same function. If a large portion of this protein were replaced by a metabolically inactive protein, e.g. virus, unable to fulfil this rôle in the normal cellular metabolism, wide-spread secondary effects on the physiology of the plant might become manifest, such as maintenance of chloroplastic protein level, as well as the ultimate stunting of the infected plants.

Since virus protein appears to be made at the expense of the normal protein in tobacco cytoplasm, the question remains as to how much of a change has occurred when the normal protein is transformed into virus protein. Does the change involve extensive breakdown followed by a new and constitutionally different synthesis of virus protein, or is the transformation process more direct, perhaps involving only a polymerization of the protein? Comparison of the amino acid and nucleic acid contents of the two proteins, the extent of cross reaction between the antibodies formed from the virus and the normal protein,1 and comparative analysis of the phosphorus relationships of the two proteins (16) should be useful properties in deciding between the alternatives expressed here in their extreme forms.

**SUMMARY**

Evidence is presented suggesting that tobacco mosaic virus protein is synthesized in Turkish and Havana tobacco leaf cells at the expense of a normal nucleoprotein found in the cytoplasm of the leaf cells. Virus protein can be detected by electrophoretic methods 3 days after inoculation of apical leaves and progressively increases in amount in the lower leaves up to the 12th day after inoculation. The process of virus synthesis appears to reach a static level about 12 days after infection. There is a simultaneous and proportional decrease in the normal nucleoprotein as virus protein is formed.

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THE PROTEINS OF GREEN LEAVES: III. EVIDENCE OF THE FORMATION OF TOBACCO MOSAIC VIRUS PROTEIN AT THE EXPENSE OF A MAIN PROTEIN COMPONENT IN TOBACCO LEAF CYTOPLASM

Sam G. Wildman, C. C. Cheo and James Bonner


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