Changes in Certain Water-Soluble Nitrogenous Constituents of Burley Tobacco During Curing

James R. Young and R. N. Jeffrey

(with two figures)

Introduction

An investigation of the chemical changes that take place in tobacco during curing is in progress at the Kentucky Agricultural Experiment Station. Attempts are being made to determine what the normal changes are and how they are influenced by such variables as temperature and relative humidity, which have been shown to influence the final quality of tobacco. A study of this type adds to the knowledge of leaf metabolism during starvation and drying. Knowledge of the chemistry of curing may lead to methods of producing high quality tobacco which have not been found empirically.

This paper reports the results of experiments on the changes that occur in certain water-soluble, nitrogenous constituents of the tobacco leaf during curing and of the effect of relative humidity upon these changes in chemical composition. Relative humidity was used as the variable rather than temperature because the work of Jeffrey (3) showed that differences in relative humidity cause larger differences in quality of the tobacco than do differences in temperature, within the limits encountered in practical Burley tobacco curing.

Chemical investigations of the tobacco plant have been made in many regions where tobacco is an important cash crop but very little of this work has dealt with the chemistry of curing. The most complete studies of the chemical changes that occur during the curing of tobacco were made by Vickery (10, 13) and his associates at the Connecticut Agricultural Experiment Station. Primed leaves of a Connecticut shade-grown variety of cigar tobacco were cured in a curing shed and analyses were made of the leaves at different stages of curing. The analyses included insoluble nitrogen, several fractions of the soluble nitrogen, ash, carbohydrates, and ether extract.

In 1914 Garner (2), of the Bureau of Plant Industry, reported on some chemical studies of tobacco curing. Both primed and stalk-cured leaves of several Connecticut cigar varieties were analyzed before and after curing. Analyses included several carbohydrate fractions, several organic acid fractions, several soluble nitrogen fractions, ash, and protein nitrogen. Ash and dry matter were expressed as grams per 100 leaves. The other analyses were expressed as percentage of dry weight.

Basis for reporting results

Since both the total sample weight and the dry weight of the leaves change during curing, neither of these forms a satisfactory basis for express-

1 The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.
ing results. Some investigators have expressed their findings as weight of component in a given number of leaves. Because of the great variation in size of Burley tobacco leaves, even if taken from the same stalk position, it would be necessary to take a very large sample to make the sampling error negligible. Since it was planned to cure the tobacco in curing chambers under controlled conditions, such large samples could not be used.

VICKERY et al. (10, 13) working with primed tobacco expressed their results as weight of a component per unit weight of the fresh leaves. Their method could not be used in this study because the tobacco leaves were cured on the stalk. Some investigators have expressed their data as weight of a component per unit area of the fresh leaf. This method is the one used in the present paper as it is possible to estimate the area of tobacco leaves while they are still attached to the stalk.

Methods

DETERMINATION OF LEAF AREA

The length and width of each of the four largest undamaged leaves on each plant were measured to the nearest centimeter before the plants were cut and a tag was attached to each leaf indicating its dimensions. Just before the plants were cut for the curing chambers, the leaves to be used in determining the regression equation relating the product of the length and the width to the area were cut from the plants and the midribs removed. Different methods of determining the relationship between these dimensions and the leaf area were used in the two years in which the study was conducted. In 1939 the lamina (leaf minus midrib) was weighed and plugs of known area were removed by means of a cork borer and weighed. The area of the leaf was calculated by multiplying the weight of the leaf by the ratio of area to weight of the plugs. The constants in the regression equation were evaluated by the method of least squares on data from 42 leaves. The regression equation is:

\[ y = \frac{0.76x}{1000^2} - 0.015 \]

where \( x \) = the product of the length and width of the leaf expressed in centimeters, and \( y \) = the leaf area in square meters. The standard error of estimate \( \sigma \) and the coefficient of correlation \( r_{xy} \), were found to be:

\[ \sigma_x = 0.009 \text{ m}^2 \]
\[ r_{xy} = 0.96 \]

Due to the difficulties involved in taking plugs representative of the lamina, a different method was used in 1940. The length and width of the leaves were measured as before but the area was determined by blue-printing the lamina after the midrib had been removed and determining the area of the blueprints by weighing them. The regression equation, standard error of estimate, and the coefficient of correlation found from the data obtained from 100 leaves are:
THOUGH the constants of these two regression equations are of different magnitude, the lines which they represent cross near the average leaf area so that the results obtained with the two equations are not widely different. Each equation, however, was used in calculating the corresponding year's results.

WOLF and Gross (15) studied structural responses induced by topping and suckering. They gave data for 50 tobacco leaves which included measurements of length, width, and area. From their data the following regression equation may be calculated:

\[
y = \frac{0.63x}{1000} + 0.03
\]

\[
r_{xy} = 0.99
\]

WOLF and Gross worked with a variety of flue-cured tobacco. One would not expect the regression equation to be the same for different varieties but the magnitude of the coefficient of correlation indicates that there is a high degree of linear correlation between the area of the tobacco leaf and the product of its length and width.

**Sampling**

The analyses were made of leaves from plants of Kentucky Experiment Station Burley no. 16 tobacco grown on the Station farm during the seasons of 1939 and 1940. In 1939 the plants were cut and speared on August 30, but were piled in the field until the next day to wilt. A fresh leaf sample was taken at the time of cutting. The tobacco was housed in the air-conditioned chambers described by O'BANNON (6) and used by JEFFREY (3) in studies of the effect of different air conditions during curing upon the quality of cured tobacco. Since these studies showed that the relative humidity was more critical than the temperature in controlling the quality of the cured tobacco, three different levels of relative humidity were used: one at about the optimum (70 per cent.); one above (78 per cent.); and one below (59 per cent.). The same temperature was maintained in all cases (75° F. or 23.9° C.).

The tagged leaves from the 6 plants on one stick constituted a sample. Since each tier supported 6 sticks it was possible to take one sample from each chamber at each of 6 different times during curing, in addition to the fresh-leaf sample. The time of sampling for each expressed as hours from cutting to sampling is recorded in the first column of Table I.

When the samples were taken, the tagged leaves were examined and those damaged in housing were not included in the sample. After the tagged leaves were removed, the rest of the stick of tobacco was returned to the chamber in order to change the conditions surrounding the next sample as
<table>
<thead>
<tr>
<th>Time</th>
<th>Relative Humidity (%)</th>
<th>Dry Matter</th>
<th>Sulphated Ash</th>
<th>Total Nitrogen (%)</th>
<th>Nicotine Nitrogen (%)</th>
<th>Asparagine Nitrogen (%)</th>
<th>Glutamine Nitrogen (%)</th>
<th>Ammonia Nitrogen (%)</th>
<th>Amino Acid N (by Diff.) (%)</th>
<th>Residual Nitrogen (%)</th>
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* Barn-cured samples.
TABLE II

Concentration of certain constituents of Burley tobacco leaf wet at different times during the air-curing process, 1940 crop, stated as percentage of the dry weight and as weight per square meter of leaf area.

<table>
<thead>
<tr>
<th>TIME</th>
<th>RELATIVE HUMIDITY</th>
<th>DRY MATTER</th>
<th>SULPHATED ASH</th>
<th>TOTAL NITROGEN</th>
<th>NICOTINE NITROGEN</th>
<th>ASPARAGINE NITROGEN</th>
<th>GLUTAMINE NITROGEN</th>
<th>AMMONIA NITROGEN</th>
<th>AMINO ACID N (BY DIFF.)</th>
<th>RESIDUAL NITROGEN</th>
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<tr>
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<td>gm.</td>
<td>%</td>
<td>gm.</td>
<td>%</td>
<td>gm.</td>
<td>%</td>
<td>gm.</td>
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<td>21.0</td>
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<td>0.42</td>
<td>0.12</td>
<td>0.10</td>
</tr>
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</table>

* Barn-cured samples.
little as possible. The midrib was removed from the leaves of the sample and the web was dried as rapidly as possible in a current of air at 67° C. The dried leaves were weighed, ground, and stored in waxed paper friction-top cartons. Moisture determinations were made and the dry matter per square meter of fresh leaf was calculated. Link (5) found that in plants dried at 65° C. the only significant alteration of the nitrogenous constituents was a decrease in the amount of soluble nitrogen due to coagulation. None of the substances determined in this study are coagulated by heat. Link did not determine ammonia or amide nitrogen in his study of the effect of drying.

Harvesting and sampling of the 1940 crop followed the same plan; only the points of difference will be given. On September 3, 1940 the plants were split and cut. The relative humidity of the three chambers was maintained at 50, 69, and 86 per cent. In 1939, no significant differences were observed in the composition of the samples cured at the different humidities; for this reason a wider range of relative humidities was selected for curing the samples from the 1940 crop. The results are given in table II. The analyses of the 1939 samples indicated that the chemical changes occurred more rapidly in the early stages of curing. Therefore most of the samples of the 1940 crop were taken during the early stages of curing. At cutting time two additional sticks were cut after their leaves had been measured as above and were placed in the barn to constitute the 1940 barn-cured samples.

**ANALYSES**

**DRY MATTER.**—The dry weight per square meter of leaf area was calculated from the leaf area of each sample, and the oven-dry weight of the leaf web of the sample.

**SULPHATED ASH.**—One gram of air-dry ground tobacco was weighed into an ignited and weighed porcelain crucible. Ten drops of a 5 to 3 solution of sulphuric acid in water were dropped on the sample. The sulphuric acid was added to prevent loss of potash by volatilization. The crucibles were placed in a cold muffle which was slowly heated to 700° C. Moisture was determined on the samples; and the grams of ash per 100 grams of oven-dry material (percentage) and the grams of ash per square meter of fresh leaf were calculated. A third determination was made if the duplicates did not agree within 3.5 per cent.

**TOTAL NITROGEN.**—A semi-micro Kjeldahl method was used. The digestion was made by a modification of the official Gunning method of the Association of Official Agricultural Chemists (7). Fifty milligrams of air-dry, ground tobacco was introduced into a 100-ml. Kjeldahl flask. Three ml. of concentrated sulphuric acid containing 0.1 gm. of salicylic acid were added and the material was allowed to stand for 30 minutes with occasional shaking. One-half gram of sodium thiosulphate was added to effect the reduction of the nitrate nitrogen, and the material was warmed on an electric hot plate for 5 minutes. After cooling, 1 gm. of potassium sulphate and a small
piece of selenium were added and the mixture was digested 2 hours. On cooling, 10 ml. of water were added and the digested sample was washed into the Kirk apparatus (4), followed by 6 ml. of 40 per cent. sodium hydroxide. The ammonia liberated was steam distilled into a receiver charged with 5 ml. of 0.1 N sulphuric acid. The excess acid was back titrated with 0.05 N sodium hydroxide. After moisture determinations were made on the air-dry samples by drying duplicate 2-gm. samples for 16 hours in a vacuum oven at 70° C., the nitrogen was calculated as percentage of oven-dry material and as grams of nitrogen per square meter of fresh leaf area. Results were not considered acceptable unless the duplicates agreed within 3 per cent. It was necessary to have the samples finely ground in order to obtain this agreement.

Nicotine Nitrogen.—Nicotine was determined by the Avens and Pearce (1) modification of the silicotungstic acid method. It was necessary to distill about 45 minutes instead of 30 minutes as recommended by Avens and Pearce in order to get duplicates agreeing within 2 per cent.

Amide Nitrogen.—Glutamine N and asparagine N were determined by the methods of Vickery, Pucher, and others (12). The amide N was determined on a hot-water extract of the dry tobacco prepared by heating at 80° C. for 10 minutes (14). In the case of both amides a third determination was made if the duplicates failed to agree within 6 per cent.

Ammonia Nitrogen.—Ammonia N was determined by the method of Pucher and co-workers (9). Nicotine interfered with the determination by forming a finely divided crystalline precipitate on reaction with the Nessler's reagent. This interference was overcome by precipitating the nicotine as nicotine silicotungstate following the distillation. The precipitate was removed prior to Nesslerization by centrifuging and an aliquot was taken for the determination. The precipitate could not be removed by filtering as the filter paper contained large enough quantities of ammonia to interfere seriously with the determinations. The excess silicotungstic acid caused no interference.

The nitrogen present as ammonia was calculated as percentage of oven-dry weight and as milligrams of ammonia N per square meter of leaf area. The determination was repeated if the duplicates failed to agree within 5 per cent.

Amino Acid Nitrogen.—Amino acid nitrogen was determined by the method of Peters and Van Slyke (8) on the hot-water extract of the dry tissue. Ammonia was removed by low-pressure distillation at 40° C. with light magnesium oxide prior to the determination of amino nitrogen in the Van Slyke manometric apparatus. A third determination was made when duplicates failed to agree within 1 per cent.

Vickery and co-workers (12) found that 80 per cent. of the amide nitrogen of glutamine reacted with nitrous acid in the Van Slyke manometric apparatus. The total amino nitrogen was calculated by subtracting 80 per cent. of the amide nitrogen of glutamine from the amino nitrogen found.
by the Van Slyke method. The amino nitrogen other than that of the amides which will be designated hereafter as amino acid nitrogen was found by subtracting the amino nitrogen of the amides from the total amino nitrogen. Amino acid nitrogen was reported as the percentage of oven-dry weight and as milligrams of nitrogen per square meter of fresh leaf.

Results

Tables I and II give the results for the 1939 and 1940 crops, respectively. Some of these results are also presented in graphical form in figures 1 and 2, expressed as weight per square meter of fresh leaf. It should be noted in connection with these tables and figures that the central leaves of the plant, which were analyzed in this work, turned yellow in about 3 to 6 days, and brown in 7 to 12 days. The low relative humidity samples preceded the higher relative humidity samples in both color changes.

Analyses

Dry matter.—A marked decrease in dry matter occurred during the first seven days of curing, after which no significant change was observed. It seems evident that the regular initial sample for the 1939 crop is out of line and that the preliminary fresh leaf sample (designated as PFL) is more nearly correct; consequently, the lines in the figures are connected to this point though both values are recorded. If this value is used, it may be seen that the loss of original dry matter is about 30 per cent. in each year in which the experiment was conducted. The preliminary fresh leaf, the initial 1939, and the initial 1940 samples contained 43, 22, and 26 leaves, respectively; thus the initial 1939 sample would be expected to be the least accurate.

Vickery et al. (10, 13) have reported decreases in dry matter during the curing of the primed leaves of a Connecticut shade-grown variety, amounting to 18 and 20 per cent. Garner (2) observed decreases in dry matter of 12 and 15 per cent. during the curing of primed leaves and decreases of 25 to 30 per cent. during the curing of leaves on the stalk.

There are two reasons why stalk-cured leaves might be expected to lose more dry weight than primed leaves. One is that the stalk-cured leaves do not dry as rapidly as the primed leaves, therefore allowing greater opportunity for catabolic processes to go on which would give rise to volatile compounds. The other is that there is a chance for translocation to take place from the leaves to the stalk in tobacco cured on the stalk.

It has been found by other workers that, in plants living under adverse conditions, the growing points and meristematic tissue receive soluble nutrients at the expense of older and more mature tissue. For this reason, translocation might be expected to take place from the leaves to the stalks and thence to the living axillary buds or suckers in tobacco being cured with the leaves still attached to the stalk. It is possible to remove and plant "suckers" which have been on plants in the curing barn for as much as two months and to obtain good growth.
**Fig. 1.** Changes in the dry matter, sulphated ash, total nitrogen, and nicotine nitrogen content of Burley tobacco leaf during the air curing process.
Fig. 2. Changes in the asparagine, glutamine, ammonia, and amino acid nitrogen content of Burley tobacco leaf during the air curing process.
SULPHATED ASH.—The results obtained by this determination are variable. If the initial samples are averaged and the samples from the later stages of curing are averaged, it may be concluded that the loss in ash content of the leaf web was about 12 per cent. Garner (2), however, with fewer samples to indicate the variability of his data, attached significance to a decrease of about 9 or 10 per cent. of the original ash content of the whole leaf. He found decreases of 3.4 and 4.7 per cent. in the ash content of the leaf web.

TOTAL NITROGEN.—If the preliminary fresh leaf sample is considered to be the most accurate initial 1939 sample, as above, the loss in total nitrogen would appear to be about 38 per cent. in about 8 days in 1939 and about 41 per cent. in about 10 days in 1940.

Garner (2) observed a larger decrease in total nitrogen in stalk-cured leaves than in primed leaves but he obtained evidence of loss of total nitrogen even in primed leaves; he attributed this to volatilization of ammonia. The additional decrease in total nitrogen in the case of stalk-cured leaves was attributed to translocation.

Vickery (10) reported a decrease in total nitrogen in primed leaves of 15 per cent. This loss occurred before the leaves had all become brown. In a later investigation (13), his data did not indicate any decrease in total nitrogen during the same stages of curing.

NICOTINE NITROGEN.—The nicotine content of the samples expressed on a leaf area basis shows a slight downward trend as curing progresses. This change is probably too small to be significant except in the final 86 per cent. relative humidity sample; this had undergone "houseburn," which includes attack by microorganisms as well as further autolytic changes by the plant enzymes. This indicates that nicotine probably did not enter actively into the metabolism of the curing process. The nicotine values here reported are higher than similar values appearing in the literature for Burley tobacco. No satisfactory explanation can be offered for this difference. The method used was checked by means of samples previously run by another laboratory that used the A.O.A.C. method, and satisfactory agreement was obtained.

ASPARAGINE NITROGEN.—In 1939 the asparagine content of the curing tobacco increased to almost six times its initial value in the first three days of curing. After this no significant change was observed. No significant difference was found between the results at the different relative humidities. In 1940 the asparagine nitrogen increased to over eight times its initial value in about four days and then remained constant except for the 86 per cent. relative humidity sample. These statements do not imply that there was no further production of asparagine after the third or fourth day, but that the rate of destruction equaled the rate of production if it did continue. In the 86 per cent. relative humidity sample, the destruction of asparagine apparently continued after the production had ceased, resulting in a value about as low as the initial value.

GLUTAMINE NITROGEN.—The method for the determination of glutamine is rather inaccurate as only small amounts are present and it is calculated
by difference between two determinations neither of which was consistently duplicated within much less than 6 per cent. In 1939, the amount of glutamine nitrogen approximately doubled in the first three days of curing and then held approximately constant until about two weeks from the start of curing, after which it gradually decreased to about the initial value. The result obtained for the sample cured at 70 per cent. relative humidity for 473 hours (19.7 days) cannot be explained except as an experimental error.

In 1940, the glutamine, like the asparagine and to a lesser degree some of the other nitrogenous constituents, reached higher values than in 1939. The glutamine accumulated to over four times the original value and twice the maximum of 1939 in about four days and then gradually fell to about the same value as in 1939 except in the very low humidity sample. If the high final value on this sample is significant, it may be because this sample became very dry while the high glutamine content still existed, inhibiting further chemical change.

The values found for amide content of leaf web were much more variable during the later stages of curing than in the early stages; it does not seem likely that analytical errors are altogether responsible.

VICKERY (10) found that the total amide nitrogen of the hot-water extract of whole tobacco leaves increased from 0.04 gm. per 1000 gm. of fresh leaf to about 0.4 gm. during curing. Later (13), he found that the total amide nitrogen increased from 0.2 gm. per 1000 gm. of fresh leaf to about 1.0 gm. during the same stages of curing.

There are two views concerning the formation of amides in plants. The view of VICKERY (11) is that amide metabolism is a phase of the general respiratory activity of the tissues. The older view of PRIANICHNIKOW was that the function of amides was to provide a means of disposal of ammonia which otherwise might accumulate in high concentrations and prove toxic. The nitrogen content of the leaves was greater in the 1940 than in the 1939 crop. Also a greater decrease in total nitrogen occurred in the samples from the 1940 crop. This means that probably more catabolic changes occurred which might give rise to ammonia, a form of nitrogen thought to be essential in amide synthesis. A much larger quantity of amides was found in the 1940 samples but this could be due to decomposition in the 1939 samples as previously indicated. If the amount of amides formed in 1940

Since this work was completed, glutamine has been re-determined on some of the 1940 cured samples. The values did not agree with those previously found; they were so low that they stimulated speculation as to the stability of the amides in air-dry plant tissues when stored for considerable lengths of time. Ten months elapsed from the time the samples were taken from the 1939 crop until these samples were analyzed for amides. Only 4 months elapsed from the time the 1940 samples were taken until they were analyzed for amides. The values for amides of the 1939 crop were much lower than for the 1940 crop and, in view of the values obtained for the 1940 crop nine months after sampling, one is led to the conclusion that the amides may decompose during storage under such conditions. This casts doubt upon the results of this experiment and possibly upon VICKERY's results (14).
was larger because of the increased protein decomposition it still does not prove conclusively whether or not detoxification was actually responsible.

The increase in amide nitrogen appears to have been more immediate than the increase in ammonia nitrogen, particularly in the results from the 1940 crop, where the early samples were taken at more frequent intervals and the analyses are generally more reliable. It may be that the amide nitrogen ceased to rise after about the fifth day because of failure of the supply of α keto acids.

**Ammonia Nitrogen.**—During curing of the 1939 crop, the ammonia nitrogen increased to about six times its original value, in the first week, and then remained about constant in all samples. In 1940 the same level was reached after ten days. A high value was obtained for the final 1940 sample at 60 per cent. relative humidity, but this is probably an error. The sample cured at 86 per cent. relative humidity lost ammonia in the later stages of curing, similar to its loss of other soluble nitrogenous constituents.

**Amino Acid Nitrogen.**—During curing of the 1939 and 1940 crops, the amino nitrogen other than that contributed by the amides reached a maximum in about three days, after which it decreased, reaching values as low or lower than the initial in another three days in 1939 and somewhat more slowly in 1940. There is probably no other significant change except in the final 1940 high-humidity sample, which is low in amino acid nitrogen as well as other soluble nitrogen components. The hydrolysis of part of the protein to amino acids is one of the first changes that take place in the nitrogen compounds of the curing leaf; this might be expected. It is followed by a rapid conversion of the amino acids to other forms.

During curing of the 1940 crop a decrease of amino nitrogen (other than that of amides) was observed between the third and tenth days, amounting to about 60 mg. per square meter. An increase of two-thirds this amount was observed in the nitrogen of amide groups of asparagine and glutamine. The remainder of the decrease in total amino nitrogen can be accounted for only by translocation or volatilization. During curing of the 1939 crop, a decrease in amino acid nitrogen of about 75 mg. occurred. As practically no net synthesis of amides was detected during this period, the loss probably occurred by volatilization or translocation or both.

**Residual Nitrogen.**—If the sum of the asparagine N, glutamine N, ammonia N, amino acid N, and nicotine nitrogen is deducted from the total N, a figure is obtained which is here designated as the residual N. This residual N is probably chiefly protein N and nitrate N. It decreased by about 32 per cent. in the 1939 crop and by about 60 per cent. in the 1940 crop, which had a higher initial nitrogen content. The final value reached is not far different in the two years. In this, as in most other cases, the loss all occurred in the first ten days.

**Barn-Cured Samples.**—The barn-cured samples were analyzed for the same components as were the samples cured in the curing chambers. The
findings are given in the tables along with the other data, designated BC. The values found agreed, for the most part, with those for the samples cured in the chambers.

Discussion

If we attempt to draw general conclusions from the data presented in the graphs, it will be seen that no significant differences are evident between the tobacco cured at different relative humidities with the single exception of the final sample cured at 86 per cent. This sample was so badly injured that even a person unfamiliar with tobacco would pick it out immediately. The other samples were sufficiently different to be distinguishable by any good tobacco grader. Since the data do not show any such difference, it is obvious that the analyses did not include those substances which are responsible for quality as determined by commercial grade. On the other hand, they did include substances which changed markedly during the curing process. It would appear, as one might expect, that the first change was a hydrolysis of the protein to amino acids which, in turn, were either translocated or yielded ammonia and amides. The increase in ammonia concentration was rather slow at first, but it would appear that ammonia was being produced, as it was probably an intermediate in the formation of the amides. It is also the most volatile nitrogen compound whose presence would be expected. In the 86 per cent. relative humidity sample the loss of all soluble nitrogen compounds was the most complete. Fungus growth was visible on this sample. Probably various microorganisms combined with the plant enzymes to produce the more complete autolysis in this sample.

It is evident that the loss of ash occurred by translocation. Although it cannot be assumed that the movements of inorganic salts and soluble nitrogen compounds would necessarily be the same, the total nitrogen content of the leaves fell by about 40 per cent. while the ash content was falling about 12 per cent.; consequently, it seems possible that some of the nitrogen was lost by volatilization.

After the amide concentration had ceased to rise at about the fourth day, the ammonia continued to rise until about the eighth or tenth day. About this time all significant changes ceased except in the sample which underwent "house-burn." This sample lost additional nitrogen in all soluble forms which were determined, including nicotine. By the end of the curing period, this tobacco was dark colored, had the characteristic odor of "house-burn" known to all tobacco men, and had fungus colonies on it. It is possible that microorganisms may have used the soluble nitrogen compounds in the synthesis of insoluble compounds or undetermined soluble compounds. There was no significant change in the undetermined forms reported in the tables as residual nitrogen. This indicates either that no further attack was made by the plant enzymes or the microorganisms on the leaf protein, or that the amount of protein formed by the microorganisms from soluble sources was about equal to the amount broken down.
Summary and conclusions

This study was made to determine some of the chemical changes that occur in leaves of White Burley tobacco during curing on the stalk, under automatically controlled conditions of temperature and relative humidity. Samples taken at different stages of curing were analyzed for ash, total nitrogen, nicotine nitrogen, asparagine nitrogen, glutamine nitrogen, ammonia nitrogen and amino acid nitrogen. The results are expressed as weight per square meter of fresh leaf. The following conclusions are justified:

1. The dry matter content of the leaf web decreased by about 30 per cent. during the early stages of curing.
2. The total nitrogen decreased by about 40 per cent. of the original amount in the early stages of curing.
3. A rapid increase occurred during the early stages of curing in all forms of soluble nitrogen determined except nicotine.
4. The asparagine, glutamine, and ammonia nitrogen remained high except in leaves cured at very high relative humidity (86 per cent.).
5. The amino acid nitrogen determined by subtracting the amino nitrogen of the amides from the total amino nitrogen decreased from its maximum to below its original concentration. Some of this amino acid nitrogen was used in amide synthesis, some was translocated and some may have been volatilized.
6. The nicotine content remained practically constant throughout the cure except in the "houseburned" sample, indicating that nicotine does not enter actively into the normal metabolism of the curing process.
7. The leaves cured at 86 per cent. relative humidity lost most of their soluble nitrogen during the later stages of curing. This loss is probably due in part to the action of the enzymes of the plant and in part to the action of microorganisms growing on the leaves.
8. Except for the sample cured at 86 per cent. relative humidity, there is no evidence of change in composition after the leaf has burned brown. This indicates that changes due to plant enzymes and microorganisms are both nearly stopped as soon as the tissue has become dry.

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LITERATURE CITED