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Chemical Investigations of the Tobacco Plant VII. Chemical Changes That Occur in Stalks During Culture in Light and in Darkness

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INTRODUCTION

EXPERIMENTS were described in the preceding bulletin of this series (19) in which samples of leaves from the tobacco plant were subjected to culture in water. Analyses of the leaves at different intervals of time permitted many of the chemical changes that took place in continuous light and also in darkness to be followed. The changes that occur under these circumstances have interest because they reveal something with respect to the nature of the metabolic reactions of leaf tissue. To what extent the observations can be applied to the explanation of the behavior of the living leaf while still attached to the plant is problematical, but it is certain that the leaves are living and actively metabolizing organisms for many hours subsequent to their excision from the plant. The nature of the chemical changes that occur gradually alters with the lapse of time and factors of an obviously irreversible nature become increasingly significant as the cells become moribund. Although apparently unchanged for some time, the originally green and turgid tissue ultimately becomes flaccid; the green pigments and, later, the yellow pigments are destroyed; the leaves rapidly lose water and the chemical changes that can then be recognized are almost entirely those of autolysis. At exactly what point the chemical changes should no longer be regarded as illustrative of those that can occur in normal living leaves is uncertain, and the difficulties are increased by the fact that the period of survival in a manifestly healthy condition is considerably affected by light or darkness, by the composition of the culture solution in which they are placed, and by such conditions as temperature, humidity, and by the age of the leaves when collected.

The analytical methods available for these studies enabled us to follow the behavior of the simple carbohydrates, the organic acids, and many of the nitrogenous components. The leaves exposed to continuous illumination showed evidence of extensive photosynthesis; the organic solids increased materially, but only about half the increase could be accounted for as water-soluble reducing substances calculated as glucose. The nature of the remaining portion of the increase is entirely unknown; it may represent photosynthetic sugar, which was subsequently converted into substances that no longer responded to the sugar reagent, or it may represent products of direct photosynthesis of substances that were not sugars. The greater part of this new material was soluble in water, but evidence was secured that some of it was not.

Note: The chemical investigations of tobacco herein described were carried out as part of a general project under the title "Cell Chemistry," by the Department of Biochemistry of the Connecticut Agricultural Experiment Station, New Haven, Conn. The Department has enjoyed the benefit of close cooperation from the Tobacco Substation at Windsor. The expenses were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington.

The leaves cultured in complete darkness behaved quite differently. There was a rapid and profound loss of organic solids, and this was interpreted as a rough measure of the effect of respiration; the soluble carbohydrates in particular were rapidly consumed, but much of the loss fell upon substances of some other nature.

In addition to the changes in carbohydrates and organic solids, extensive alterations in the nitrogenous constituents of the tissue were observed both in light and in darkness. A part of the protein underwent rapid digestion into water-soluble substances, and the amino acids produced were apparently extensively deaminized by an oxidative process. The ammonia thereby formed was employed for the synthesis of one or both of the amides asparagine and glutamine. The increase in the quantity of nitrogen combined in these specific substances could be quantitatively accounted for in terms of the α-amino nitrogen removed from the products of hydrolysis of the protein, and of the relatively smaller amount of nitrate nitrogen which also disappeared. Asparagine alone was synthesized in substantial quantities during culture of the leaves in darkness, but both glutamine and asparagine, the former in somewhat greater amount, were synthesized during culture in light. Inferences with respect to the presence of appreciable amounts of a non-nitrogenous precursor of asparagine in normal leaves and to the ready formation of a non-nitrogenous precursor of glutamine under the influence of light could therefore be drawn.

The general sequence of reactions corresponded with the views of protein and amide metabolism expressed many years ago by Schulze (11), and more recently by Prjanischnikow (6), but the quantitative nature of the experiments permitted a greater degree of precision in the interpretation than has hitherto been possible.

The changes in the organic acids of the leaves were likewise studied, and evidence was secured that these substances do not undergo extensive alterations during culture in light. Organic acids were therefore not produced directly nor indirectly by photosynthesis, nor were they formed in significant quantities from the decomposition products of the proteins or carbohydrates. In darkness, however, there was a profound loss of malic acid and an equally striking increase in citric acid, and considerable probability was shown to attach to the view that much of the malic acid is converted into citric acid in this tissue during culture in darkness.

Our observations on the behavior of excised tobacco leaf tissue during culture encouraged us to believe that the study of stalk tissue, under analogous conditions, might lead to a fuller understanding of certain details of the chemical physiology of this species. Although there is a considerable literature that deals with the composition of the normal stalk tissue of plants, little appears to have been recorded regarding the behavior of isolated stalk tissue under conditions of culture. Borodin (1) was probably the first to inquire into the nature of the chemical changes that occur in excised leaves or shoots of plants when these are cultured in water. His experiments were repeated and extended by Schulze (9, 10, 12) and also by Müller (4), but these investigators employed for their experiments shoots with leaves still attached, or young twigs the buds of which were allowed to develop in culture. Their chemical investigations were confined mainly to the consideration of the evidence for the conversion of protein into asparagine, and to the study of allantoin formation in the shoots of the plane tree

(Platanus orientalis) and in one or two other species. Furthermore the presence of leaf tissue in their samples rendered it impossible to discriminate between the contributions made to the chemical changes by the stalk tissue as distinct from the leaf or developing bud tissue.

There are occasional references in the literature to the behavior of very young stalk tissue in culture solution. For example, Zaleski (20) cultured the stems of etiolated bean seedlings on glucose solution in the light and obtained evidence for an increase in protein. These experiments are difficult to interpret because of the failure to provide controls. It would appear, therefore, that little information is available as to what may be expected to happen when relatively mature stalk tissue is cultured in water. It might be anticipated, however, that many of the chemical changes would be less striking and extensive than those observed in leaves when the data are expressed upon a similar basis. Leaf tissue is structurally adapted to the promotion of reactions in which light energy plays a part. The ratio of surface to mass is high, and the proportion of the total mass of tissue devoted to purely structural purposes is low; accordingly, reactions such as those of photosynthesis and respiration, which involve the passage of gases through surfaces, have full scope. Stalk tissue, on the other hand, is mainly adapted to the support of the leaves, and consists very largely of elements that have a mechanical function; the ratio of surface to mass is low.[1] Nevertheless the cells of the cortex of the stalk of the tobacco plant are provided with chlorophyll, and it seems highly probable that this part of the stalk may to some extent fulfil functions that resemble those of the leaves.

Aside from its purely structural function, the stalk also serves as the path of transport of elaborated food material from the leaves to the growing points of the terminal bud and of the roots. In addition, it acts as a storehouse in which excess of food material over the immediate requirements for growth is temporarily retained. The behavior of such stores, under the stress of starvation in water culture, is not without interest.

PREPARATION OF MATERIAL

The plants studied were grown under shade tents at a farm adjoining the Tobacco Substation at Windsor, Connecticut, in the season of 1936. The variety was the same as that from which the leaves described in Bulletin 399 (19) were obtained, Cuban Shade or Connecticut shade-grown tobacco, and the agricultural conditions under which the crop was grown were similar. On July 21[2] the plants were denuded of leaves in the field, and the stalks were cut at the ground level and transported to the laboratory where all traces of the basal parts of the leaves still attached to the stalk were carefully cut away. Flower buds, where present, were individually removed from their pedicels. A sufficient number of samples of 10 stalks each was then selected with care to exclude stalks of unusual size and to obtain samples of initially equal total weight. A thin layer of tissue was cut from the base of each stalk, and the samples were supported vertically in enamel-ware pails that contained a shallow layer of distilled water.

[1] The area of 1 kilo of fresh tobacco leaf tissue is somewhat greater than 3 sq. m. (18), and the total surface is therefore, of the order of 6 sq. m. The total surface of 1 kilo of fresh stalk tissue, calculated as cones on a base 2.5 cm. in diameter, of height 200 cm. (4.5 stalks per kilo), is about 0.5 sq. m.

[2] The plants were about 55 days old and had been somewhat retarded by a dry season, but the leaves were well developed and a few plants were beginning to blossom. Rain (0.44 inch) fell during the 24 hours preceding the collection, but the total precipitation in the month of July up to the 21st was only 0.86 inches.

Seven samples were placed in a greenhouse where provision for continuous illumination was made, and seven were placed in a dark room. Two samples were immediately cut transversely by machine into thin slices which were dried in a ventilated oven at 80°. The "crude dry weight" was ascertained, and the tissue was finally ground in a Wiley mill and preserved for analysis in closed containers.

The technique of culture management was closely similar to that described in Bulletin 399 (19) and the same analytical methods were employed. The series of dried samples obtained represented material that had been subjected to various periods up to 332 hours of culture in water either in continuous light or in complete darkness.

GENERAL BEHAVIOR

At the expiration of 160 hours, all of the stalks in the light showed evidences of green sprouts at the upper nodes, and about half of the stalks in the dark had formed colorless outgrowths at similar points. At the expiration of 332 hours, small green leaves, 2 to 3 cm. long, had developed at many nodes on the stalks in light, while about half of those in the dark bore small, elongated, etiolated branches with minute, undeveloped leaflets. These sprouts were not removed before preparing the tissue for analysis.

The analytical data recorded in the tables are expressed in grams per kilo of the original fresh weight of each sample before culture. The methods of calculation, from the results of analysis of the dry powdered sample, of the quantity of each constituent in terms of one kilo of original fresh weight have been described in Bulletin 399. In describing the progress of chemical changes in these samples of stalks, it is assumed that the composition at the start was, within the limits of sampling error, identical for each sample.

The samples in light lost about 5 percent of their water promptly (Table 1), remained at this level for many hours and finally slowly lost an additional 5 percent. The samples in darkness gradually gained a small amount of water, retained this excess for nearly 100 hours, but ultimately slowly lost about 9 percent. The changes were sluggish and were not extensive, comments that apply equally well to many of the detailed chemical conversions that were observed in this tissue. The data for organic solids, although slightly irregular, doubtless due to minor sampling errors, indicate a scarcely significant alteration in the light over the entire period[1], but there was a significant loss in the dark. This observation is in marked contrast to the behavior of leaves which, under similar circumstances, show a prompt and extensive increase in organic solids in light, and a profound loss in the dark.

AMMONIA AND AMIDE METABOLISM

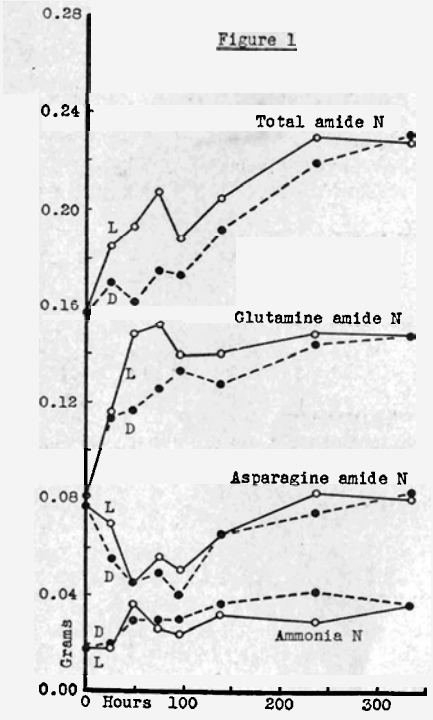
The data for ammonia nitrogen (Table 1 and Figure 1) and, accordingly, also for the amides may be affected by a systematic error the order of magnitude of which it is difficult to assess. As we have pointed out in connection with our studies of tobacco leaves, the free ammonia determined on dried samples of tissue is somewhat less than that found in similar samples of fresh tissue that are extracted with cold water after cytolysis with ether. The difference is negligible with freshly picked leaves, but increases progressively to significance during culture of the leaves in light.

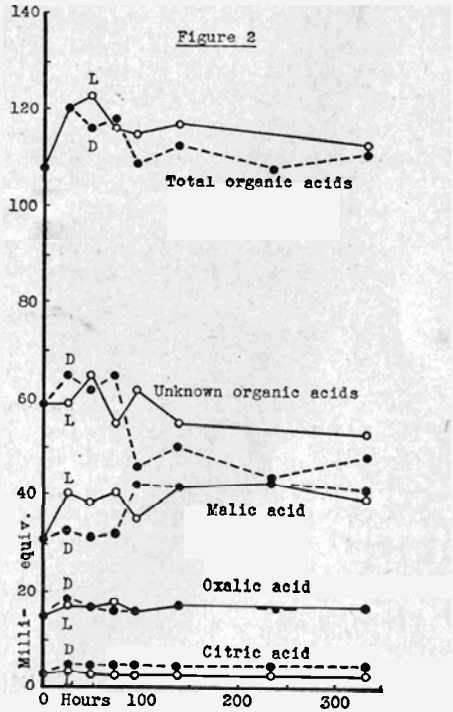
[1] The results of calculation of the best straight line that expresses these data are given on p. 125.

No information with respect to the possible occurrence of this phenomenon in the present samples of stalk tissue has been secured owing to the difficulty of preparing extracts with cold water after cytolysis with ether.[1]

The quantities of ammonia nitrogen found in the dried stalk tissue were small, being of essentially the same order of magnitude as those in leaf tissue similarly prepared, and the slow increase during the culture period is closely like the change that occurs in leaf tissue cultured in light. It is of interest to note that there is very little difference in the rate of increase of the ammonia whether the stalk tissue was cultured in light or in darkness. Leaf tissue cultured in the dark, on the other hand, soon reaches a point at which a sudden acceleration in the rate of ammonia production occurs.

The total amide nitrogen (Figure 1) increased materially during culture of the stalk tissue. The increase appeared to be somewhat more rapid in the early stages in the light than in darkness, but the final value reached





was approximately the same in both cases. Much of the increase was due to the formation of glutamine which occurred especially promptly in the light culture. Both glutamine and asparagine were present in the stalk

[1] Our previous studies of tobacco stalk tissue (17) revealed the presence of substances which were decomposed with the production of ammonia during the preparation of extracts with boiling water. Although a part of this ammonia was obviously derived from glutamine, it was equally clear that much of it had some other origin. The quantities of glutamine found in parallel samples of fresh tissue that had been dried were invariably much smaller than would be expected from the quantities of ammonia set free during the operation of preparing the boiling-water extract. This operation is obviously inadmissible when dealing with tobacco stalk tissue. The problem of preparing a representative extract of the water-soluble substances in tobacco stalk tissue without subjecting it to conditions that might alter its constituents presents many serious difficulties.

TABLE 1. THE WATER, ORGANIC SOLIDS, ASH, AND NITROGENOUS COMPONENTS OF TOBACCO STALKS DURING CULTURE IN WATER, IN DARKNESS AND IN LIGHT

(Data are expressed in grams per kilo of original fresh weight of each sample. The more important data in this and subsequent tables are plotted in the figures.)

Hours	0	24	48	72	95	139	236	332
Water								
Dark	896	907	923	921	893	886	853	815
Light	896	848	850	855	851	844	791	812
Total solids								
Dark	104	113	108	106	107	106	99.7	102
Light	104	117	108	109	107	109	112	105
Ash								
Dark	10.9	11.1	10.9	10.9	10.7	10.2	10.3	11.1
Light	10.9	12.3	9.2	10.8	9.9	10.1	10.7	10.3
Organic solids								
Dark	93.7	102.1	96.8	95.0	95.9	95.4	89.4	91.1
Light	93.7	105.0	98.7	97.8	96.9	98.8	101.0	94.4
Ammonia nitrogen								
Dark	0.0175	0.0200	0.0298	0.0299	0.0298	0.0364	0.0408	0.0359
Light	0.0175	0.0174	0.0361	0.0263	0.0240	0.0317	0.0285	0.0358
Total amide nitrogen								
Dark	0.157	0.170	0.162	0.175	0.173	0.194	0.219	0.231
Light	0.157	0.185	0.193	0.207	0.188	0.205	0.230	0.228
Glutamine amide nitrogen								
Dark	0.0803	0.114	0.117	0.126	0.133	0.128	0.144	0.148
Light	0.0803	0.116	0.148	0.152	0.139	0.140	0.148	0.148
Asparagine amide nitrogen								
Dark	0.0766	0.0553	0.0446	0.0487	0.0397	0.0662	0.0744	0.0825
Light	0.0766	0.0690	0.0451	0.0555	0.0499	0.0646	0.0817	0.0795
Water soluble nitrogen								
Dark	1.96	1.94	1.87	1.93			2.02	
Light	1.96	1.96	2.05	2.03			2.06	
Soluble amino nitrogen								
Dark	0.199	0.238	0.242	0.262			0.409	
Light	0.199	0.220	0.239	0.267			0.404	
Nitrate nitrogen								
Dark	1.07	1.07	1.08	1.13	1.04	1.15	1.04	1.04
Light	1.07	1.17	1.12	1.11	1.05	1.02	1.09	1.01
Protein nitrogen								
Dark	0.640	0.749	0.688	0.638	0.633	0.617	0.559	0.554
Light	0.640	0.710	0.614	0.600	0.620	0.605	0.600	0.579
Total nitrogen								
Dark	2.50	2.73	2.48	2.51	2.45	2.33	2.50	2.53
Light	2.50	2.60	2.41	2.49	2.43	2.46	2.48	2.43

tissue in considerably larger amounts than we have observed in leaf tissue, but the changes that occur during culture are far less striking and are accordingly more difficult to interpret. It would appear from the present data that glutamine alone is formed in significant amounts during culture of the stalk either in light or darkness.

The apparent behavior of the asparagine is difficult to explain. Asparagine is determined by difference, and the values secured may include other substances which yield ammonia on acid hydrolysis (16); too much emphasis can not safely be placed upon the identification of this substance when indirect methods only are employed, and this is particularly important when the quantity of apparent asparagine is relatively small. If, however, the present results are actually an expression of the behavior of asparagine in tobacco stalk tissue during culture, it would appear (Figure 1) that the quantity present diminished materially in the early stages but later, as the vegetative processes that resulted in the growth of the axillary buds became established, increased again to its initial concentration.[1]

The synthesis of amides in leaf tissue could be quantitatively accounted for in terms of transformation of the protein into amino acids and the oxidative deamination of these substances. In the case of the stalk tissue, it is much more difficult to render such an accounting because of the relatively larger experimental errors involved in the determination of the small quantities present. The distribution of the nitrogen in the stalk tissue is entirely different from that of leaves. The main nitrogenous constituents of the stalks before subjection to culture are summarized in Table 2.

TABLE 2. DISTRIBUTION OF NITROGEN IN TOBACCO STALK TISSUE

(Data in grams per kilo of fresh tissue)

Total N	2.50
Soluble N	1.96
Nitrate N	1.07
Organic N	1.43
Soluble organic N	0.89
Protein N	0.64

The nitrate nitrogen was 42.8 percent of the total nitrogen and 54.6 percent of the soluble nitrogen. The protein nitrogen was 44.7 percent of the organic nitrogen of the tissue. A typical leaf sample may have 16 percent of its total nitrogen as nitrate nitrogen and 67 percent of its organic nitrogen as protein. Stalk tissue is much less rich in organic nitrogenous compounds than leaf tissue, and the extraordinarily high proportion of nitrate nitrogen that may be present is an illustration of storage. This particular sample was indeed unusually high in nitrate, but in general the proportion of nitrate in this tissue fluctuates between wide limits and is a

[1] Glutamine, when hydrolyzed at 100° in a solution buffered at pH 7.0, is quantitatively converted into ammonia and pyrrolidone carboxylic acid. The data presented in this Bulletin were obtained from estimations of the ammonia so produced. A method is now under development in this laboratory whereby the pyrrolidone carboxylic acid produced can be independently determined. The results in most cases furnish an accurate check upon the results of ammonia determination. When applied to the present series of samples, the new method confirms the old with respect to the synthesis of glutamine in tobacco stalks during culture in light, but indicates little change during culture in darkness. Inasmuch as asparagine is calculated by difference, the new method therefore suggests that much of the increase in amide nitrogen during culture in darkness is due to asparagine formation, and accordingly the behavior of the stalk tissue resembles that of the leaf. Pending the further development of this new method and more intensive study of the behavior of the amides in tobacco stalk tissue, it seems best to restrict the discussion to a presentation of the main features of the data. In any case the amide and protein metabolism of stalk tissue is small and is quantitatively of less importance than the organic acid and carbohydrate metabolism.

function not only of the distribution of nitrate in the soil but also of the weather conditions immediately preceding the collection. As we have previously noted (17), nitrate may accumulate rapidly in the tobacco plant immediately after rain, provided the stores in the soil are adequate.

The protein nitrogen of mature tobacco leaves is usually of the order of magnitude of 2.2 to 2.7 gm. per kilo of fresh tissue, that of the present sample of stalk tissue was 0.64 gm. But the soluble organic nitrogen of leaves (that is, soluble nitrogen exclusive of nitrate) is not materially greater than that of the stalk. A typical figure for leaf tissue is of the order of 1 gm. per kilo; the present sample of stalk tissue contained 0.89 gm. of soluble organic nitrogen per kilo. Accordingly, although leaves contain proportionately far more protein in terms of fresh weight than stalks, the concentration of soluble organic nitrogenous substances is not greatly different in the two parts of the plant. This result might be anticipated; the soluble nitrogenous substances in general represent a mixture of the components of the cell sap and of the fluids from the circulatory systems, that is, the fluids that bathe the protoplasm or represent elaborated products undergoing transport in solution, and such a mixture might be expected to have a somewhat similar composition throughout the organism. The low proportion of protein in the stalk tissue, on the other hand, is a result of the smaller proportion of physiologically active cells present.

The metabolism of the protein in stalk tissue during culture is probably not essentially unlike that of the protein of leaves under the same conditions. The quantities involved are, however, smaller, and it is much more difficult to demonstrate the path followed than is the case with leaves. In general, however, it is clear that the protein is slowly decomposed and that soluble amino nitrogen is produced. The increase in amino nitrogen amounted to approximately 0.200 gm. in 236 hours (Table 1) and was similar both in light and in darkness. As has been pointed out, the asparagine changed very little in this period, but the glutamine amide nitrogen increased by 0.064 to 0.07 gm. in the two experiments. Glutamine yields 180 percent of its amino nitrogen as gas in the Van Slyke apparatus and, accordingly, considerably more than half of the apparent increase in amino nitrogen can be accounted for by the increase in glutamine alone. Unfortunately the determinations of protein nitrogen are too irregular to permit of accurate interpretation.[1] The standard error of the determination of the total nitrogen itself is $\pm$ 0.1 gm. in the dark experiment and $\pm$ 0.07 in the light experiment, and no greater confidence than this may be placed in the determinations of the protein nitrogen. The amount of protein decomposed was of about this order of magnitude and, accordingly, it cannot be definitely asserted that the increased quantities of soluble amino nitrogen, of glutamine, and of ammonia observed originated from protein decomposition. But from analogy with the behavior of leaves in culture, there is little doubt that this is the case.

NITRATE METABOLISM

The quantity of nitrate in the present series of samples of tobacco stalk tissue was unusually high. It amounted to somewhat more than half the soluble nitrogen, doubtless as a result of the weather conditions immediately

[1] The plot of the straight line calculated by the method of least squares which best represents the data for protein nitrogen indicates a loss of 0.148 gm. of protein nitrogen in 300 hours in the dark, and a loss of 0.069 gm. in the same period in the light.

preceding the time of sampling. Dry weather brings about an increase of nitrate in the soil and this is rendered rapidly available to the roots by a rain. The nitrate of the stalks represents the excess over the immediate demands of the organism for nitrogenous food absorbed by the roots, and the quantity in these samples gives a clear idea of the great capacity of this species to store nitrate under favorable conditions. The stalks of seedlings at the time of transplantation may contain nitrate nitrogen to the extent of nearly 90 percent of the soluble nitrogen and 66 percent of the total nitrogen (17), a condition related to the extremely rich soil employed in the seedbeds. During growth, however, the nitrate rapidly diminishes to a much lower level.

Examination of the data in Table I shows that very little if any change took place in the quantity of nitrate in the stalks during culture in water. There are slight irregularities, possibly the result of sampling errors, and a clearer idea of the behavior of the nitrate may perhaps be obtained if it is assumed that the change is truly linear with time. The best straight line calculated from the data by the method of least squares then furnishes by its slope an indication of the tendency of the data as a whole. The data for the stalks cultured in darkness give a line which intersects the axis at zero time at 1.091 gm. At 300 hours the line has dropped to 1.056 gm., and the data therefore indicate that approximately 0.035 gm. of nitrate disappeared during the interval. The data for the stalks cultured in light give a line that starts at 1.113 gm. at zero time and drops to 1.027 gm. in 300 hours, implying that 0.086 gm. of nitrate disappeared. It is possible, therefore, that a little of the store of nitrate present in the stalk entered into the metabolism during the culture period, the quantity being slightly greater in light than in darkness. The order of magnitude of the total change is, however, only a little greater than the standard error of the determinations of the nitrate in the light experiment ($\pm$ 0.054 gm.) and is less than the standard error of the determinations in the dark experiment ($\pm$ 0.042 gm.). Accordingly it would appear that the most that can be said is that a barely significant utilization of nitrate occurred in light but that no significant utilization was detected in darkness.

This conclusion conforms to what has already been pointed out in connection with the nitrogen metabolism of stalk tissue during water culture. The changes which involve proteins, amides, and amino acids are relatively small in magnitude when expressed in terms of the total mass of tissue and, although the proportion of nitrate present is as high or higher than is frequently the case in leaf tissue, this substance shares to only an insignificant extent in the somewhat sluggish metabolism that obtains under the conditions studied. The function of nitrate as a storage substance for nitrogen, and the function of the stalk as a storehouse for this nitrogen are clearly manifest. Nitrate is undoubtedly a highly reactive metabolite under conditions where growth of new tissue is a predominant factor, but it is obviously little concerned in the changes that occur in stalks during the conditions of starvation involved in water culture. In fact, it is by no means improbable that the small quantity of nitrate that apparently was utilized was really involved in the chemical reactions attendant upon the production of small sprouts from the axillary buds towards the end of the period of culture.

ORGANIC ACID METABOLISM

The total organic acids of the tobacco stalks (Table 3, Figure 2) apparently increased promptly both in light and in darkness by approximately 11 percent of the amount originally present, and subsequently diminished throughout the culture period of 332 hours to approximately the amount present at the start. In the light, the change in the quantity of acid was slow; in the dark, it was complete in about 100 hours. The changes in general are small and border on the limits of accuracy to be expected of such measurements, and there is little distinction to be drawn between the behavior in light and in darkness. The changes in terms of total mass of substance metabolized are insignificant and indicate that the organic acids do not take any important part in the respiration of the tissues, resembling in this the behavior of the organic acids of the leaf.

The oxalic acid of the stalks, like that of the leaves, underwent no material change. Citric acid increased promptly during culture in the dark by about 50 percent of the amount originally present and remained substantially constant at this higher level throughout the period studied. In light, on the other hand, save for an apparent small increase at the start, it diminished by about 15 percent of the amount originally present and, after 70 hours, remained constant at this lower level. The quantity present initially in the stalk tissue is, however, very small, and the change in relation to the total quantity of organic acids present is not important. Nevertheless, the marked proportional increase during culture in the dark resembles that observed in leaf tissue and was even more prompt. It would appear that citric acid, although present in amounts much smaller than either oxalic or malic acid, is definitely involved in the chemical changes that occur.

TABLE 3. THE ORGANIC ACIDS OF TOBACCO STALKS

(Data are expressed in milliequivalents per kilo of original fresh weight of each sample)

Hours	0	24	48	72	95	139	236	332
Total organic acids								
Dark	108	121	116	118	109	113	108	111
Light	108	120	123	116	115	117		113
Oxalic acid								
Dark	15.1	18.4	17.2	15.8	15.7	16.7	16.5	16.5
Light	15.1	16.9	16.7	17.7	15.5	17.2		17.0
Citric acid								
Dark	3.2	5.2	5.0	5.0	4.8	4.7	4.7	4.9
Light	3.2	3.7	3.1	2.7	2.6	2.8	2.7	2.9
Malic acid								
Dark	30.9	32.5	31.5	32.1	42.2	41.5	43.2	40.9
Light	30.9	40.1	38.3	40.6	35.2	41.3	42.9	39.4
Unknown acids								
Dark	58.8	65.3	62.1	65.1	46.0	50.3	43.3	48.1
Light	58.8	59.3	65.2	55.3	62.0	55.6		53.4

The behavior of the malic acid of the stalk tissue during culture is distinctly different from that of leaves. Both in light and in darkness, there was an increase of approximately 11 milliequivalents, or more than 30 percent of that present at the start; the increase was prompt in the light but was delayed for some 70 hours in darkness. Evidence was presented in connection with the studies of the organic acids of leaves that indicated that the citric acid newly formed during culture in the dark was derived from chemical transformation of malic acid. This reaction, involving a marked loss of malic acid, if it occurred at all in the stalk tissue, was completely masked by the much larger quantity of malic acid produced during culture both in light and in darkness. In the stalk, the increase in citric acid in the dark, although relatively large, is absolutely very small, and, if it is indeed derived from malic acid, could be accounted for by the conversion of only about 2.5 milliequivalents of this substance.

The increase in total organic acids in the light can be adequately accounted for by the contemporary increase in malic acid; the increase in malic acid in the dark, which occurred after the lapse of 72 hours, was, however, accompanied by a decrease in total organic acidity of approximately an equal order of magnitude. As a result, the calculated quantity of unknown organic acids diminished sharply at this point. That this apparent change is not entirely the result of chance variation is indicated by the subsequent values for the unknown organic acids. After the lapse of 95 hours, these remained constant at the low level then reached.

Interpretation of these results is difficult, and tentative suggestions only can be made at the present time. Inasmuch as the total organic acidity increased, it would appear that the newly formed malic acid in the stalks cultured in light arose from the oxidation of non-acidic substances, probably carbohydrates. A marked decrease in the carbohydrate content occurred during the same interval and a correlation between the two changes is highly probable on purely chemical grounds. This, indeed, is the first suggestion we have obtained that organic acids may in certain cases arise *de novo* during water culture.

The increase in malic acid that occurred between 72 and 95 hours during culture in the dark was associated with an apparently significant loss of unknown organic acids. During the same period the carbohydrates changed but little as they had already diminished to a low level. It is possible, therefore, that the delayed increase of malic acid in darkness is to be attributed rather to a conversion of a part of the unknown organic acids into malic acid than to a conversion of the carbohydrates.

In general, and with the conspicuous exception of the behavior of the malic acid, the metabolism of the organic acids of the stalk resembles that of the leaves, but the conversions involved are quantitatively and proportionally to the total organic solids of far smaller significance. The fundamental difference with respect to the metabolism of malic acid is that, whereas the malic acid of the leaves remains substantially constant during culture in light, that of the stalk increases. Translocation from other parts of the plant can play no part in this change, and conversion of some other constituent into malic acid remains the only feasible explanation. Inasmuch as the stalk contains a considerable store of carbohydrates which undergo rapid change during culture, the origin of the malic acid newly formed during culture in light may be fairly simply accounted for; the origin of that formed in the dark is not clear.

CARBOHYDRATE METABOLISM

The stalk is usually regarded as a storage organ and it is presumably here that the soluble carbohydrates not immediately drawn upon for purposes of growth accumulate. The form in which the carbohydrate is stored differs widely with different species. Shabanov (13) has recently drawn attention to the importance of the pith of tobacco stalks as the repository of monosaccharides. The soy bean (5), under light conditions that promote reproductive development, stores starch in the stalk in large amounts; the sugar cane normally stores sucrose, and other species employ still other substances.

The data on the soluble carbohydrate content of the present samples of tobacco stalk tissue include determinations of total reducing power, the increase in reducing power brought about by mild hydrolysis with acid and presumably due to the presence of sucrose, and the reducing power after treatment of the hydrolyzed solution with a suspension of yeast. These determinations were carried out on solutions prepared by extracting samples of the dry powdered tissue with 75 percent alcohol and subsequently treating an aqueous solution of the material thus obtained successively with Lloyd's reagent and with permutit. The sugar titrations were made by the method of Somogyi. The results are calculated in terms of glucose and sucrose, although it is obvious that the reducing substance that remains after treatment with yeast is not glucose.

The total water-soluble carbohydrate of the stalk tissue (Table 4, Figure 3) is initially much higher than that in leaf tissue. Whereas, in leaves from field crops, total soluble carbohydrate values of from 2.5 to 6.8 gm. per kilo of fresh tissue have been observed (19), that of the present sample of stalk tissue was 11.9 gm. per kilo, or slightly more than 12 percent of the organic solids. Soluble carbohydrates are highly reactive metabolites and the actual figure observed in any one sample at any one time may be far from representative. That this is not the case in the present instance, however, may be inferred from data obtained during an earlier study of the rate of growth of the tobacco plant (17). Stalks collected in 1933 at 47 days from setting in the field had 11.9 gm., at 54 days 11.8 and at 61 days 13.9 gm., of soluble carbohydrate per kilo of fresh weight.

On being subjected to culture in water, the soluble carbohydrate of the stalks rapidly diminished in quantity both in light and in darkness, but the rate of loss was greater in darkness than in light. It seems probable that the smaller rate of disappearance of carbohydrate in light is due to photosynthesis in the green parts of the stalk. The difference between the quantities of soluble carbohydrate in each individual sample cultured in light, and the corresponding sample in darkness is nearly constant throughout and is very close to 2 gm. If photosynthesis continued during the period of culture in light, the soluble carbohydrate would be expected to increase. This is what occurs in the leaf, the level finally attained (19) in one experiment being 18.8 gm. per kilo of fresh weight (190 hours) or 17 percent of the organic solids, a strikingly high value, and one much greater than we have found in tobacco stalks under any conditions.

The rapid loss of soluble carbohydrate from the stalk tissue in light is thus in complete contrast to the behavior of the leaf; respiration evidently

dominates the situation in the former case. The only direct evidence for photosynthesis that has been secured is the somewhat slower rate of loss in light than in darkness.

TABLE 4. THE CARBOHYDRATES OF TOBACCO STALKS

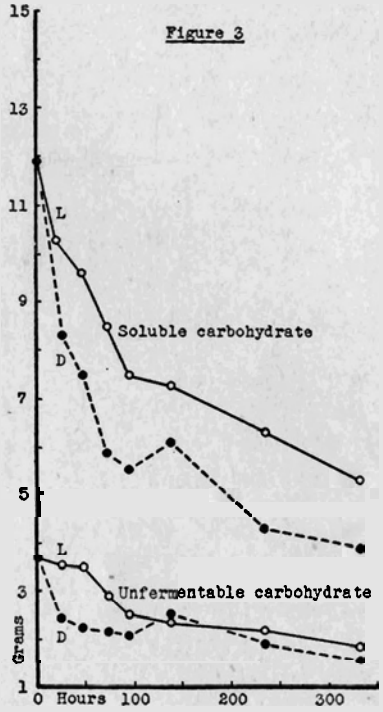
(Data not otherwise designated are expressed in grams per kilo of original fresh weight of each sample)

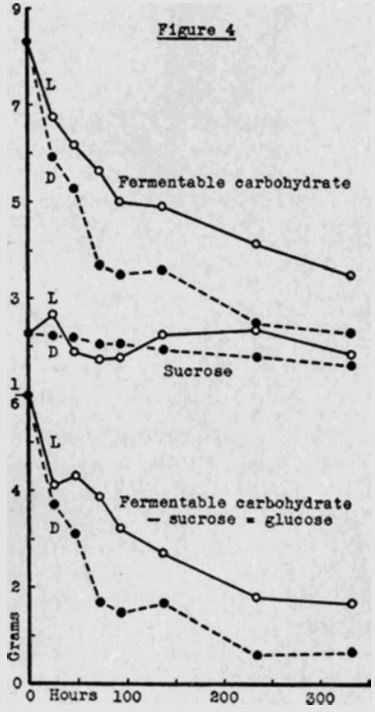
Hours	0	24	48	72	95	139	236	332
Total water-soluble carbohydrate (as glucose)								
Dark	11.9	8.33	7.48	5.85	5.56	6.11	4.31	3.88
Light	11.9	10.3	9.62	8.54	7.52	7.28	6.33	5.32
Fermentable carbohydrate (as glucose)								
Dark	8.30	5.93	5.25	3.69	3.49	3.59	2.41	2.30
Light	8.30	6.76	6.18	5.62	4.99	4.93	4.14	3.50
Unfermentable carbohydrate (as glucose)								
Dark	3.65	2.40	2.23	2.16	2.06	2.53	1.90	1.58
Light	3.65	3.50	3.45	2.92	2.53	2.35	2.18	1.82
Proportion of total carbohydrate fermentable (percent)								
Dark	69.4	71.2	70.3	63.0	62.9	58.6	55.9	59.4
Light	69.4	65.9	64.2	65.8	66.4	67.8	65.5	65.8
Sucrose								
Dark	2.25	2.21	2.18	2.03	2.05	1.95	1.80	1.62
Light	2.25	2.66	1.89	1.76	1.79	2.23	2.36	1.83
Glucose (fermentable carbohydrate—sucrose)								
Dark	6.05	3.72	3.07	1.66	1.44	1.64	0.61	0.68
Light	6.05	4.10	4.29	3.86	3.20	2.70	1.78	1.67

The relationships of these processes can be better understood if the diverse nature and functions of the different parts of the stalk tissue are considered. The chlorophyll-bearing cells of the cortex alone can be regarded as the site of photosynthesis, and the relative proportion of this type of tissue in the whole mass of the stalk is small. The analytical data are calculated in terms of the whole mass and the effect of photosynthesis in a small part is accordingly minimized. It would appear that the whole of the soluble carbohydrate of the tissue is involved in the oxidative reactions that occur during culture, and this effect far overbalances the increase that can take place only in the cortical cells. These relations can be clarified only when analyses of the different tissues of the stalk have been carried out, but it would be surprising if the chemical behavior of the green parts of the cortex were fundamentally different from that already observed in leaves, and still more surprising if the chemical functions of the woody cylinder and pith resembled those of the cortex very closely.

It is of interest to see if the changes in the carbohydrates of the stalk tissue can be correlated in any way with the changes in organic solids. It may be assumed that loss of organic solids during culture is an effect of respiration and, indeed, that it provides an approximate measure of the total amount of respiration during the period studied. It is usually assumed that the carbohydrates furnish much of the material oxidized during respiration, and consequently one might expect to find the fluctuations in carbo-

hydrates and in organic solids in darkness to be similar in direction and extent. The difficulty, however, is that the estimations of the organic solids contain the experimental errors of the moisture and ash determinations as well as the error in the assumption of equal initial composition of the samples, and the data accordingly are less satisfactory than the more direct and accurate determination of the reducing power. However, the stalk tissue cultured in light lost 6.6 gm. of total soluble carbohydrate, while that in darkness lost 8.0 gm. over the entire period. The curves that represent the rate of loss are very nearly logarithmic for the first 100 hours; about half the total loss occurred within 72 hours. If the changes are to be interpreted as the effect of oxidation of the carbohydrates to volatile end products, this oxidation proceeded with maximum velocity at the start and





resulted in the loss of 6 gm. of carbohydrate in 72 hours in the dark and of 3.3 gm. in the light—quantities that should be large enough to be detected in the determinations of organic solids. But the total organic solids behaved in a markedly different manner. The value at zero time is derived from two independent samples, the two values being 92.0 and 95.3 gm. The total organic solids apparently *increased* to 102 and 105 gm. in 24 hours in darkness and in light respectively and thereafter diminished somewhat slowly.

Further investigation alone will determine to what extent the apparent sudden increase in organic solids and subsequent slow diminution represent the events that actually occurred. The smoothness of many of the curves for the analytical data derived from this set of samples indicates that the

sampling errors were not excessive but, with respect to the organic solids, it seems best to make use of an expression which reveals the tendency of the data as a whole rather than to depend on the individual determinations. The simplest method is that involved in the assumption that the changes in organic solids are a continuous linear function. The calculation by the method of least squares of the straight line that best expresses the data will then reveal the manner in which the organic solids change, due emphasis being given to each observation.

The line calculated from the determinations of organic solids in the samples cultured in the dark starts at approximately 97.8 gm. and slopes downward to 90.6 gm. at 300 hours and indicates a loss of about 7 gm. of organic solids. This is appreciably greater than the standard error of the determinations. If the deviations of the individual determinations from the best straight line are calculated, the mean deviation and its standard error are 1.97 ± 2.8. Accordingly a total change of over 7 gm. has significance.

The line for the samples cultured in the light starts at 99 gm. at zero time, and drops to 97.2 gm. at 300 hours. The apparent change of 1.8 gm. is within the error of the determinations.

The apparent loss of organic solids in darkness (7 gm.) corresponds sufficiently closely with the loss of soluble carbohydrates (8 gm.) to suggest that these substances were largely involved in the respiration, and, in fact, indicates that none of the other components of the tissues were to any extent concerned. With respect to the behavior of the organic solids and the carbohydrates in light, however, we are faced with a dilemma. The soluble carbohydrates diminished by about 6.6 gm. and the data fall upon a relatively smooth curve with a slope that leaves little doubt of the order of magnitude of the change. This occurred in spite of an exposure of the tissue to light under conditions which, with leaves, gave rise to photosynthesis and rapid storage of soluble carbohydrates. The organic solids in the stalks on the other hand did not change detectably, and it seems obvious that losses due to respiration were compensated by assimilation of carbon. The problem is how to account for the fact that the soluble carbohydrates actually decreased during a period when photosynthesis was apparently active.

A purely tentative explanation may be given in terms of analogies with the behavior of leaf tissue during culture in light. It has been found (19) that the increase in organic solids may amount to as much as 35 gm. per kilo during 235 hours of culture in water, but of this quantity only about 16 gm. at most could be accounted for as soluble reducing carbohydrates. Starch synthesis was negligible, being of the order of 1 gm.[1] Accordingly the products of photosynthesis, which are generally assumed to be primarily soluble carbohydrates, must have been to a considerable extent metabolized into substances no longer detectable by sugar titration methods. In the leaves, with a large area of tissue specially adapted to photosynthetic reactions, the products of assimilation of carbon increased more rapidly than they could be disposed of by respiration. Even the true sugars accumulated in substantial amounts in spite of the probable utilization of a portion for this purpose.

[1] Mention of the method (7) employed in this laboratory for the determination of starch was inadvertently overlooked in Bulletin 399.

In the stalks, on the other hand, with a limited proportion of the tissue adapted to photosynthesis, the demands of respiration removed not only the soluble carbohydrates provided by photosynthesis, but made severe inroads upon the store already present at the start of the culture. Only that part of the newly formed carbohydrate that was utilized for the formation of non-reducing organic substances escaped the oxidative processes, and it turns out that this was sufficient to compensate approximately for the substances used up in respiration during the period of culture studied. Thus the organic solids as a whole remained very nearly unchanged, although the soluble reducing carbohydrates diminished.

This explanation is based upon the assumptions that the first recognizable and stable product of photosynthesis is a sugar, and that the chief substances oxidized during respiration are sugars, the entire products of this oxidation being volatile. These assumptions conform with present day views of the two processes involved. But the possibility must not be overlooked that photosynthesis may give rise to products other than reducing carbohydrates. The question has been discussed by Spoehr (14) who quotes results of Saposchnikoff (8) and of Kraschenninnikoff (3) that suggest that a very appreciable fraction of the carbon dioxide assimilated by leaves may be employed for the synthesis of organic substances other than carbohydrates. In particular, the latter investigator found that the amount of carbohydrates formed in tobacco leaves under his experimental conditions was only 57 percent of the increase in dry weight. This result is remarkably close to our own results with tobacco leaves in water culture.

One further point should be mentioned. In the respiration of leaves during culture in the dark, the loss of organic solids involved a very material fraction of the total organic solids of the tissue, and of this only one-half to one-third or even less could be accounted for in terms of the loss of carbohydrates. Respiration in the leaves, therefore, drew heavily upon substances other than carbohydrates. In the stalk, on the other hand, nearly the whole of the loss of organic solids through respiration could be accounted for by the loss of carbohydrates. This is an interesting fundamental difference in the behavior of the two tissues and may be considered in relationship to the wide difference in respiration rate expressed in terms of the surface area through which the gaseous products of respiration must escape. The data for soluble carbohydrates are the most satisfactory to use for this comparison as it seems probable that this part of the carbohydrate is completely oxidized to carbon dioxide and water. In Table 5 are shown the data for the quantities of soluble carbohydrate that disappeared in a period of 95 hours in darkness, expressed in grams per kilo for both leaf and stalk tissue. The carbon dioxide equivalent, calculated on the assumption that the substance oxidized is glucose, is also shown. The figure for the leaf tissue is the largest we have observed, values of about half this magnitude being more common. The value for stalk tissue is equivalent to 0.098 gm. of carbon dioxide per kilo of fresh weight per hour, and is of about the order of magnitude observed by Drain (2) for the carbon dioxide produced by apples in storage at a temperature similar to that of our culture experiment. The total surface area of our samples of tobacco stalks was approximately 0.5 sq. m. and, accordingly, the rate of respiration was approximately 0.19 gm. per sq. m. per hour. This is a rate of respiration comparable in order of magnitude with that observed by Spoehr and McGee (15) for helianthus leaves freshly picked from a mature, vigorous plant.

The rate of respiration of the leaf tissue is, however, of a far smaller order of magnitude than that of the stalk when the data are expressed in terms of surface area. Our 1-kilo samples of tobacco leaves have an area of approximately 3 sq. m. (18) and the total surface is 6 sq. m. Accordingly the rate of respiration is of the order of 0.012 gm. of carbon dioxide per sq. m. per hour.

TABLE 5. LOSSES PRESUMABLY DUE TO RESPIRATION IN 95 HOURS OF CULTURE IN THE DARK

(Data in grams per kilo of original fresh weight)

	Leaves[1] gm.	Stalks gm.
Soluble carbohydrate	4.49	6.34
Organic solids	12.7	
CO_2 equivalent	6.6	9.3
CO_2 per sq. m.	1.1	18.6

The actual rate of evolution of carbon dioxide from the leaves must have been two to three times as great as this since the carbohydrate that disappeared is considerably less than half of the organic solids lost from the leaves during the culture period, but even so, the rate of respiration of these leaves was very small. Spoehr and McGee give data on an excised bean leaf the rate of respiration of which varied from 0.063 to 0.022 gm. per sq. m. per hour, this being the smallest respiration rate they mention; helianthus leaves respired much more copiously.

The point in connection with the present investigation is that the leaves of tobacco, in spite of their great area and special adaptation for gaseous exchange, respired at a rate far less than the stalks. The stalks apparently evolved carbon dioxide at a rate commensurable with that observed in other massive tissues such as fruits or tubers; the leaves respired much less rapidly. The losses of carbohydrate or of organic solids in terms of total mass of tissue oxidized were comparable, but in terms of surface area they were not.

It seems clear, therefore, that internal factors in the two tissues played a dominant rôle in controlling respiration, and that the surface exposed to gaseous exchange is of minor import. Whether it is a matter of the demand for energy in terms of area, or whether chemical factors such as distribution of active sugar, enzymes, or co-enzymes in the cells, or availability of oxygen are significant, cannot be decided from the present data.

The soluble reducing carbohydrate, as determined by the chemical methods employed in this laboratory, is a complex mixture. Three clearly differentiated factors can be distinguished: fermentable sugar determined after inversion, sucrose, and unfermentable reducing carbohydrate the nature of which is unknown although the data are calculated in terms of glucose. The loss of soluble carbohydrate during culture of the tobacco stalks fell more heavily upon the fermentable portion than upon the unfermentable (Figure 3), and examination of the data for sucrose (Figure 4) shows that this sugar underwent very little change either in light or dark-

[1] These data are calculated from results shown in full in Bulletin 399, Table 20, p. 832, sample DG

ness. If the sucrose determinations are individually subtracted from the fermentable sugar determinations, a measure is obtained of the quantity of monosaccharide (probably glucose) in the tissue at each stage. More accurately, the sucrose values should be corrected by a factor of 1.05 before subtraction since sucrose gives 105 percent of reducing sugar after inversion, but this correction is of minor importance in the present connection. The curves plotted in Figure 4 show the values obtained. The constancy of the sucrose is of special interest. This sugar apparently plays a small part in the carbohydrate metabolism of the stalk during culture in water and, even toward the end of the period, when the stalks were throwing out buds and, in the light, had developed a small area of green leaf, there is no significant evidence of an effect on the sucrose. The glucose, however, underwent rapid oxidation and diminished to a very low level in the dark within 95 hours. In the light, the rate of loss was less, and attention has been drawn already to this slower rate as an evidence of photosynthesis in the cortical cells.

The unfermentable carbohydrate values plotted in Figure 3 reveal two important points. There is evidence that this material underwent oxidation during culture both in light and in darkness. The behavior in darkness resembles that of the unfermentable carbohydrate in leaves (19, p. 820). It is significant that the loss was prompt and that, after 24 hours, the quantity present changed but little until the extreme end of the period studied. It would appear that this factor is complex, a part being readily oxidized in darkness, the rest being resistant to further change.

The behavior in light is somewhat different. No substantial part of the unfermentable carbohydrate was oxidized until 48 hours had elapsed, but soon thereafter it dropped to a level indistinguishable from that promptly reached in the dark culture. Whether or not the delay in oxidation is an effect of synthesis of substances belonging to this group during the early stage of culture in the light cannot be definitely determined. Synthesis of such substances undoubtedly occurs in leaf tissue during culture in light and may perhaps be expected, though to a smaller extent, in the stalk. The total quantity of unfermentable carbohydrate present in this sample of stalk tissue at the start of the culture was higher than that characteristic of leaf tissue (19, p. 832; 0.56, 1.76, and 0.48 gm. per kilo), but corresponds to the quantities observed in other samples of stalk tissue of similar age (17, calculated from data of Tables 2 and 5; 47 days, 3.38 gm.; 54 days, 3.00 gm.; 61 days, 3.27 gm. per kilo). It is evident that a part of the substances in this group is involved in the carbohydrate metabolism of the stalk during culture.

This conclusion can be presented from another point of view if the relative proportions of fermentable and unfermentable carbohydrate are considered. At the start, 69 percent of the soluble carbohydrate was present as fermentable sugar. In the dark, after 48 hours, this proportion dropped steadily to about 58 percent, indicating that a somewhat larger part of the fermentable sugar was oxidized than of the unfermentable. In the light, the proportion of fermentable sugar remained nearly constant, the lowest value of 64 percent being reached in 48 hours. Obviously the oxidation of the two kinds of carbohydrate proceeded at essentially similar rates in terms of the quantity present. This can be even more clearly appreciated from the following figures. In the dark, nearly 67 percent of the

total quantity of fermentable sugar was oxidized and 57 percent of the unfermentable carbohydrate disappeared. In the light, the corresponding figures were respectively 57 and 50 percent.

SUMMARY

A series of samples of stalks of tobacco plants, denuded of all leaf tissue, was subjected to culture in distilled water either in continuous light or in complete darkness. Analysis of the samples at intervals up to 332 hours permitted many of the chemical changes that occur under these conditions to be followed. Towards the end of the period of study, both in light and in darkness, shoots appeared at many of the upper nodes of the stalks, those in light developing into small leaves, those in darkness being elongated, colorless and stalk-like with rudimentary leaflets.

The water content of the stalks changed very little, there being a prompt loss of about 5 percent in light and a barely significant gain in darkness. At the end, both series had lost about 10 percent of the water originally present.

The organic solids diminished appreciably in darkness but there was no significant change in light. The loss in darkness was doubtless due to respiration, the apparent constancy in light indicates that photosynthesis occurred to an extent sufficient to compensate for the losses due to respiration.

There was a prompt increase in the amide nitrogen and in the soluble amino nitrogen both in light and in darkness, and a slow but significant increase in ammonia. Evidence was secured of synthesis of glutamine in light and it is probable that the metabolism of glutamine and asparagine is not essentially different from that of tobacco leaves; quantitatively in relationship to the total mass of tissue it is of minor importance.

The total organic acids increased slightly early in the period of culture and thereafter slowly diminished to the quantity present at the start. Oxalic acid did not change in amount either in light or in darkness. Citric acid is present only in small amounts, but promptly increased in the dark by about 50 percent of the amount originally present and subsequently remained unchanged. In the light, on the other hand it diminished slightly. Malic acid is present in considerable amounts in tobacco stalk tissue and increased both in darkness and in light during culture, though more slowly in darkness than in light. Its behavior in darkness is entirely different from the behavior of the malic acid of tobacco leaves under similar conditions.

The carbohydrate metabolism of the stalk tissue of the tobacco plant presents many contrasts to that of the leaf. In the leaf, the carbohydrates rapidly increased during culture in the light until over 16 percent of the organic solids present consisted of soluble reducing substances. In the dark, the carbohydrates rapidly dropped to a very low level. In the stalk, both in light and in darkness, the soluble carbohydrate diminished with considerable rapidity. The initial value was far higher than that found in leaves under ordinary conditions, the final value reached was comparable

to values observed in normal leaves. The only apparent effect of illumination was to delay the disappearance of the carbohydrate, an effect that can be most easily interpreted to indicate a small amount of compensatory photosynthesis. The oxidation processes affected both fermentable and unfermentable sugar in a substantially similar manner but did not appear to affect the sucrose to any significant extent.

BIBLIOGRAPHY

1. Borodin, J., Bot. Zeitung, **36**: 802. 1878.
2. Drain, B. D., Bot. Gaz., **82**: 183. 1926.
3. Krascheninnikoff, Accumulation of Solar Energy in Plants (Russian). (Moscow) 1901.
4. Müller, C. O., Landw. Vers. Sta., **33**: 311. 1887.
5. Murneek, A. E., Missouri Agr. Expt. Sta. Research Bul. **268**. 1937.
6. Prjanischnikow, D., Biochem. Ztschr., **150**: 407. 1924.
7. Pucher, G. W., and Vickery, H. B., Indus. and Engin. Chem., Anal. Ed., **8**: 92. 1936.
8. Saposchnikoff, W., Ber. deut. bot. Gesell., **8**: 233. 1890.
9. Schulze, E., Landw. Jahrb., **9**: 689. 1880.
10. Schulze, E., Landw. Jahrb., **17**: 683. 1888.
11. Schulze, E., Landw. Jahrb., **35**: 621. 1906.
12. Schulze, E., and Bosshard, E., Ztschr. physiol. Chem., **9**: 420. 1885.
13. Shabanov, I., Proc. in Tobacco Chemistry, Krasnodar (Russian), **7**: 86. 1937.
14. Spoehr, H. A., Photosynthesis, 220. (New York) 1926.
15. Spoehr, H. A., and McGee, J. M., Studies in Plant Respiration and Photosynthesis. Carnegie Inst. Wash., Pub. 325. 1923.
16. Vickery, H. B., and Pucher, G. W., Biochem. Ztschr., **293**: 427. 1937.
17. Vickery, H. B., Pucher, G. W., Leavenworth, C. S., and Wakeman, A. J., Conn. Agr. Expt. Sta. Bul. **374**. 1935.
18. Vickery, H. B., Pucher, G. W., Wakeman, A. J., and Leavenworth, C. S., Chemical Investigations of the Tobacco Plant. Carnegie Inst. Wash., Pub. **445**. 1933.
19. Vickery, H. B., Pucher, G. W., Wakeman, A. J., and Leavenworth, C. S., Conn. Agr. Expt. Sta. Bul. **399**. 1937.
20. Zaleski, W., Ber. deut. bot. Gesell., **27**: 56. 1909