Transformations of Labeled Formic Acid, Formaldehyde, Methanol, & CO_2 Absorbed by Bean & Barley Leaves From Air[1]

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The lower layers of the atmosphere are known to contain some volatile organic substances, formed as a result of the activity of various organisms, industrial activity of man, and geochemical processes.

Though usually the amount of these substances in the air is insignificant, their presence is, nevertheless, a practically constant factor affecting in some manner, directly or indirectly, vital activities of organisms (19, 23, 11).

According to the data of Kholodny (11), volatile organic substances are strongly absorbed by the soil and affect the microflora and root systems of plants. However, the uptake of such substances by the aerial parts of plants was hardly taken into consideration at all, although it seems that volatile organic substances, present in the atmosphere, should affect first of all the leaves, as the organs specialized in aerial nutrition. It could be expected that in these organs which perform most intensively various synthetic processes, the greatest possibilities should exist for the conversion of various organic substances present in the air.

It is also evident that not only the roots, but also the leaves have preserved some features of the older, heterotrophic metabolism, and are capable of feeding on various organic substances and combining this nutrition with photoassimilation.

In the work presented here, a comparative study was carried out of the uptake from the atmosphere of highly diluted C^{14} labelled monocarbon compounds having a different degree of reduction, such as methanol, formaldehyde, formic acid, and carbon dioxide. The transformation of these substances can serve as a model for the assimilation of many other substances; it is also of great interest because of the probable participation of monocarbon fragments in various transfer reactions. As shown by the development of biochemistry in recent years, reactions of this kind are widely distributed and play an important part in the metabolism of plant and animal organisms.

The study of the paths of conversion of the abovementioned monocarbon compounds is of peculiar interest also because all of them, and formic acid in particular, are biochemically active substances known to occur in plant tissues.

Thus, formic acid is accumulated in raspberry (15), in the leaves of stinging-nettle (14), and the fruit and needles of the fir tree (13). There are data reporting that traces of formaldehyde occur in leaves (12). The presence of methanol was observed in fruits (25) and leaves (10).

The metabolism of these compounds in plant tissues has drawn the attention of many investigators. Already the first experiments showed that carbon from the labeled formic acid enters malic, succinic, oxalic, citric, glycolic, and other acids 40 minutes after the infiltration of this acid into tobacco leaves (27). When sucking $HC^{14}OOH$ through petioles into the leaves of tobacco (16) and barley (24, 14), an increased radioactivity, along with that of the usual photosynthetic products, was found in serine (16), glyceric acid, choline, and phosphorylcholine (24, 14).

Formic acid also participates in the methylation of several alkaloids (3, 1).

As to formaldehyde, there are data showing that it is rapidly consumed in leaves (22, 14). When adding labeled formaldehyde to beet leaf homogenates, 1-phosphoerythrulose-4-C^{14} is formed (20).

Methanol introduced into the pulp is also able to undergo conversion (21).

However, none among the considerable number of papers devoted to the conversion of these substances in plant tissues permits one to follow the whole path of their conversions in leaves under normal conditions, in particular, the early parts of this path.

Materials & Methods

The leaves of bean (*Phaseolus vulgaris,* var. Sax) and of barley (*Hordeum vulgare,* var. Krasnoyarsky) which differ in metabolic characteristics, were used for investigation.

An aliquot of fresh leaves was placed into a special network holder and introduced into a transparent chamber floating over mercury (7); this chamber was filled with air and dilute vapor of one of the labeled compounds—formic acid, formaldehyde, methanol, or $C^{14}O_2$ (fig 1).

The concentration of the labeled compounds in the chamber was determined by direct measurement of radioactivity of gaseous samples, and by calcula-

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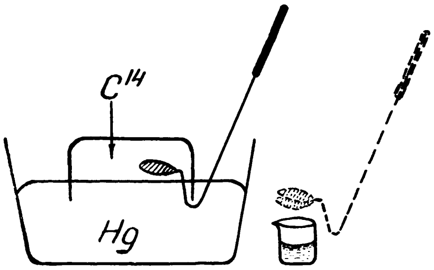


Fig. 1. Scheme of apparatus for exposure of leaves to light in presence of C^{14}-labeled volatile substances.

tion from the specific activity of the initial preparations. A known amount of the gas was introduced into a closed vessel for complete absorption of radioactive vapor by an appropriate absorbent. Formic acid was absorbed by $Ca(OH)_2$ solution; formaldehyde, by dimedone solution; methanol, by metallic sodium. The radioactivity of the suspensions of calcium formate, dimedone formaldehyde, and sodium alcoholate (as well as of other samples) was measured by an end-window counter tube, after evaporating the solvent on plates, taking into account the self absorption in each substance.

Table I

Change in Radioactivity of Sodium Methylalcoholate Samples*

Conditions	Time intervals between countings in days			
	0	3	6	9
Samples kept dry	100	98.9	97.9	96.8
Samples 3 times moistened with methanol	100	97.8	95.2	92.0
Samples 3 times moistened with water	100	88.5	76.9	65.5

* In percentage of initial radioactivity.

Since the radioactivity measurement of sodium methylalcoholate on plates, unlike that of other samples, could be strongly distorted by its hydrolysis under the action of water vapors present in the air and release of radioactive methanol, preliminary experiments had to be carried out to test the efficiency of the adopted procedure. With this aim in view, sodium methylalcoholate was suspended in methanol. Aliquots of the suspension were transferred onto discs, and after drying in a desiccator over NaOH, their radioactivity was measured by a counter. Water (0.25 ml) was then added to one part of the samples, while the same amount of methanol was added to the other part; the samples were dried and counted after 3 days. Moistening with water and methanol was repeated several times. The results of these measurements of radioactivity deposited on plates are given in table I.

Table I shows that satisfactory measurement of radioactivity of sodium alcoholate suspensions on plates is possible; even after a 9-day-long storage of samples in a desiccator their radioactivity diminished only by 3.2 %, and by some 2 to 3 % when moistened with methanol and counted within 3 days.

The main experiments on the absorption of the above-mentioned labeled volatile compounds by leaves were carried out under an illumination of 2,000 lux, and in the dark, in an atmosphere free from CO_2, and in the presence of 5 % CO_2. Air temperature was 25 to 26 C. The exposure of leaves in the cell lasted 10, 60, or 300 seconds, respectively. Special experiments carried out with intact leaves showed that such a short exposure did not affect their subsequent growth and development.

After exposure the leaves were quickly killed by immersion into 10 ml of boiling 0.5 % water solution of formic acid. The soluble material extracted twice with 10 ml of 0.5 % formic acid after boiling for 1 minute and then washed three times with the same acid at room temperature. The material was taken up, fractionated, and analyzed by radiochromatography (6, 8).

Results & Discussion

Table II shows the concentrations of the radioactive substances in the chamber and the rate of their absorption by bean and barley leaves in light. Using the same concentrations of all the tested substances in the atmosphere would have been the best way to

Table II

Comparison of Light Assimilation of Labeled Monocarbon Compounds from the Atmosphere by Leaves

Labeled compound	Conc in the chamber (vol %)	Absorption in 10 sec/10 g of leaves × mmole × 10^{-5}		Would be absorbed in 10 sec/g of bean leaves if vapor content were 0.2 % in volume for all substances	
		Beans	Barley	mmole × 10^{-5}	% to CO_2
Formic acid	0.02	3.1	2.4	31.0	63.6
Formaldehyde	1.1	16.6	20.6	3.0	6.2
Methanol	1.0	6.6	4.9	1.3	2.7
CO_2	0.2	49.1		49.1	100.0

compare their absorption, however, it was inadvisable to do so, in the first place, because the specific radioactivity of the several preparations differed greatly. Therefore, on the basis of preliminary data, we had to adjust the concentrations of the different radioactive substances in the atmosphere to reach optimal conditions for the experiment.

It can be suggested that, by analogy with CO_2 assimilation, the absorption of volatile organic compounds by the leaves will be, within certain limits (2), proportional to their concentration in the atmosphere. If we make this assumption for the low concentrations used in our experiment, and on this basis calculate the rate of absorption of each of them with the concentration of one of them chosen as standard, e.g. that of CO_2, then the relative absorbability of the substances tested should become clear. Such calculations lead to results presented in the two last columns of table II. This shows that leaves are capable of more or less rapid absorption of various organic monocarbon compounds from the atmosphere.

Comparing the absorption rates, we find that carbon dioxide is first followed by formic acid; then, lagging conspicuously behind, comes formaldehyde and, last, methanol.

These results were obtained after very short exposure. It should be noted however that, although in concentrations employed the substances did not produce conspicuous injuries affecting the growth of plants, these concentrations turned out to be too high for continuous absorption. No linear correlation of assimilation with time could be observed in prolonged exposure of leaves in the chambers; the assimilation rate was not constant. This can be seen from table III, and figure 2.

The data in table III and figure 2 show a great difference in the effect of carbon dioxide upon the assimilation rate of the monocarbon derivatives of different reduction level. The greatest inhibiting effect of CO_2 is shown by the assimilation of formic acid vapor in light followed by that of formaldehyde. In the presence of 5 % CO_2, methanol assimilation by barley leaves remains practically unaffected or even increases (in the case of bean leaves). This peculiarity of methanol assimilation is hard to explain. It should be noted that dark assimilation by leaves of all the substances tested is several times smaller than that in light; and that no inhibiting action of CO_2 is found in dark fixation of formic acid, formaldehyde, or methanol.

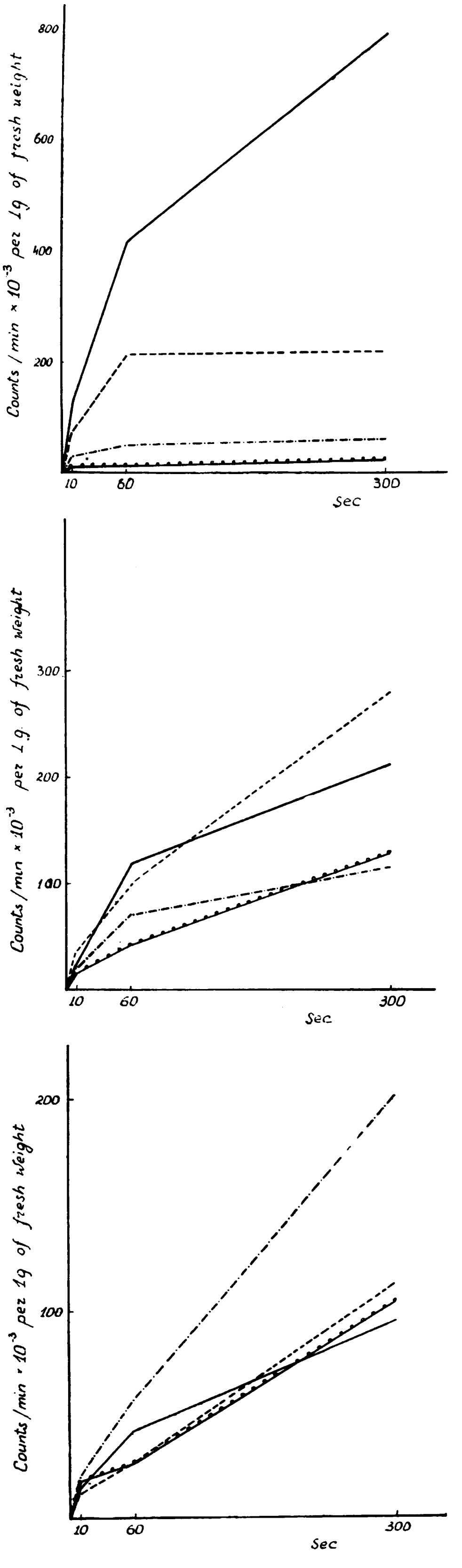
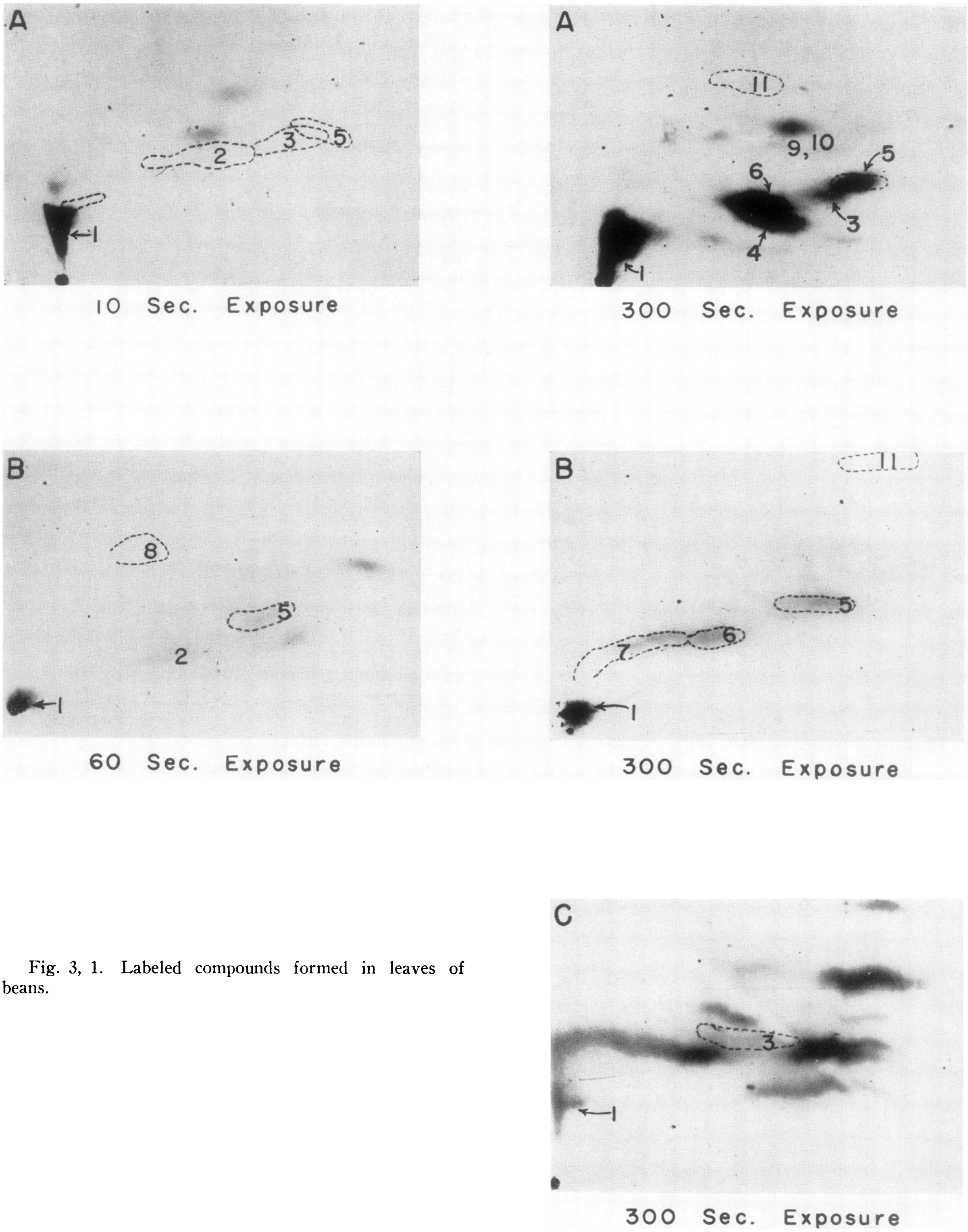


Fig. 2. Photoassimilation curves of the vapors of formic acid (A), formaldehyde (B), and methanol (C) by bean and barley leaves.

Designations: ———— beans in the absence of CO_2; —·—·—· beans in the presence of CO_2; — — — barley in the absence of CO_2; . . . . barley in the presence of CO_2.



10 Sec. Exposure

300 Sec. Exposure

60 Sec. Exposure

300 Sec. Exposure

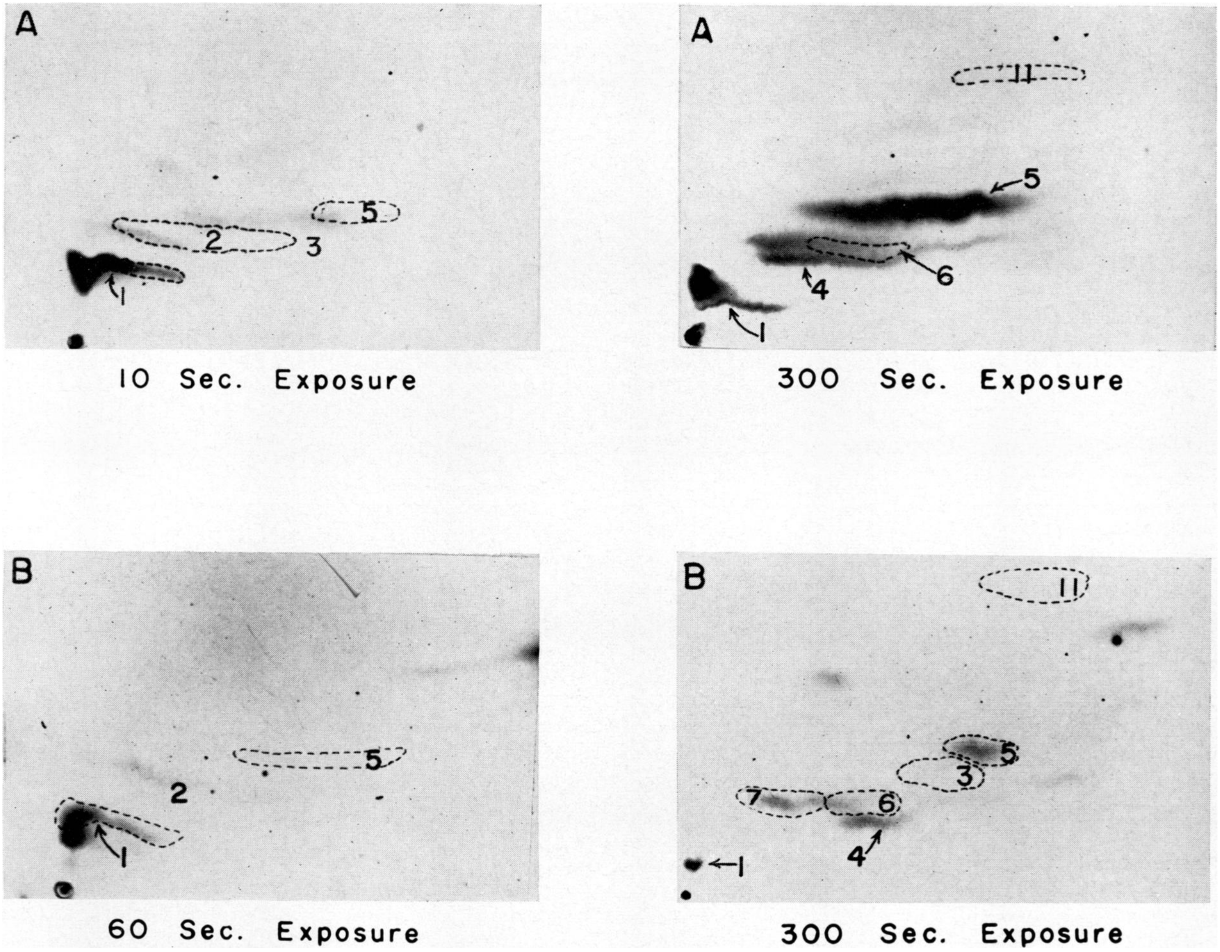
300 Sec. Exposure

Fig. 3, 1. Labeled compounds formed in leaves of beans.

Fig. 3. Labeled compounds formed in leaves of beans (fig 3, 1) and barley (fig 3, 2 *opposite page*) by photo-assimilation of vapors of C^{14}-labeled formic acid (A), formaldehyde (B), and methanol (C).

Solvents: *Horizontally, from left to right*: phenol saturated with 0.1 M formic acid; *vertically, from bottom to top*: butanol : glacial acetic acid : water (78 : 20 : 50).

Designations: 1, zone of phosphate esters and PGA; 2, glucose; 3, fructose; 4, sucrose; 5, α-alanine; 6, serine; 7, aspartic acid; 8, malic acid; 9, glyceric acid; 10, glycolic acid; 11, succinic acid.



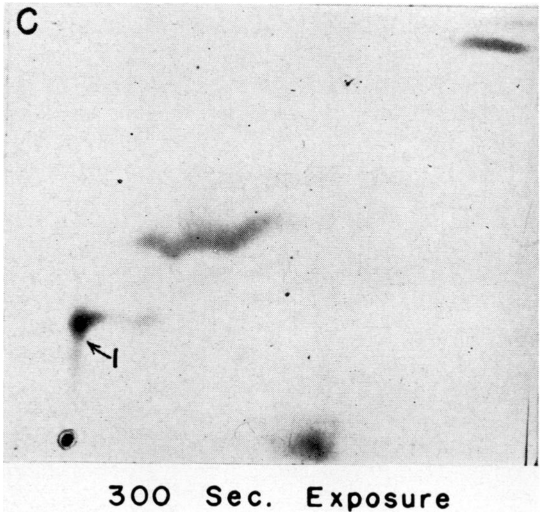
10 Sec. Exposure

300 Sec. Exposure

60 Sec. Exposure

300 Sec. Exposure

Fig. 3, 2. Labeled compounds formed in leaves of barley. (*For further explanation, see legend on opposite page.*)



300 Sec. Exposure

Table III

Assimilation of Labeled Monocarbon Organic Compounds by Leaves from the Atmosphere

Compound	Exposition conditions	Radioactivity/g of leaves (dry wt) cpm $\times 10^{-3}$			
		Beans		Barley	
		$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$
Formic acid	10 sec light	126.3	28.5	72.6	6.7
	60 sec light	416.0	45.1	210.4	9.6
	300 sec light	803.2	57.6	212.0	23.6
	300 sec darkness	106.0	48.8	17.7	16.0
Formaldehyde	10 sec light	22.7	20.4	27.7	17.0
	60 sec light	119.8	69.3	99.0	40.5
	300 sec light	211.6	114.4	279.1	127.5
	300 sec darkness	21.8	22.6	31.7	44.9
Methanol	10 sec light	14.7	18.2	10.7	17.7
	60 sec light	41.6	62.7	26.9	26.9
	300 sec light	94.7	201.2	112.5	104.5
	300 sec darkness	73.9	49.5	88.4	60.2

A study of the rate of assimilation of formic acid, formaldehyde, and methanol in relation to illumination and CO_2 present in the air suggests that both common and specific paths of conversion of these substances exist in plant tissues. This hypothesis was substantiated by data obtained from radiochromatography and ion exchange analyses.

Thus, after 10 seconds of photoassimilation of formic acid vapor in the absence of CO_2, the major fraction of radioactivity was found in phosphate esters. When exposure was prolonged to 1 or 5 minutes, radioactivity was found also in glucose, α-alanine, sucrose, fructose, aspartic acid, serine, glycolic, and glyceric acids, and in several unidentified substances. The increase in the percentage of radioactivity in aspartic acid of bean leaves, and in α-alanine of barley leaves is very characteristic, and is accompanied by a sharp decline in the percentage of labeled substances destroyed in the pores of a strongly-acid cation exchanger, KU-I. These regularities were clearly illustrated by radioautographs of corresponding chromatograms, some of which are shown in figure 3.

Quantitative expression of these regularities is presented in figure 4.

When comparing the data in this table with similar data obtained in photosynthetic CO_2 assimilation, in the case of photosynthesis, greater radioactivity invariably was found in the fraction of substances not absorbed by the cation exchanger, KU-I, namely, in sugars, organic acids, and phosphate esters. For example, after 5 minutes of photosynthetic $C^{14}O_2$ assimilation, the radioactivity of this fraction of bean leaves amounted to 77.0 % of that of all dissolved substances, while in barley leaves it was 66.6 % (9); in the case of photoassimilation of labeled formic acid, this radioactivity amounted to 61.9 and 51.5 %, respectively.

The presence of CO_2 in the atmosphere sharply inhibits the incorporation of formic acid into phosphate esters and later into sugars. Therefore, after short exposures, the radioactivity found on the chromatograms in phosphate esters, glycolic acid, and serine is almost the same. In dark experiments, radioactivity is first found in glycolic acid, serine, and aspartic acid.

The facts presented concerning the conversions of formic acid bear witness to its assimilation being realized mainly along the photosynthetic path, after preliminary oxidation to CO_2 by an active enzyme of the formicodehydrogenase type found in the seeds of Leguminosae (5, 18). Apart from this main path of formic acid conversion, a considerable portion is being assimilated by direct incorporation into serine (26).

After short photoassimilation of labeled formaldehyde, a considerable portion of its radioactivity also is concentrated in phosphate esters. Later, the label accumulates in α-alanine, serine, aspartic acid, and in several unidentified substances located on chromatograms in the zone of rapidly moving amino acids, such as phenylalanine and leucine (cf. fig 3). The presence of CO_2 in the atmosphere has in this case but a feeble effect upon the distribution of radioactivity between the products. In the dark, unidentified products which move far on the chromatogram, become intensely labeled.

Among the first photoassimilation products of labeled methanol in bean and barley leaves were (as in the foregoing experiments) the phosphate esters; labeled amino acids appeared later. An unidentified product moving very far on the chromatograms is formed very early. The same substance is responsible for the major part of radioactivity of the extract after dark fixation of labeled methanol.

Thus, bean and barley leaves assimilate vapors

Table IV

Radioactivity (%) of Substances Produced by Photoassimilation of Formic Acid Vapor by Bean & Barley Leaves

Plant	Exposure in sec	Phosphoric esters & sugars	Amino acids				Substances dissimilating at cation changer	Organic acids			Insoluble residue
			Serine	Alanine	Aspartic acid	Other amino acids		Glyceric acid	Glycolic acid	Other acids	
Beans	10	45.44	0.99	0.59	1.39	1.98	38.91	5.44	0.00	4.26	1.00
	60	43.83	2.46	1.58	3.74	5.81	26.79	1.28	1.58	11.43	1.50
	300	44.28	2.72	2.06	8.35	9.19	13.41	2.35	0.28	11.16	6.20
Barley	10	41.55	0.99	0.79	3.85	2.07	40.76	3.36	1.97	3.36	1.30
	60	46.08	2.42	10.06	5.03	6.70	15.36	1.30	1.12	5.03	6.90
	300	32.67	3.35	19.37	2.81	7.51	11.22	3.89	3.98	5.70	3.50

of formic acid, formaldehyde, and methanol with the formation of products of about the same qualitative composition and about the same sequence of appearances as observed in CO_2 assimilation. Apart from the main oxidative path of the assimilation of these substances, each of them also undergoes other characteristic transformation which seems to take place in the dark.

The considerable number of spots with a very weak radioactivity, observed on chromatograms obtained in experiments with short exposure of leaves in the presence of vapors of labeled formic acid, formaldehyde, or methanol, seems to be related to the existence of several independent paths for their involvement in metabolism. Among these paths is likely to be found some characteristic of animal organisms, where they are more clearly expressed and therefore better known. The ability of green leaves to assimilate certain organic compounds in light may represent a combination of vestigial heterotrophy with photoassimilation. The relatively rapid assimilation rate of these volatile organic substances, particularly of formic acid, by green plants can be of considerable importance for the purification of air from impurities caused by industrial processes and motorized transportation since formic acid is one of the most important of these impurities (10).

Summary

I. Plant leaves are capable of photosynthetic assimilation, in light, of the vapors of organic substances such as formic acid, formaldehyde, and methanol.

II. Assimilation of these substances proceeds also in the dark, but at a considerably slower rate than in the light.

III. Investigations of the dynamics of formation of the labeled products, and identification of many of them, show the prevalence of the oxidative path of assimilation of these compounds with subsequent assimilation of the CO_2 formed by normal photosynthesis.

IV. Apart from the main path of conversion of HCOOH, HCOH, and CH_3OH, certain other transformations take place leading to the formation of serine, as well as of certain other substances. The relative importance of these paths depends on biochemical peculiarities of the chosen object.

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