Table 1. Comparison of percentage $CO_2$ obtained by the critical orifice analyzer and the Haldane gas analyzer (seven samples).

| Haldane gas analyzer (%) | Critical orifice analyzer (%) | Critical orifice analyzer minus Haldane gas analyzer (%) |
|---|---|---|
| 2.97 | 2.94 | −0.03 |
| 3.56 | 3.54 | − .02 |
| 6.45 | 6.48 | + .03 |
| 1.12 | 1.08 | − .04 |
| 6.66 | 6.73 | + .07 |
| 1.37 | 1.33 | − .04 |
| 6.80 | 6.86 | + .06 |

uring orifice pressure drop, the influence of ambient pressure must be taken into account. It was found empirically, from measurements made over a 19-mm range of ambient pressure (756–775 mm-Hg), that a 1-mm change in pressure was equivalent to about 0.04 percent change in $CO_2$. By adjusting the reservoir level so that on room air, passed through Ascarite before entering the analyzer, an arbitrary zero point in the scale was maintained, the variation with ambient pressure in the range from 3 to 6 percent $CO_2$ was reduced to less than 0.008 percent per mm-Hg change in atmospheric pressure. In this way small changes in ambient pressure could be neglected, and a single calibration curve served for a day's run (*4*).

### References and Notes


1. R. T. Page, *Ind. Eng. Chem. Anal. Ed.* **7**, 355 (1935).
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3. The National Jet Co., Cumberland, Md., manufactures microdrilling equipment. They drill holes down to 0.001 in. in diameter in blanks for a nominal cost.


4. I wish to acknowledge the assistance of my father, W. J. Mead, who constructed the manometer, and of Libby Servello, who performed the Haldane analyses. E. M. Landis made possible the use of the analyzer in student exercises and William B. Kinter assisted in supervising its use.

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## Auxin Effects on the Utilization of $C^{14}$-Labeled Acetate by Wheat Roots


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Several years ago we observed production of acetaldehyde by wheat roots incubated in solutions of auxins (*1*). This effect suggests that the search for a specific chemical event underlying auxin action ought to include a study of reactions of the acetyl group, and prompted the work with acetate-1-$C^{14}$ reported here (*2*).

Roots excised from wheat seedlings (White Federation 38) grown for 4 days in distilled water (*3*) were used. For the 2-hr experiment, 19 g of roots were incubated at 21.5°C in 100 ml of $6 \times 10^{-4}M$ acetate solutions having activities of 242.5 and 229.0 μc, respectively, for the control and experimental treatments. For the 30-min experiment, 3 g of roots were incubated at 23°C in 35 ml of approximately $1 \times 10^{-4}M$ acetate solution having 30 μc of activity. In both experiments, indoleacetic acid (IAA) was used as an auxin at $2.5 \times 10^{-5}M$. Acetate solutions were adjusted to *p*H 4.7 with KOH.

During the experiments, $CO_2$-free air passed through closed vessels containing the roots, then through a U-tube held at −80°C to trap volatile products, and finally through a gas-washing bottle containing 0.1*N* NaOH to retain $CO_2$. At the end of the experiment the roots were separated from the solutions and washed. Combined solutions and washings from roots and cold traps were analyzed for $C^{14}$. These values, together with those for radioactivity in the original solutions and in the $CO_2$ produced by respiration were used to calculate the total quantity of $C^{14}$ absorbed by the roots in the 2-hr experiment. No measure of $C^{14}$ absorption is given for the 30-min experiment because the decrease in activity of the solution during the course of the experiment was about equal to the analytic error.

Root material in the 2-hr experiment was blended in the cold with water. After filtration this process was repeated three times on the solid material. An aliquot of the combined filtrate (designated "Total water-soluble fraction" in Table 1) was assayed for activity by a wet oxidation procedure (*4*). In this method the organic material is oxidized by chromic acid (*5*), and the gaseous products are carried to an evacuated ion chamber by a stream of $CO_2$. Activity in the chamber is measured on a vibrating reed electrometer (*6*). Values in Tables 1 and 2 represent averages for two determinations, and in each case duplicate values agree within 3 percent.

Another aliquot of the filtrate was acidified to *p*H 1.0 with sulfuric acid and was extracted for a period of 96 hr with ether. Titration of the ether extract

Table 1. Utilization of acetate-1-$C^{14}$ by wheat roots in 2 hr.

| | Control roots (μc) | Roots in IAA $2.5 \times 10^{-5}M$ (μc) | IAA/control times absorption factor* |
|---|---|---|---|
| $C^{14}$ absorbed | 214.40 | 178.97 | 100 |
| Total water-soluble fraction | 121.00 | 129.50 | 129 |
| Lipids | 1.92 | 2.15 | 135 |
| Acidic substances | 77.25 | 89.00 | 139 |
| Acetic acid (calculated) | 71.78 | 85.65 | 144 |
| Nonvolatile acids | 5.47 | 3.35 | 74 |
| $CO_2$ of respiration | 15.70 | 13.96 | 101 |

* Absorption factor equals 100 times the ratio of uptake of $C^{14}$ in the control to that of the auxin treatment.

Table 2. Utilization of acetate-1-$C^{14}$ by wheat roots in 30 min.

| | Control roots (μc) | Roots in IAA $2.5 \times 10^{-5}M$ (μc) | IAA/ control $\times 100$ |
|---|---|---|---|
| Total 80 percent EtOH extract | 0.491 | 0.461 | 94 |
| Lipids | .007 | .013 | 186 |
| Ether-insoluble substances | .484 | .448 | 93 |
| Acetic acid | .029 | .107 | 369 |
| Residue of EtOH extract | .467 | .317 | 68 |
| $CO_2$ of respiration | .018 | .011 | 61 |

with an aqueous solution of NaOH to *p*H 8.5 effected the separation of an "Acidic substances" (Table 1) fraction that was analyzed for radioactivity. The "Lipids" of Table 1 are materials remaining in the ether. Chromatographic analysis revealed that most of the activity in the acids extracted by ether was due to acetic acid. Aliquots of the solutions of the acidic substances were lyophilized and then acidified with dry HCl in a vacuum line. Volatile acids released in this fashion were distilled into tubes to which excess $NH_4OH$ was added later. Chromatograms of the ammonium salts (*7*) and their radioautographs established that all activity in the volatile-acid fraction resided in acetic acid. The residual, nonvolatile acids were freed of NaCl by lyophylization in the presence of $H_3Po_4$ and then assayed for radioactivity. Values for acetate in Table 1 were calculated from those for total acidic substances and nonvolatile acids.

Roots from the 30-min experiment were extracted in a closed system with five successive 100-ml portions of boiling 80-percent ethanol that were drawn by suction into a flask containing 40 ml of 0.01*N* NaOH. After removal of the ethanol, this alkaline solution was extracted with ether for a period of 96 hr. An aliquot of the extract, labeled "Lipids" in Table 2, was assayed. Samples of the aqueous residue were assayed and are identified as "Ether-insoluble substances" in Table 2. Another aliquot of the residue was acidified, and steam was distilled to isolate the volatile acids. Residual solutions and steam distillates were assayed, and the radioactive material of the steam distillate was identified by radioautography as acetate.

It is evident that acetate utilization is inhibited by IAA at $2.5 \times 10^{-5}M$, a concentration that also inhibits root growth (*8*). In the 2-hr experiment (Table 1), the auxin-treated roots accumulate more radioactivity in the water-soluble constituents than the control roots do, but it is seen that this difference is due to the higher level of free acetic acid in the former. Acids other than acetic, however, have greater activity in the control tissues, indicating obvious inhibition of acetate utilization by IAA.

It is of interest to see (Table 2) that the inhibitory effect of IAA is evident 30 min after exposure of the roots to auxin. In the IAA-treated roots, there is much more activity that can be assigned to acetic acid than there is in the control roots. The latter have greater activity in the 80-percent ethanol-soluble materials exclusive of acetic acid. One fraction of the 80-percent ethanol extract, probably lipids, extracted from an aqueous, alkaline solution by ether, shows greater activity in the IAA-treated roots.

Boroughs and Bonner (*9*) describe experiments with *Avena* coleoptile tissues in which the effects of IAA on the conversion of acetate-1-$C^{14}$ and other labeled compounds to a number of plant substances were studied. Of all the cellular material analyzed, only the synthesis of noncellulosic polysaccharides is impressively stimulated by IAA. A small stimulation of lipid synthesis occurs, but the synthesis of other cell-wall materials, proteins, and a variety of soluble substances were not appreciably affected.

Our experiments appear to confirm a suggestion arising from studies of auxin-induced acetaldehyde evolution that IAA inhibits the synthesis of citric acid from oxalacetic acid and the acetyl group (*1*). The results may be interpreted as evidence that IAA controls growth by regulating the metabolism of acetyl groups in some fashion, or it may be argued that these effects are, as most auxin responses must be, of a secondary nature and are the consequence of some more profound auxin action. The possibilities for a primary auxin effect involving enzymes of acetylation appear to be strengthened by the recent work of Siegel and Galston (*10*) and of Leopold and Guernsey (*11*) in which evidence for the formation of a thioester of IAA and coenzyme A is presented.

### References and Notes


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