There remains little doubt that members of this family, and of the genus *Atrichopogon* in particular, are responsible for most natural pollination of *H. brasiliensis* in Brazil. It is of interest that members of two of these genera (*Atrichopogon* and *Dasyhelea*) were also identified as among the pollinating agents in Puerto Rico. In Puerto Rico, however, members of the genus *Dasyhelea* were found to be most abundant, whereas in Brazil individuals of the genus *Atrichopogon* were most abundant. This difference in relative numbers of insects is believed to be related to the reduced number of stigmas bearing insect bristles in Brazil. Specimens of the predominant form in Brazil, which is yellowish in color, were found to shed few if any bristles when rubbed over the stigmas of fresh Hevea flowers. The dark form, most common in Puerto Rico, was found to shed bristles readily.

An insect belonging to the family Culicidae, tentatively identified as *Mansonia* sp., may also be responsible for some pollination in Brazil. This insect is somewhat larger than the midges and has wing veins and margins marked by a fringe of scales. These scales, or ones similar to them, have occasionally been found on Hevea stigmas and appear to be associated with the presence of pollen grains.

In Puerto Rico, thrips were found to be the most numerous insects around Hevea flowers and were shown to be agents of pollination, although not as important as the midges. In Brazil, thrips of three different genera (*Frankliniella, Scirtothrips,* and *Heterothrips*[4]) were collected from Hevea flowers. The actual numbers of flower thrips observed in Brazil at the time of these studies were so small, however, that these insects were probably of little or no importance as pollinating agents.

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[4] Identified by J. D. Hood, of Cornell University.

The Rate of Endogenous Respiration as Affected by the Oxidation of Exogenous Substrates[1]

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Many manometric data in the literature are corrected on the premise that the endogenous respiration continues as its normal rate during the simultaneous oxidation of exogenous substrates. This assumption, unless valid, may lead to erroneous interpretations, especially in the case of such organisms as *Penicillium chrysogenum* Q-176, which possess a relatively high endogenous respiration as compared to their total respiration with a substrate. Inasmuch as it is impossible to distinguish directly between the oxygen needed for the metabolism of endogenous and exogenous materials, some information on the course of the endogenous respiration can be obtained indirectly by comparing the release of isotopically labeled CO_2 from labeled resting cells in the presence or absence of unlabeled substrates (*1–5*). This approach presupposes (*a*) that CO_2 production is a function of oxygen consumption and (*b*) that $C^{14}O_2$ release is representative of the total endogenous CO_2 production. This preliminary note illustrates some of the difficulties encountered in the use of this method.

Vegetative cells of *P. chrysogenum* Q-176 were grown from a spore inoculum at 25° C in 500 ml Erlenmeyer flasks containing 100 ml of a medium in which acetate was the main source of carbon (*6, 7*); the flasks were agitated on a reciprocating shaking machine, which made 90 4-in. strokes/min. After 42 hr of growth, the cells from 6 flasks were harvested, washed with *M*/15 phosphate buffer (pH 6), and added to a flask of fresh medium that also contained 2.0 mg each of 1-C^{14}- and 2-C^{14}-acetate with specific activities of 1 mc/mM. This flask was placed in a closed system similar to the one described by Martin and Wilson (*8*); air was circulated by a small diaphragm pump and freed of CO_2 by passage through alkali. The cells were allowed to grow for 6 hr, while the system was being shaken on a reciprocating shaker. The cells were then harvested, washed with buffer, minced for 15 sec in a Waring blendor, and diluted with buffer to give a cell concentration of about 2–4 mg (dry weight)/ml. Washing the cells after mincing rather than before had no effect on the results. Two ml aliquots of cells were placed in Warburg flasks containing 0.2 ml of 20% carbonate-free NaOH in the center well (without filter paper), 0.5 ml substrate or buffer in one side arm, and 0.5 ml 72% perchloric acid in the other. The experiment was started by the addition of substrate or buffer to the main compartment and stopped, at desired intervals of time, by the addition of perchloric acid. The temperature used was 30° C, and the gas phase was air. One hour or more after the completion of the experiment the alkali was removed quantitatively from the center wells. $BaCl_2$ was added to the alkali to precipitate the carbonate; the $BaCO_3$ was collected by centrifugation, washed twice with 95% ethanol, and then plated on a microporous disk. The radioactivity on the disk was measured by standard techniques, using a thin mica window Geiger-Müller counter. Counts were corrected, by a graphical method, to the activity at 0 self-absorption (*9*).

Fig. 1 shows the results of an experiment with 0.1 *M* glucose or sodium acetate as substrates; the values are averages from two or more determinations. Although the oxidation of glucose did not affect the rate at which $C^{14}O_2$ was released, the oxidation of acetate eventually suppressed the release of $C^{14}O_2$ almost com-

[1] Supported in part by the Atomic Energy Commission. Presented at the 51st General Meeting of the Society of American Bacteriologists in Chicago (*1*).

[2] Predoctoral fellow of the Atomic Energy Commission.

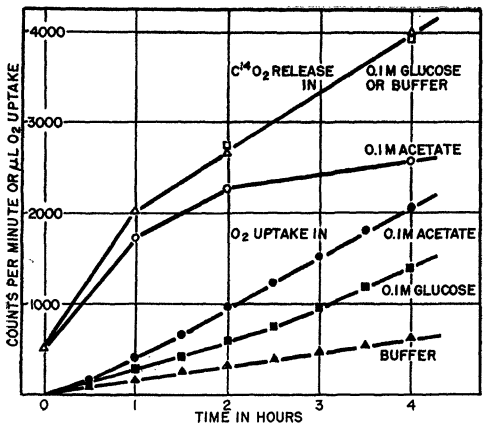

FIG. 1. The effect of glucose or acetate on the uptake of O_2 and release of $C^{14}O_2$ from cells of *P. chrysogenum* Q-176 labeled by growth on 1- and 2-C^{14}-acetate as the main source of carbon. Manometric readings were made every 10 min, but not all of them are recorded.

pletely. These results were observed consistently, even when the cells were labeled by growth on 2-C^{14}-acetate as the main source of carbon rather than a mixture of carboxy- and methyl-labeled acetate.

Parallel experiments using the 2-flask manometric method (*10*) showed that the endogenous RQ was relatively constant over a 4-hr period (0.74 ± 0.04); this fulfills the first prerequisite on which this method is based—namely, that the determination of CO_2 be an indirect measure of the oxygen consumed. However, is the release of $C^{14}O_2$ a true measure of the total endogenous CO_2 released? Inasmuch as the ratio of CO_2/O_2 remained uniform during the experiment, the ratio of $C^{14}O_2$ should also be constant, if this question is to be answered in the affirmative. Fig. 1, however, shows that this ratio became constant only during the last 2 hr of the experiment. This means that $C^{14}O_2$ release could have been completely representative of the total endogenous release of CO_2 only during the second half of the experiment.

The extent to which liberation of labeled CO_2 measures over-all endogenous production of CO_2 depends on the distribution of labeled carbon within the cell. If all cellular components have the same specific activity, the $C^{14}O_2$ evolved will allow a realistic estimate of the total endogenous respiration. If, however, the specific activities of the cellular components vary, the determination of labeled CO_2 may give an incomplete and often misleading picture. This is illustrated by an experiment in which *P. chrysogenum* Q-176 was labeled by allowing the organism, after it had been starved for 2 hr in phosphate buffer at pH 6, to oxidize 2-C^{14}-acetate for 2 hr in the phosphate buffer (Fig. 2). Of the total $C^{14}O_2$ released by resting cells during a 3-hr period, over half was released during the first 30 min. Obviously, the $C^{14}O_2$ evolved by cells labeled in this manner was only representative of a small portion of the total endogenous respiration.

The best way to obtain uniformly labeled cells is to use a small inoculum and to grow the cells on a uniformly labeled substrate as the sole source of carbon. When this procedure is applied to microorganisms a good deal of radioactivity is lost in waste products. A less costly way is to allow an already developed culture to continue growing for a limited period of time on uniformly labeled substrates as the sole source of carbon. Whether such cells are homogeneously labeled can then be ascertained according to the criteria already mentioned and also by determination of the specific activities of the cellular carbon and the liberated CO_2-carbon. If the cells are labeled uniformly the specific activities of the cellular carbon and the CO_2-carbon should be the same, and the radioactive CO_2 actually represents the endogenous respiration.

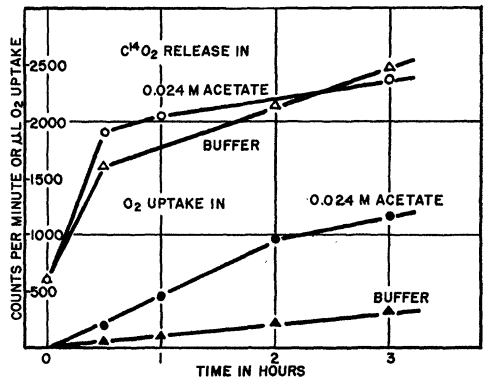

FIG. 2. The effect of acetate on the uptake of O_2 and release of $C^{14}O_2$ from cells of *P. chrysogenum* Q-176 labeled by oxidative assimilation of 2-C^{14}-acetate. Manometric readings were made every 10 min, but not all of them are recorded.

The data in Fig. 1 exemplify the danger of using the total amount of $C^{14}O_2$ released within a given time as an expression of the endogenous respiration. For example, if only single determinations had been made in this experiment instead of kinetic studies, the data might have led, with equal lack of justification, to the conclusion that acetate inhibited the release of $C^{14}O_2$ (*a*) greatly, (*b*) slightly, or (*c*) not at all, depending on the time when the measurements were made. Unfortunately, not even all kinetic data can be interpreted unequivocally. For instance, from the data described it seems justifiable to conclude that the rate of endogenous respiration of acetate-grown *P. chrysogenum* is not affected by the concurrent oxidation of glucose; yet, as will be discussed more fully elsewhere, it is premature to assert, without auxiliary experiments, that the endogenous respiration is inhibited by the oxidation of acetate.

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Radiochemical Preparation of Heptadecene-8[1]

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During the course of studies concerned with the role of radioactivity in petroleum genesis, a highly purified sample of oleic acid was bombarded with 14 mev deuterons in the MIT cyclotron (*1*). Previous investigations (*2–4*) had shown that decarboxylation is a predominant reaction during the exposure of fatty or naphthenic acids to α-particles or to deuterons. Heptadecene-8 was, therefore, anticipated as a major product from the irradiation of oleic acid.

$$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH \rightarrow CH_3(CH_2)_7CH{=}CH(CH_2)_6CH_3 + CO_2$$

Following bombardment of 56.8 g of the purified oleic acid (free from stearic acid) for 2 hr with an average beam intensity of 9 μamp, using the gold-plated chamber described by Honig (*5*), the recovered viscous liquid was saponified by refluxing for 3 hr with alcoholic sodium hydroxide. The solution was then extracted with ether, and the extract was dried over sodium sulfate and evaporated on a steam bath. The yellow, viscous, nonsaponifiable residue amounted to 10% of the oleic acid originally bombarded. Part of this material (950 mg) was dissolved in 100 ml pentane and passed through a 12 × 800 mm column packed with a 5:1 Florosil-Celite 545 mixture. The effluent and wash pentane fluoresced deep blue under ultraviolet light. After the pentane was evaporated, a light, almost colorless liquid remained which had a melting point of 15°–20° C. On preliminary micro vacuum distillation using a simple U-type still, a solid residue remained. This material, which was not further investigated, amounted to about 0.5%, based on the original oleic acid, and had a melting point of 50°–60° C. Hydrogenation of a portion of the distillate (3% of the original oleic acid bombarded) indicated the liquid to contain 92% of mono-olefin based on the molecular weight (238) of the anticipated heptadecene.

Fractionation of a second portion of the chromatographed material (300 mg) in a micro still described by Craig (*6*) led to the recovery of a number of fractions, of which 5 had approximately the same boiling points and refractive indices. These 5 fractions were combined, and the physical properties of the resulting liquid are shown in Table 1.

An effort was made to establish the position of the double bond chemically. Approximately 75 mg of the material was ozonized in pentane at −30° C, and the resulting ozonides were hydrolized. The acids thus obtained were isolated and converted to the anilides with the intention of obtaining an x-ray powder pattern by which to identify the individual acids present (*7*). Unfortunately, impurities in the anilides caused a depression of the melting point so that the mixture could not be crystallized at room temperature. Attempts to purify the product further were not successful, and insufficient material was available for repetition of the work.

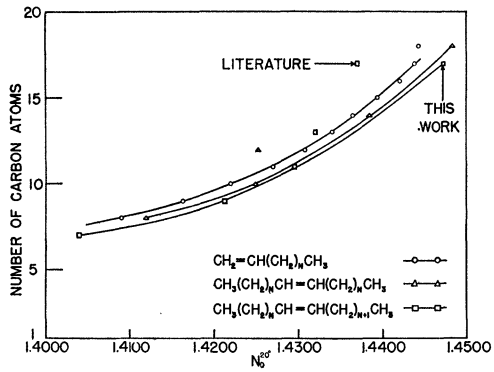

FIG. 1. Refractive indices of the isomeric normal monoolefins.

As indicated in Table 1, the refractive index and melting point of the unsaturated hydrocarbon obtained by radiochemical decarboxylation of oleic acid differ considerably from the values reported in the literature for heptadecene-8.

It has repeatedly been shown (*8–10*) that both the density and refractive index of an olefin increase as the double bond is shifted to the center of the molecule. Although heptadecene-1 has been reported as having n_D^{20} 1.4438 (*11*), the value for heptadecene-8 has been given as n_D^{20} 1.437 (*10*). The value for heptadecene-8

TABLE 1

PHYSICAL PROPERTIES OF HEPTADECENE-8

	Observed	Literature
D_4^{20}	0.802	0.795/25° (*13*)
N_D^{20}	1.472	1.437 (*11*)
Boiling point (corr), °C	297–298	173/16 mm (*13*)
Melting point (corr), °C	−12.5 to −11.5	−50 (*13*)
Double bonds/molecule (catalytic hydrogenation)	1.02 ± 0.05	1*
Carbon (%)	85.9	85.7*
Hydrogen (%)	14.1	14.3*

* Calculated.

[1] This paper is a contribution from American Petroleum Institute Research Project 43C, located at the Massachusetts Institute of Technology; W. L. Whitehead, director; Clark Goodman, physical director.
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