from the wild possessed this type of hemoglobin. It is impossible, without crossing animals of this phenotype with monkeys of other hemoglobin types, to determine whether this isolated case is a single mutation or whether this variant occurs at a low frequency in the population. These data further document the presence of biochemical variation in the genus *Macaca* (*4*, *5*).

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1. The conditions under which laboratory monkeys are imported make it impossible to determine the exact geographic origin of individuals, or the statistical relation of laboratory to wild populations. Many circumstances indicate, however, that a laboratory group constitutes a highly select, rather than a random, sample of a species.
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6. Supported by grant FR00166 from the National Institutes of Health; the sponsorship of Dr. Eloise Giblett, associate director of the King County Blood Bank and Visiting Research Affiliate of the Regional Primate Research Center at the University of Washington, is acknowledged.

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Alkanes in Fungal Spores

Abstract. *The chlamydospores of* Ustilago maydis, U. nuda, *and* Sphacelotheca reiliana *were analyzed by gas chromatography and mass spectrometry for their hydrocarbon contents. For the first time we observed that they contain paraffinic hydrocarbons; the average contents were 42, 58, and 146 parts per million, respectively.* n-*Alkanes having odd numbers of carbon atoms predominate, with carbon-chain lengths ranging from* C_{14} *to* C_{37}. *The major alkanes are* n-C_{27} *in* U. maydis, n-C_{27} *and* n-C_{35} *in* U. nuda, *and* n-C_{29} *in* S. reiliana. *Each type of spore carried a distinctly characteristic population of hydrocarbons.*

Paraffinic hydrocarbons have recently been extensively investigated in a wide variety of higher-plant tissues (*1*, *2*). It is well established that such hydrocarbons reside almost universally on the external surfaces of leaves, stems, and other structures and are principally normal alkanes with predominantly odd chain lengths ranging from C_{25} to C_{35}. Several bacteria also contain paraffinic hydrocarbons, but generally shorter in chain length than those of higher plants (*3*). We have analyzed the chlamydospores of several closely related fungi for their hydrocarbon content; to our knowledge, our data provide the first evidence of presence of hydrocarbons in fungal spores.

Spores of *Ustilago maydis, U. nuda,* and *Sphacelotheca reiliana* (*4*) were obtained from various sources during 1964–65 (*5*). Samples were passed through a No. 100 sieve (*6*) before microscopic verification of the type of spores present and the purity; they were stored at 5°C.

Our methods of extraction and analysis were modifications of those reported (*7*). Initial extractions were made with 50 ml of a 3:1 mixture of benzene and methanol; for secondary extractions we used 50 ml of *n*-heptane. Each extraction was for 30 minutes at 50°C, with frequent stirring in an open beaker, and spores were separated from solvents by low-speed centrifugation. The combined extracts were evaporated at 40°C under a stream of purified nitrogen; the residue was taken up in a series of 5-ml portions of *n*-heptane and transferred to the top of a silica-gel column (1 × 20 cm).

The silica gel was prepared by treatment in an electric furnace for 10 hours at 425°C before washing with four volumes of *n*-heptane immediately before use. The column was eluted with 20 ml of *n*-heptane, and the collected fraction was dried at 40°C under a stream of purified nitrogen. The residue from the *n*-heptane fraction was dissolved in 10 to 20 μl of benzene from which 1- to 2-μl portions were injected into a gas chromatograph incorporating a 30 m by 0.025-cm stainless steel capillary column; Apiezon L (*9*) was the stationary phase.

Major alkane components were identified by cochromatography techniques and by comparison with the retention times of authentic alkanes chromatographed under identical conditions; they were additionally verified by analysis with a combination of LKB-9000 gas chromatograph and mass spectrometer (*10*). Throughout this study, all procedures were checked by use of suitable solvent blanks.

Ustilago maydis (Fig. 1A) from two sources (*5*) contained on average 42 parts of hydrocarbon per million by weight, with alkanes ranging in carbon-chain length from C_{14} to C_{32}; the ma-

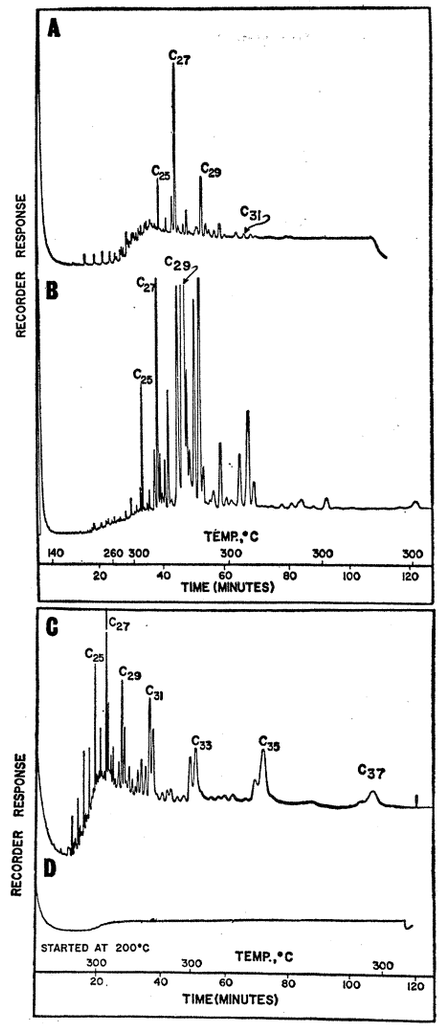

Fig. 1. Gas-chromatographic separations of extracts of chlamydospores; nitrogen carrier gas. No split was used and attenuations are as indicated. (A) Extract of 2.10 g of *U. maydis* spores; approximately 5 percent of *n*-heptane extract injected; nitrogen carrier pressure, 2430 g/cm. Programmed at approximately 6°C per minute from 140° to 300°C. (B) Extract of 0.34 g of *U. nuda* spores; approximately one-third of *n*-heptane extract injected; nitrogen carrier pressure, 3490 g/cm. Programmed at approximately 6°C per minute from 200° to 300°C. (C) Extract of 2.45 g of *S. reiliana* spores; approximately one-tenth of *n*-heptane extract injected; nitrogen carrier pressure, 2430 g/cm. Programming as for (A). (D) Extraction and gas chromatographic–analysis blank; approximately one-tenth of *n*-heptane extract injected; nitrogen carrier pressure, 2430 g/cm. Programming as for (A).

jor components were normal C_{25}, C_{27}, and C_{29} alkanes. The source or age of the spores, within the limits of our study, appeared to have little influence on the type of pattern obtained for *U. maydis*. When leaves adjacent to the infected area, the cob material, and the remaining unaltered seeds of the corn host were analyzed in the same manner, the hydrocarbon patterns were different from those of *U. maydis*. Uninfected seeds, leaves, and cob material of fresh corn obtained from two different sources provided almost identical hydrocarbon patterns that differed from those of host and of *U. maydis* spores.

On the other hand, *U. nuda* (Fig. 1C) yielded approximately the same quantity of hydrocarbon material (58 ppm) as did *U. maydis*, but the distribution was markedly different: the first population of alkanes ranged from C_{25} to C_{29}; the preponderance ranged from C_{31} to C_{37}, with C_{35}—the major contributor—representing approximately 18 percent of the total weight of hydrocarbons. As determined by gas chromatography of standards and partly by mass spectrometry, the major components were normal alkanes having odd numbers of carbon atoms. The chromatographic pattern of *U. nuda* indicated the presence of considerably more branched-chain alkanes than in *U. maydis*, on the basis of identification inferred from their respective retention times.

The predominant hydrocarbon present in *S. reiliana* (Fig. 1B) is *n*-nonacosane, which comprises over 34 percent of the total weight of hydrocarbons; *S. reiliana* yielded the largest quantity of hydrocarbons of the three spores studied: 146 ppm by weight.

Although the extraction procedure was rather rigorous, no attempt was made to disrupt the individual spores; therefore the hydrocarbons were assumed to be principally on the surface. As for the function of this outer hydrocarbon, it may serve as a protective covering to assist in controlling internal water balances and to resist microbial attack—as has been suggested for higher plants (*11*). Such mechanisms could help to explain the excellent environmental resistance of most fungal spores.

For several species of higher plants, the hydrocarbon distribution patterns are fairly consistent (*2, 12*) and may be of taxonomic significance. The positive identification of a given fungus, especially if several closely related types possess similar physical or biologic properties, is often difficult and time-consuming. It would seem that the readily distinguishable hydrocarbon patterns that we observed may offer a method of chemotaxonomic differentiation similar to that proposed for higher plants. Although these preliminary data suggest such a possibility, one should use caution in invoking such an hypothesis without consideration of the possible influences of variations in environment and host metabolism.

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Carbamylation and Binding Constants for the Inhibition of Acetylcholinesterase by Physostigmine (Eserine)

Abstract. *The kinetic treatment and experimental procedures which have been used to evaluate the binding and phosphorylation rate constants of organophosphate inhibitors were applied to the carbamate inhibitor eserine. The binding constant* K_I *and carbamylation rate constant* k_{2c} *for the inhibition of acetylcholinesterase by eserine were successfully evaluated.*

Wilson and co-workers (*1*) have proposed the following scheme for the reaction of carbamate inhibitors (I) and esterases (E):

$$I + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.I \xrightarrow{k_{2c}} E' \xrightarrow{k_3} E + \text{carbamic acid} \quad (1)$$

(E.I) is a reversible complex whose formation is controlled by the equilibrium affinity constant $K_I = (k_{-1}/k_1)$ and (E′) is the carbamylated active site of the esterase. Carbamylation is controlled by the carbamylation rate constant k_{2c} and decarbamylation by the rate constant k_3. The methods used have not permitted separate evaluations of K_I or k_{2c} to be made but have given a measure of the overall rate of formation of E′ through k'_2 where $k'_2 = (k_{2c}/K_I)$.

The significance of carbamylation rates as compared to binding seemed of interest since eserine has been regarded simply as a reversible competitive inhibitor having great affinity for the active site of cholinesterases (*2*).

Experimental techniques have been developed for the determination of K_a, the affinity constant, and k_{2p}, the phosphorylation constant for the inhibition of acetylcholinesterases by organophosphates, where K_a is precisely equivalent to K_I and k_{2p} is analogous with k_{2c} (*3*). The kinetic treatment used is based on the assumption that $k_3 = 0$ in scheme 1. The reaction is then assumed to be irreversible. But experimentally this condition has only to be approximated, and it is sufficient if k_{2c} or $k_{2p} \gg k_3$.

Since the mechanism of the carbamate reaction is considered to be identical with that of organophosphates, it seemed of interest to see if the kinetic treatment and experimental procedures giving k_{2p} and K_a would also apply to carbamates and give k_{2c} and K_I. Eserine was chosen for historical reasons and because of its solubility.

The experimental design and interpretation of results were based on the equation

$$\frac{[I]\ \Delta t}{2.3\ \Delta \log v} = \frac{[I]}{k_{2c}} + \frac{1}{k'_2} \quad (2)$$

where $[I]$ is the concentration of eserine and $(\Delta t/2.3\ \Delta \log v)$ is the first-