

agreement with our data. These patients have shown reduction in REM sleep time, drop in the frequency of eye movements, and absence of compensatory rebound after shock (14).

Electrical damage to brain tissue may be ruled out as a possible explanation of these results since shock administered to anesthetized animals, as well as repeated subconvulsive shock to unanesthetized animals, had no effect on REM sleep time. A convulsion induced by intravenous injection of metrazol, however, resulted in a lowering of REM time to levels comparable to those obtained with electroconvulsive shock, which suggests that some property of the convulsion itself leads to reduction of REM sleep time without producing the usual REM deprivation effect of compensatory rebounds. There is growing evidence that REM sleep is biochemically mediated (15-19). If electrically induced brain damage is ruled out, it is plausible that the intense activity accompanying the seizures in some manner altered the brain levels of substances, or their precursors, that induce REM sleep.

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20. Supported by grant MH 08185 from the National Institute of Mental Health, research career development award 1-K3-MH-5804 from PHS, postdoctoral fellowship award 5T1-MH-8304 from the National Institute of Mental Health, and grant from the Epilepsy Foundation.
21. We thank Richard Duncan for assistance.

25 July 1966

Hemoglobin Polymorphism in *Macaca nemestrina*

Abstract. Four hemoglobin phenotypes have been noted in the pigtailed monkey (*Macaca nemestrina*). Pedigree studies suggest a simple codominant Mendelian explanation for inheritance of three of these phenotypes, including one electrophoretically identical with human type A. The fourth type occurred in only one animal in this series.

An unusual hemoglobin polymorphism has been observed during an electrophoretic study of biochemical variants in primates. A number of distinct patterns were inscribed by hemoglobins from pigtailed monkeys (*Macaca nemestrina*). No intraspecific variation has been reported for this species to date.

In all, 75 *Macaca nemestrina* were tested. A number of these represented families of a wild-caught male and female and an offspring born at the Regional Primate Research Center at the University of Washington, but the majority of the experimental subjects had been captured in the wild. The

latter animals had been obtained in a number of shipments presumably containing animals from several local populations (1).

Hemoglobin was analyzed by vertical starch-gel electrophoresis on a continuous buffer system at pH 8.6 with tris-ethylenediaminetetraacetic acid (EDTA)-borate stock solution. The concentration of this buffer was 0.9M tris, 0.5M boric acid and 0.2M EDTA (2). The percentage of alkaline resistance was measured by the method of Betke *et al.* (3).

Four hemoglobin phenotypes were noted on the basis of electrophoretic mobility and staining intensity (Fig.

1). The first type, a band of the identical mobility as human A, was observed in 12 of the 75 animals. The second type, characteristically faster than the normal human A type, occurred in 43 monkeys. Two bands of equal staining intensity and of mobilities suggesting the presence of both of the first two types characterize a third phenotype. The third type was found in 19 monkeys. The fourth type, a possible variant of the third, has a two-component system of the same speed as the third but of differing staining intensity. The third type displayed an equal distribution of hemoglobin between the two bands. In the fourth type, the ratio was 75 to 25 percent in the intensity of the staining. These unusual proportions appeared consistently in four determinations made on two fresh samples obtained several days apart. The observed gene frequencies at this locus differ significantly from the expected. This difference is probably due to sampling error. No A₂ component—the minor hemoglobin fraction characteristic of man and higher primates—has been found in macaques tested to date.

The percentage of alkaline-resistant hemoglobin ranged from 0 to 1.8, but there was no significant relationship between this characteristic and any of the four electrophoretically determined types. The percentage range of alkaline-resistant hemoglobin in this study confirmed the findings of Tuttle *et al.* (4).

Pedigree studies suggested a simple codominant Mendelian explanation for the inheritance of the first three of these phenotypes. For example, when a male with the first phenotype was bred with a female of that phenotype, their offspring was of that type. When the same male was bred with a female of the second phenotype, their offspring was of the third phenotype (which, as noted, appeared to indicate presence of the first two types of hemoglobin in equal quantities).

No pedigree data are available for the fourth type, as only one animal

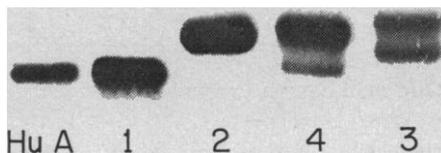


Fig. 1. Four hemoglobin types in *Macaca nemestrina* compared to human A, designated Hu A; other terminology devised for this paper. (Composite of three starch gels.)

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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References and Notes

1. The conditions under which laboratory monkeys are imported make it impossible to determine the exact geographic origin of in-

dividuals, 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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- 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.

23 August 1966

Alkanes in Fungal Spores

Abstract. *The chlamydo spores 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 C₁₄ to C₃₇. The major alkanes are n-C₂₇ in U. maydis, n-C₂₇ and n-C₃₅ in U. nuda, and n-C₂₉ 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 C₂₅ to C₃₅. Several bacteria also contain paraffinic hydrocarbons, but generally shorter in chain length than those of higher plants (3). We have analyzed the chlamydo spores 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 C₁₄ to C₃₂; the ma-

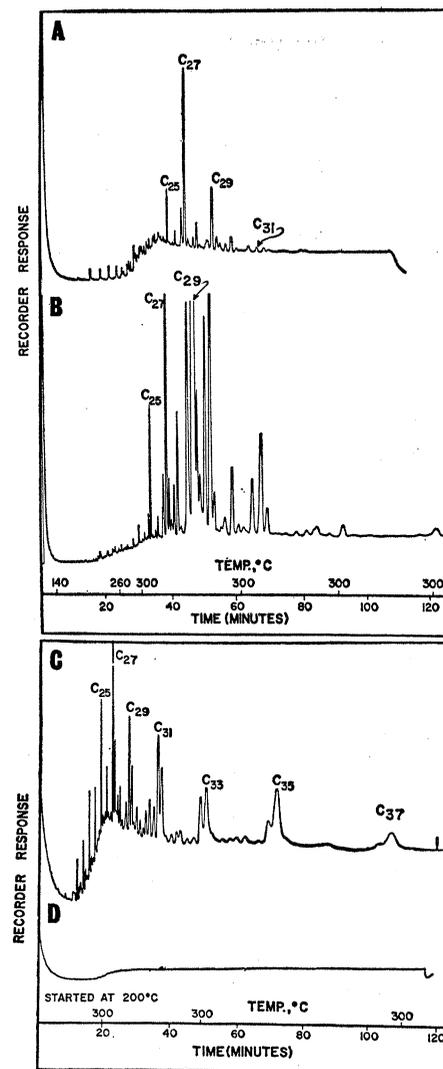


Fig. 1. Gas-chromatographic separations of extracts of chlamydo spores; nitrogen carrier gas. No split was used and attenuations are as indicated. (A) Extract of 2.10 g of *U. maydis* spores; approximately 5 percent of *n*-heptane extract injected; nitrogen carrier pressure, 2430 g/cm. Programmed at 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).