

References and Notes

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26 November 1963

Pyrene and Fluoranthene in Manganese Nodules

Abstract. *The polynuclear aromatic hydrocarbons pyrene and fluoranthene have been isolated from manganese nodules of the western North Atlantic.*

Polynuclear aromatic hydrocarbons occur in many geological materials, including recent marine, inshore sediments (1). The hydrocarbon content of a marine mineral deposit of high redox potential has now been investi-

gated and the four-ring aromatics pyrene and fluoranthene have been isolated.

The manganese nodules used in this investigation were collected from the Blake Plateau (2). Two nodules were examined separately, giving similar results (Table 1).

After grinding to less than 200 mesh, samples were extracted with chloroform and the extracts were chromatographed on activated alumina with graded eluents composed of isooctane, benzene, and diethylether. An ultraviolet spectrum (3) (Fig. 1) of the fraction eluted with a mixture of isooctane-benzene (4:1) showed principal maxima for pyrene and fluoranthene at 334 and 287 m μ .

A gas chromatogram (4) (Fig. 2) indicated many compounds in this fraction. Shoulder A had the retention time of synthetic fluoranthene, and peak B corresponded to pyrene. Each of these components was collected by condensation in glass capillary tubes inserted into the gas chromatograph outlet. The capillaries were then rinsed with isooctane. Ultraviolet absorption spectra (Fig. 1) of the resulting solutions confirmed the presence of pyrene and fluoranthene. Incomplete chromatographic separation accounts for additional peaks in the spectra.

All concentrations given in the table are based on ultraviolet spectra of the total chromatographic fraction containing both hydrocarbons. The absorption maxima mentioned above were used for the calculations, after a correction for high background absorption.

Spectra of all fractions in the alumina chromatogram were examined for the presence of other hydrocarbons; however, none were detected. The other compounds indicated in the gas chromatogram, some in significant concentrations, were not further investigated. They lacked characteristic ultraviolet spectra, which made possible the identification of the hydrocarbons on a microgram scale.

Pyrene and fluoranthene are the two most abundant polynuclear hydrocarbons in sea water, with the latter in larger concentration (5). These compounds may have been adsorbed from the sea water during the formation of the manganese nodules. However, the possibility that they were formed *in situ* by organisms living on the nodules, such as bacteria, cannot be excluded. This finding extends the known range

Table 1. Hydrocarbon concentrations.

Manganese nodules (g)	Hydrocarbons (10 ⁻³ ppm)	
	Pyrene	Fluoranthene
283	2	4
338	6	8

of natural occurrence of polynuclear hydrocarbons and suggests that compounds of this type can be well preserved in strongly oxidized sediments deposited under high redox potentials.

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2. 30°53'N, 78°47'W, 445 fathoms, *Atlantis* cruise No. 266.
3. Cary recording spectrophotometer, Model 14.
4. Aerograph HyFI, model 600, with hydrogen flame detector and linear temperature programmer. Column: 0.3 cm by 1.8 m of 5 percent Dow Corning RTV 502 on 80/100 mesh acid-washed, silanized Chromosorb W.
5. M. Blumer, unpublished data.
6. Supported by research contracts with the U.S. Navy Bureau of Ships and the U.S. Office of Naval Research [Nonr 2196 (00)] and by the grant of a Woods Hole Oceanographic Institution summer student fellowship to one of us (D.W.T.). The manganese nodules were collected by T. R. Stetson. This report is contribution No. 1430 of the Woods Hole Oceanographic Institute.

10 October 1963

Hydrogen in a Tektite Vesicle

A philippinite, No. PO-301, found by E. C. T. Chao at the Rafael Ortega site, Mandalayong, Philippine Islands, was shown by P. D. Lowman, Jr., to have a specific gravity of 2.04, which, taken in conjunction with a mass of 11.10 grams and a presumed specific gravity of the stony material of 2.45 indicates an internal bubble with a volume of 0.89 cm³. When the contained gases were excited by an electrodeless discharge at the Goddard Space Flight Center by the technique of O'Keefe, Dunning, and Lowman (1), the spectrum consisted almost exclusively of the so-called second spectrum of hydrogen, that is H₂, between 6225 and 5949Å every line with an intensity 10 in Gale *et al.* (2) was found; in addition, only one other line, of intensity 8, was measured. Shortward of 5949Å the correspondence was poorer; nevertheless, all lines could be explained as due to H₂, with the possible ex-

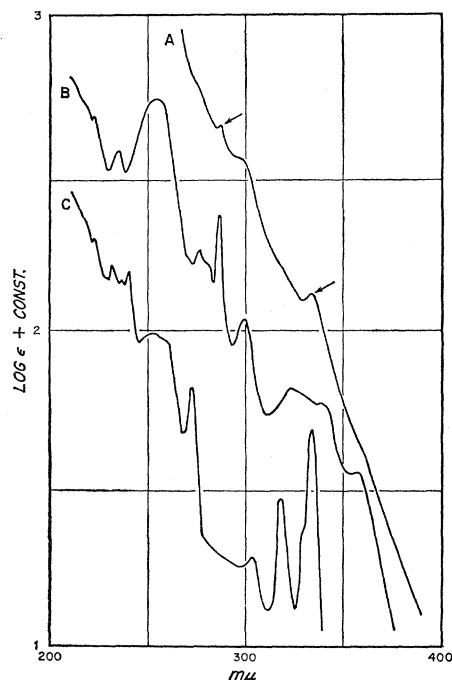


Fig. 1. A, Total isooctane-benzene fraction; B, fluoranthene from GLC; C, Pyrene from GLC.

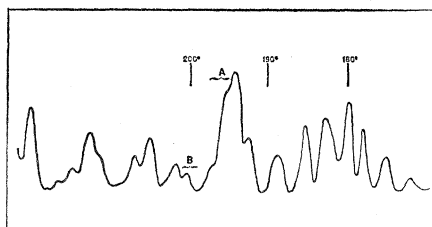


Fig. 2. Gas chromatogram of total isooctane-benzene fraction.

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ception of a line at 5876Å which was probably due to HeI.

The partial pressure of H_2 in the atmosphere is given as 0.5×10^{-6} atm (3). This is probably equilibrated by diffusion through the glass. Since a density as low as this probably could not be detected by our methods, it is likely that the hydrogen is present as a compound, possibly water vapor, which is decomposed by our discharge.

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2. H. G. Gale, G. S. Munk, K. O. Lee, *Astrophys. J.* **67**, 89 (1928).
3. *Handbook of Chemistry and Physics* (Chemical Rubber Co., Cleveland, Ohio, 1963).

11 December 1963

Xanthine Dehydrogenase: Differences in Activity among *Drosophila* Strains

Abstract. In *Drosophila melanogaster*, mutants at two loci are known to lack detectable amounts of xanthine dehydrogenase activity. These are the maroon-like eye-color locus on the X chromosome and the rosy-eye-color locus on the third chromosome ($52 \pm$). A survey was made of the xanthine dehydrogenase content of 98 wild-type strains of *D. melanogaster*. One strain with 25 percent of the xanthine dehydrogenase activity found in normal flies is described. Strains with high xanthine dehydrogenase activity have also been obtained by selection.

Screening strains for differences in amounts of protein or enzyme activity, such as reported by Lewis (1), might result in a class of mutants involved in the regulation of protein synthesis in higher organisms. Xanthine dehydrogenase in the fruit fly, *Drosophila melanogaster*, has been of special interest because of the existence of two separate gene loci, maroon-like (*ma-1*) and rosy (*ry*), which mutate independently to produce eye-color mutants that lack detectable amounts of this enzyme. In order to obtain other mutants controlling the level of xanthine dehydrogenase activity, wild-type strains

of diverse geographic origins were surveyed. In addition, some of these strains were crossed, and the progeny were selected for many generations for high or low xanthine dehydrogenase activity. This paper is a preliminary account of two strains obtained by these methods, one with low enzyme activity, and one with high activity.

Xanthine dehydrogenase was assayed by a modification of the fluorometric technique used for single flies (2). All preparative procedures were carried out at temperatures of 5°C or less. A single fly (or a pair of flies, as in the selection experiments) was homogenized in 4 ml of 0.1M tris buffer, pH 7.5, containing 5 mg of crystalline bovine serum albumin (Armour) per milliliter. Approximately 40 mg of Norite-A (Fisher) was added and the mixture was allowed to stand with occasional stirring for 1 hour. The solution was then centrifuged for 30 minutes at 30,000g. The supernatant was removed and filtered through a coarse sintered glass filter to remove the last remnants of the charcoal. One milliliter of the supernatant was placed into a fluorometer cuvette and 0.02 ml of 1×10^{-3} M methylene blue was added. The solution was allowed to stand for 4 minutes in a Thermolyne dry bath adjusted to 30°C, after which 0.01 ml of 6.67×10^{-4} M 2-amino-4-hydroxypteridine was added. The solution was then mixed and the readings were taken at 2-minute intervals for 10 minutes in a No 540 Photovolt fluorometer equipped with a 347-m μ primary filter (Photovolt) and a 405-m μ secondary filter (Turner, No. 110-812). The fluorometer was initially adjusted so that a standard solution of 1.6×10^{-6} M quinine in 0.1M sulfuric acid read 100 on the photometer scale. The high blank of the enzyme reaction mixture was suppressed by appropriate adjustment of the photometer. In all cases the enzyme assays were performed on randomly coded extracts. During the assay, the cuvettes were returned between readings to the dry bath. One unit of enzyme activity is defined as that amount of enzyme which converts 1 μ mole of 2-amino-4-hydroxypteridine to isoxanthopterin per minute. Under the conditions of the assay the rate of the reaction was linear for at least 30 minutes, and was proportional to enzyme concentration.

Figure 1 shows the distribution of the mean ($\bar{X}$) (three to four individual

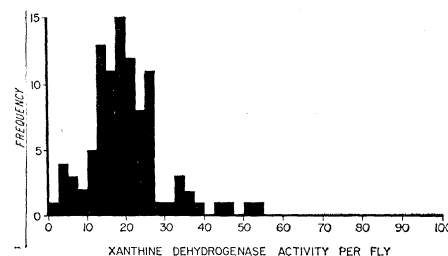


Fig. 1. Distribution of the mean activity of xanthine dehydrogenase in 98 wild type strains of *Drosophila melanogaster*. Three to four flies of each strain were assayed individually for xanthine dehydrogenase activity. The mean activity (units) per fly is plotted against the frequency of occurrence.

females assayed per strain) xanthine dehydrogenase activity in 98 wild-type strains of *D. melanogaster*.

The mean enzyme activities of the 98 wild-type strains of *D. melanogaster* and their source of origin are as follows:

Amherst College: (1) Oregon-R³⁰⁸, 19.1.
Cold Spring Harbor Laboratory: (2) Oregon-R, 16.0.

Institute of Animal Genetics: (3) Oregon-S, 16.3; (4) Pacific, 24.0.

Cancer Research Institute: (5) Amherst-3, 30.7; (6) Canton-SA, 18.2; (7) Crimea, 13.6; (8) Florida, 19.5; (9) Lansanne, 20.2; (10) Oregon-R, 18.9; (11) Oregon-RS, 11.6; (12) Samarkand, 26.0; (13) Seto, 34.4; (14) Swedish-b6, 20.5; (15) Urbana, 22.7; (16) Wageningen-A, 25.1.

Johns Hopkins University: (17) Oregon-RJ, 11.3.

Johns Innes Institute: (18) Hampton Hi U-2, 15.2; (19) Teddington-4, 23.1; (20) Samarkand-3, 14.9; (21) Bayfordbury-5, 13.3; (22) Oregon³⁵⁵, 19.5; (23) Samarkand-8, 12.2; (24) Bayfordbury-1, 25.3; (25) Bayfordbury-B6, 28.2.

Oak Ridge National Laboratory: (26) Canton-S, 44.8; (27) Oregon-R, 13.6; (28) Oregon-RC, 20.6; (29) Samarkand, 26.2; (30) Swedish-C, 25.2.

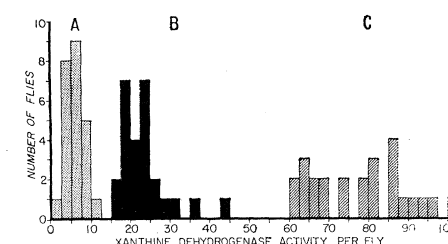


Fig. 2. The distribution of xanthine dehydrogenase activity in 25 flies from three strains of *Drosophila melanogaster*. (A) Strain with low enzyme activity (*lxd*). (B) Standard strain (Oregon-R wild-type). (C) Selected line with high enzyme activity. Twenty-five flies from each stock were assayed singly for xanthine dehydrogenase activity. The mean activity (per fly) is plotted against frequency.