calcite rather than apatite would have been deposited. The supposition that the phosphate content of the water was low is supported by the general paucity of Cretaceous fossil remains, especially of planktonic Foraminifera, in central California. A thriving marine animal community requires waters rich in phosphate. Such waters generally result from upwelling along open coasts. Hence, if the sea in the central California Cretaceous geosyncline was deficient in phosphate, as suggested by wood calcification and scarce marine fossils, it very likely was not receiving upwelled coastal marine waters. This line of reasoning tends to confirm the view of some geologists that the geosyncline was at least partially blocked off from the open sea during much of its existence.

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References and Notes

1. Ignition, under identical conditions, of black, Recent wood from another locality yielded ash that was strongly magnetic. The source of the magnetic iron is presumed to be pyrite in the original sample.
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17 April 1961

Benzpyrenes in Soil

Abstract. Benzpyrenes appear to be common and fairly abundant constituents of soils. Both the carcinogenic 3,4-benzpyrene and its inactive 1,2-isomer have been detected spectroscopically in extracts of soils from rural areas of the eastern United States.

Polynuclear aromatic hydrocarbons are commonly found in recent marine and nonmarine sediments (*1*, *2*). Evidence has now been obtained that both the strongly carcinogenic hydrocarbon 3,4-benzpyrene and its inactive isomer 1,2-benzpyrene are common and fairby abundant constituents of solids.

Table 1. 3,4-Benzpyrene in soils.

Origin and type of soil	Conc. (μg/kg)
Oak forest, West Falmouth, Cape Cod, Mass.	40
Pine Forest, West Falmouth, Cape Cod, Mass.	40
Mixed forest, West Falmouth, Cape Cod, Mass.	1300
Mixed forest, eastern Connecticut	240
Garden soil, West Falmouth, Cape Cod, Mass.	90
Plowed field, eastern Connecticut	900

A number of soils from rural areas of Massachusetts and Connecticut were analyzed. After the samples were dried to constant weight at 110°C, 50 g of each soil sample were weighed and extracted in a Soxhlet by benzene-methanol (1:1). The solvent was then removed in a rotating evaporator, and the remaining extract was treated with warm iso-octane-benzene (3:2). The soluble fraction was again dried, taken up in boiling iso-octane, and immediately adsorbed on an 8-ml column of alumina (Brockman II). The elution was carried out with a graded series of iso-octane-benzene mixtures. In some cases, yellow nonhydrocarbon materials broke through in the hydrocarbon-fractions; they were then removed by rechromatography under identical conditions. The hydrocarbons in the eluates were detected by ultraviolet spectrophometry (Cary model 14 spectrophotomer), with a Sawicki chart (*3*) and a punched-card file of hydrocarbon spectra as aids in their identification. The spectra of 3,4-benzpyrene and 1,12-benzperylene, which both occur in soils, are very similar, but the presence of fine structure in the 383-mμ band together with a high 403-mμ band constitute conclusive evidence for the presence of 3,4-benzpyrene (*3*).

The hydrocarbon assemblage in all soil samples was found to be very similar even if the concentrations varied. The 1,2- and 3,4- isomers of benzpyrene were detected in all samples. Figure 1 is the spectrum of a typical benzpyrene fraction, contaminated with some chrysene (responsible for the background absorption) and some perylene (peaks at 434 and 428 mμ). The concentration of the biochemically active 3,4-benzpyrene was estimated from the intensity of the 403-mμ band (Table 1). In addition to the benzpyrenes, the following hydrocarbons were represented in all samples: phenanthrene, fluoranthene, pyrene, chrysene, perylene, and anthanthrene. Extensive rechromatography of the combined extracts provided evidence for the additional presence of anthracene, triphenylene, benzanthracene, benzfluorene, 1,12-benzperylene, and coronene.

Some soils may contain a much higher concentration of 3,4-benzpyrene than reported here. Kern (*1*), in his paper on the discovery of chrysene in some Swiss soils, describes the isolation of two additional hydrocarbons, not identified with certainty. The reported

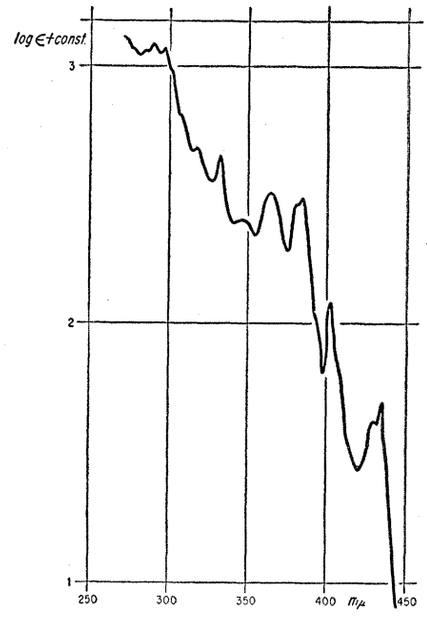

Fig. 1. Spectrum of a typical benzpyrene fraction, contaminated with some chrysene and some perylene (in iso-octane).

spectra and analytical data lead us to conclude that Kern already had isolated 3,4-benzpyrene [melting point: 171°C (benzpyrene: 177°C); molecular weight: 283 (benzpyrene: 252); log $\epsilon_{1\text{ cm}}^{1\text{ percent}}$ (at 296 mμ): 1.7×10^3 (benzpyrene: 2.2×10^3)]. According to Kern, this hydrocarbon was obtained in crystalline form with the remarkable yield of 21 mg/kg of soil.

We believe that the occurrence of such hydrocarbon concentrations in rural soils distant from major highways and industries cannot be ascribed to fallout from polluted air. More likely, these hydrocarbons are indigenous to soil. They are among the pyrolytic products of wood and might be formed in soil by related low-temperature processes, as they also occur in the transformation of plant organic matter to peat and lignite. Alternatively, the hydrocarbons might be the products of the organisms which contribute their organic matter to the soils. These genetic mechanisms—if correct—imply that man has been in contact with carcinogenic hydrocarbons, not only during the industrial epoch, but during his entire history. It remains to be examined whether the concentration and availability of the benzpyrene in soil is sufficient to be of concern to those exposed to continued contact with soil (*4*).

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4. This investigation has been supported by a research contract, NONR 2196(00), with the Office of Naval Research. This report is contribution No. 1193 of the Woods Hole Oceanographic Institution.

4 May 1961

Hemorrhagic Disease in Rodents Infected with Virus Associated with Thai Hemorrhagic Fever

Abstract. KLA 16 virus, recovered from a child with Thailand hemorrhagic fever, produces infant mouse, rat, and hamster disease that is characterized by spontaneous bleeding at multiple sites, notably in the gastrointestinal tract, and by marked abnormalities in hemostatic mechanisms. This virus differs in this respect from Chikungunya virus to which it is immunologically similar and from other Thai hemorrhagic fever viruses.

Human virus infections, characterized by spontaneous hemorrhage, are recorded from widely separated areas of Asia, Europe, and South America. Immunologically distinct agents have been implicated in the etiology of these hemorrhagic fevers, but thus far only the viruses of Kyasanur Forest Disease and Omsk, Philippine, Argentine, and Thailand hemorrhagic fevers have been propagated in laboratory hosts (*1*, *2*). These agents multiply in albino mice and produce fatal encephalitis with minimal apparent involvement of other major viscera. Recently a virus was recovered from the blood of a Thai child who contracted Thailand hemorrhagic fever in Bangkok in May 1960. This agent, KLA 16, was identified as an arthropod-borne virus of Casal's group A, closely related to Chikungunya virus and immunologically identical to TH 35 and BaH 306 viruses recovered from Thai patients by Hammon and Kitaoka, respectively (*2*, *3*). KLA 16 virus differs from these, however, in its capacity to produce hemorrhagic disease in rodents. This report describes the hemorrhage-producing characteristics of KLA 16 virus and the factors thus far known to influence production of the hemorrhagic diathesis in experimental animals.

From the patient's blood obtained on the 2nd day of disease, virus was recovered simultaneously in mice 1 to 2 days old and in trypsinized explants of hamster kidney and rhesus kidney cells. These three isolates were transmissible interchangeably in each of the three host systems and were immunologically identical. Intracerebral passage of infected brain or cell cultures resulted in subcutaneous and marked intraluminal intestinal hemorrhages in approximately 20 percent of inoculated mice. The bleeding tendency has been observed in mice inoculated with virus in as high as 12 mouse and 7 hamster kidney cell passages, with virus obtained after three terminal dilution passages in both suckling mice and hamster kidney cells.

Several factors influenced the occurrence of discernible hemorrhage after inoculation of mice (*4*). After infection with 10 to 100 intracerebral LD_{50}, several age-dependent patterns of disease were observed. Hemorrhage was seen only in mice infected before the 7th day of life. No hemorrhage, and only sporadic deaths were observed between 7 and 21 days of age, and older mice were resistant to lethal infection. The frequency and extent of hemorrhage was greatest in mice 24 to 48 hours old at inoculation. The intracerebral route of infection was more sensitive than subcutaneous or intraperitoneal inoculation for both the production of hemorrhagic disease and for quantitation of infectivity. Hemorrhage rarely followed subcutaneous or intraperitoneal inoculation of any amount of virus, and these routes were 1/100 as sensitive for detecting viable virus as intracerebral inoculation. By passing only virus from brains of hemorrhagic mice three times, it was possible to increase the frequency of overt bleeding from 20 to 90 percent. Thus intracerebral inoculation of 10 to 100 LD_{50} of this final virus seed into mice 24 to 48 hours old was found to be optimal for producing hemorrhagic disease.

Under these circumstances, normal activity of mice decreased about 72 hours after inoculation. All or portions of the small intestine were salmon pink in color, and when viewed under ×20 magnification, marked dilatation and congestion of blood vessels in the wall was seen. Within a few hours, color changed from pink to gray, and as further hemorrhage occurred into the lumen, segments or large portions of the bowel turned black (Fig. 1). Black bowel contents were strongly benzidine- and guaiac-positive; blood in the intestine was always partially digested. Subcutaneous, intra-articular, and bladder wall hemorrhages occurred occasionally. Mice which showed hemorrhagic manifestations usually died within the next 24 hours. The moribund suckling mouse was characterized by congested jugular veins, enlarged heart, progressive cyanosis, and decrease in surface temperature, but no manifestation of disease in the central nervous system was seen. Histologic examination of the bowel failed to reveal specific sites of bleeding but confirmed the congestive changes and showed vacuolar degeneration of the cytoplasm of mucosal cells

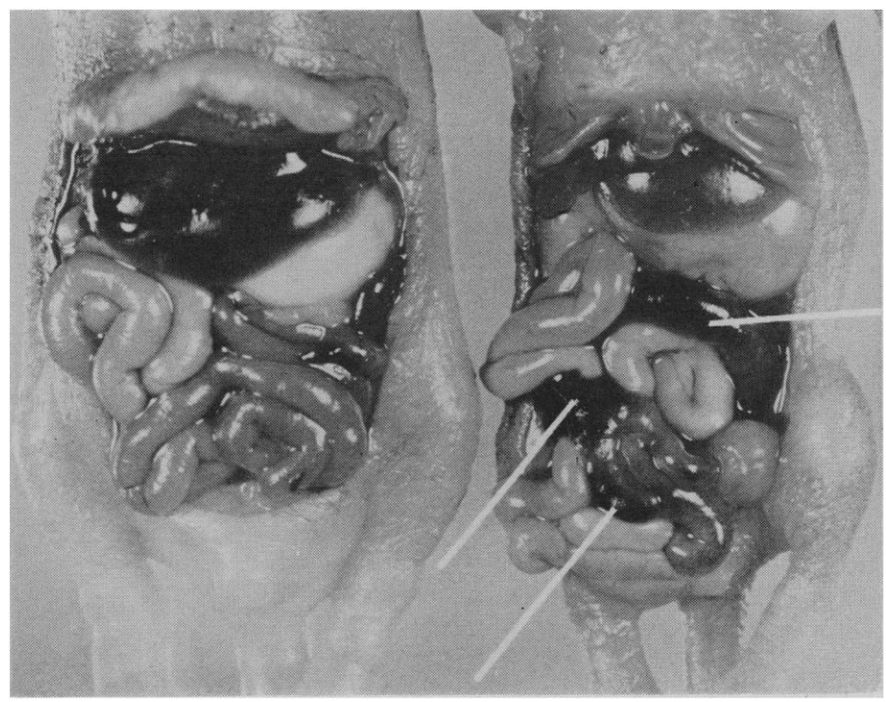

Fig. 1. Hemorrhage into isolated loops of bowel of 5-day old mouse, 72 hours after infection with KLA 16 virus (right). Normal abdominal viscera (left).