wavelengths longer than 408 nm. Inactivation and filament formation were below detection in both visible- and black-light experiments. For comparison, photodynamic mutagenesis with acridine orange also is shown in Fig. 1; addition of an acridine dye to the culture substantially increases the mutation rate from that for light alone.

The mean delay in expression of the light-induced T5-resistant mutants was approximately four generations—a delay of about 20 hours when the cell-division time was 5 hours (Fig. 1). This value is substantially greater than the mean delay of two generations observed for photodynamic mutagenesis with acridine dyes, but less than the value of 5.2 generations observed for caffeine-induced mutagenesis (*4*).

The mutation rate under anoxic conditions (95 percent N_2, 5 percent CO_2) for chemostat cultures irradiated with white fluorescent light (with a 424-nm "cut-off" filter) at an irradiance of 57 $\mu w/mm^2$ was 3.1 (S.E., ± 0.5) mutants per 10^9 cells per hour—similar to the spontaneous mutation rate under anoxia. However, with black light at an irradiance of 11 $\mu w/mm^2$ under the same anoxic conditions, the mutation rate was 12 (S.E., ± 2) mutants per 10^9 cells per hour, a value significantly greater than the dark mutation rate and one-third the rate obtained with black light at this intensity in the presence of oxygen.

This oxygen requirement may be consistent with a photodynamic mechanism of action for mutagenesis, but no conclusions are possible at present because the amount of the chromophore(s) present may be much smaller under anoxic conditions.

Effects of long-wave ultraviolet (300 to 400 nm) on DNA (*6*) and cells (*7*) have been reported. Since DNA shows some absorption at 310 nm, at least some of the effects of black light, reported by us and by others, may involve direct absorption by DNA. However, our preliminary data suggest that the major chromophore for black-light mutagenesis is not DNA. Black-light mutagenesis has also been observed in an ultraviolet-sensitive strain of *E. coli* in chemostat cultures by Kubitschek of our division (*8*).

The nature of the chromophore(s) is of special interest because of its (their) possible association with the DNA of the cell. Several components of *E. coli* are possible candidates for the chromophore. Riboflavin, a planar three-ring molecule similar to the acridines, conceivably could bind to DNA through intercalation as Lerman has suggested for other such planar molecules (*9*). Riboflavin is photodynamically active in certain systems in vitro (*10*). However, vitamin K and any of several porphyrins also are possibilities for the chromophore.

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Paramagnetic Resonance Spectra of Methyl Radicals on Porous Glass Surfaces

Abstract. *Methyl radicals stabilized on surfaces of porous Vycor glass at 77°K show three types of paramagnetic resonance spectra. One of them represents physically trapped radicals, whereas the other two types indicate interactions with surface sites.*

Recent interest in the stabilization of free radicals on solid surfaces prompts us to report further results of our electron paramagnetic resonance studies of methyl radicals, CH_3, on surfaces of porous Vycor glass (Corning 7930) at 77°K. The experimental conditions were similar to those described in our previous report (*1*).

Methyl radicals, when generated by ultraviolet photolysis of adsorbed methyl iodide, are stabilized in quantity on porous glass surfaces over a wide range of temperatures. It would be very interesting if such radicals exhibited in their electron paramagnetic resonance spectra an additional structure due to a hyperfine interaction with atomic nuclei at the trapping sites, which would help us to understand the behavior of the solid surface.

The spectrum of CH_3 usually consists of four lines with the intensity ratio 1 : 3 : 3 : 1. In the case of a monolayer of the adsorbate, an apparent asymmetrical intensity distribution was reported (*1*, *2*). However, we have come to the conclusion, after careful measurements of line widths, that the intensity ratio is actually 1 : 3 : 3 : 1. The line widths of the component lines were 1.8, 1.2, 1.1, and 1.0 ± 0.2 gauss, in order of increasing resonance field. The difference in line widths of hyperfine components may be explained as an effect of a time correlation in randomly tumbling motion of the radical in the presence of a trapping potential (*3*). However, such an explanation does not help to reveal the nature of the surface site. The observed hyperfine constant $A_1 = 23.4 \pm 0.2$ gauss, and g-factor $g_1 = 2.0024 \pm 0.0001$ are very much the same as those for CH_3 in an inert-gas matrix (*4*); therefore, such CH_3 radicals are believed to be "physically" trapped on the surfaces.

When a very small quantity of methyl iodide (less than 10 percent of a monolayer) was adsorbed and photolyzed at 77°K, two other types of spectra were observed in addition to the spectrum due to physically trapped CH_3. These extra spectra, denoted by Me′ and X in Fujimoto *et al.* (*1*), were apparently independent, and in Fig. 1, showing a typical spectrum, a superposition of three spectra is indicated.

The spectrum X appeared most significantly on glass samples outgassed at about 450°C prior to adsorption, but almost undetectable on samples preheated at 700°C or above for a prolonged period of time. It was also recognized that the intensity of the spectrum X relative to the main quartet spectrum became smaller as the surface coverage exceeded 10 percent. The spectrum X is interpreted as being due to a CH_3 radical interacting with a nucleus of spin 3/2, which is most likely the boron-11 isotope, B^{11} (natural abundance 81.17 percent), on Vycor surfaces.

The Vycor glass used for this experiment contains B_2O_3 as a major impurity (approximately 3 percent). Boron impurities may form active sites or inactive boron oxide networks on silicate surfaces, the former being responsible for catalytic activities (*5*). A methyl radical, when trapped at such

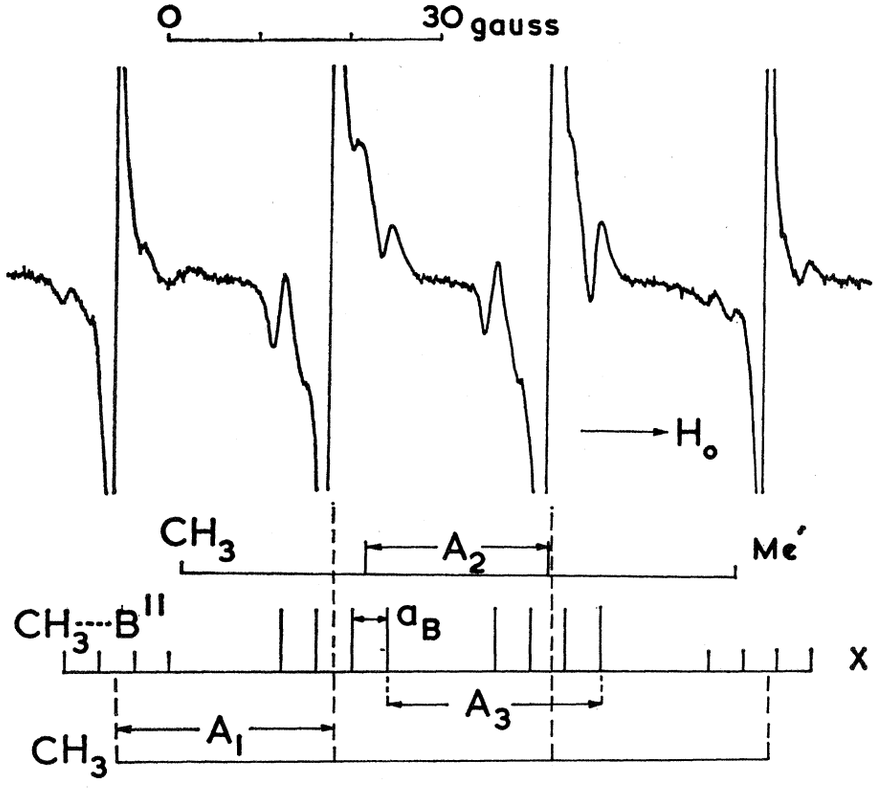

Fig. 1. Electron paramagnetic resonance spectrum of radicals from the photolysis of CH_3I on porous Vycor surfaces, preheated to 450°C for 4 hours. The surface coverage was 2 to 3 percent. Note that there is an indication of another type of CH_3 (Me'), which could be observed more clearly at early stages of irradiation.

an active site, may form a weak one-electron bond with the boron, giving rise to an additional four-line structure on each proton hyperfine line of CH_3. The observed hyperfine constants for such a radical X are $A_3 = 23.1 \pm 0.2$ gauss for the proton coupling, and the $a_B = 2.6 \pm 0.2$ gauss for the boron coupling constant. Note that the proton constant is slightly smaller than A_1, indicating a shift of a small amount of spin density to the B^{11} nucleus.

The above interpretation seems reasonable, judging from the catalytic property of silica-alumina in which aluminum impurities play essential roles. The maximum catalytic power is obtained when the surface of silica-alumina is dehydrated at about 450°C. One may thus conclude that some CH_3 radicals are interacting with such active boron sites.

A separate experiment was also carried out on a porous Vycor surface that was reacted with boron trichloride, and subsequently the B–Cl bonds hydrolyzed to B–OH and then dehydrated. The boron atoms thus introduced onto Vycor surfaces were presumably inactive, and electron paramagnetic resonance spectra of CH_3 showed no indication of boron interaction. It is also noted that there is no spectrum of Me' radicals in this case.

The presence of a boron hyperfine interaction in Vycor glass has already been demonstrated by Muha and Yates (*6*) in their papers on γ-irradiated Vycor. Therefore, our finding of a boron interaction in CH_3 spectra is not surprising.

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Alkaline Phosphatase and Leucine Aminopeptidase Association in Plasma of the Chicken

Abstract. *Two electrophoretic variants of leucine aminopeptidase show direct association with two genetically controlled forms of alkaline phosphatase. Treatment of plasma with neuraminidase converted the faster-migrating form of both enzymes to slower-moving forms, but plasmas with slower-migrating forms were unaffected by this treatment. The two forms of the enzymes may be due to the presence or absence of a single gene controlling the attachment of sialic acid to the enzyme molecules.*

Alkaline phosphatase in the plasma of the chicken has two genetically controlled variants when examined by starch-gel electrophoresis (*1*). The observation that the gene controlling the faster-moving form (Ap^2) was dominant over the slower-moving form (Ap^1) has been confirmed (*2*). Continuing efforts to elucidate genetically controlled variations in plasma enzymes have shown that different molecular forms of leucine aminopeptidase also exist among plasma samples from chickens of inbred lines. Leucine aminopeptidase is defined as an enzyme capable of hydrolyzing 1-leucyl-β-naphthylamide-HCl (*3*). Zymograms (*4*) show two characteristic zones of activity within a single sample. A leading band appears uniform in all chicken plasmas. A second band is seen in one of two positions, one closer to the origin than the other.

Electrophoresis was carried out for both leucine aminopeptidase and alkaline phosphatase of the blood from 750 chickens from several inbred lines and closed populations. In all samples, the classification of the forms of leucine aminopeptidase corresponded to the classification of mobility of alkaline phosphatase; that is, all samples showing the fast form of leucine aminopeptidase also exhibited the fast form of alkaline phosphatase (Ap^2). To verify that the two staining patterns were the result of independent reactions, some gels were stained consecutively with both substrate mixtures to give a doubly stained zymogram (Fig. 1). These tests clearly showed that the two enzymes had similar but distinctly different locations after electrophoresis. The fast form of alkaline phosphatase was slightly anodal to the fast form of leucine aminopeptidase. As observed in