Free-Radical Concentrations and Other Properties of Pile-Irradiated Coals

Abstract. Five coals reacted quite differently when they were exposed to pile-irradiation. Little or no change was found in free-radical content for the three coals of lowest carbon content, whereas the two coals of highest carbon content were found to have a considerable increase in free-radical content. The infrared spectra and the apparent hardness of the irradiated coals of higher carbon content indicate that polymerization occurred. Radiation of these coals in chemical reagents may promote reactivity.

Five coals ranging in rank from lignite to low-volatile bituminous coal have been exposed to radiation from the reactor at Brookhaven National Laboratory (*1*). Small samples (5 gm) of each coal were exposed under vacuum in aluminum cylinders for approximately 120 days to an integrated flux of 2.29×10^{19} slow neutrons per square centimeter. During this period the pile was operated at 24 Mw, the flux at the exposure site was 2.5×10^{12} neutrons per square centimeter per second, and exposure temperature was below 50°C. Irradiation was primarily by slow and fast neutrons with attendant hard beta- and gamma-radiation; presumably gamma-heating of the coals was slight. Data for the coals are shown in Table 1.

Ingram and Uebersfeld and their coworkers (*2*), carried out electron paramagnetic resonance measurements on coals and ascribed their results to the occurrence of free radicals. The irradiated coals have similarly been examined by electron paramagnetic resonance to determine the effects of radiation on their respective free-radical contents. Measurements were made on a conventional Varian EPR spectrometer at a frequency of 9.5×10^3 Mcy/sec with fields of around 3300 gauss. Various microwave power outputs down to 1 mw were used in order to avoid errors due to saturation of the sample.

The effects of radiation on the free-radical content of the low-rank coals—those having less than 83 percent carbon—appear to be negligible under the conditions described. Comparatively large increases in free-radical content were observed for coals of higher rank—those containing 89.3 and 90.5 percent carbon. The structures of these coals are apparently amenable to the formation of permanent free radicals by irradiation. The difference in irradiation effects between the high- and low-rank coals is not explained; there are, however, definite differences in both physical structure and chemical behavior between coals of 90-percent carbon content and coals of lower rank.

Widths of the electron resonance absorption lines can give information on the environment of the free-radical electron or on changes in that environment with irradiation. But no large changes in line width were found as a result of irradiation. The greatest percentage decrease in line width produced by irradiation was found for the 90.5-percent carbon coal, 1.4 to 1.1 gauss (*in vacuo*). In the presence of air the 90.5-percent carbon coal had a line width of 6.0 gauss, presumably because of interaction of molecular oxygen and the free-radical electrons. All other coals showed only slight differences in line width with the presence or absence of air.

Features of infrared spectra of coals have been discussed by Brown (*3*) and by Friedel and Queiser (*4*). Infrared spectra of the irradiated coals (Fig. 1) were more diffuse than those of the unirradiated samples, but the wavelengths and the relative intensities of the absorption bands were unchanged; this finding indicated that no appreciable changes in rank had occurred.

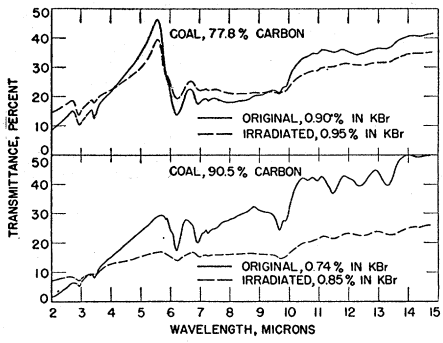

Fig. 1. Infrared spectra of irradiated coals.

The diffuseness of the spectra indicates appreciable polymerization. Radiation had the least effect on the infrared spectra of lignite (69.9 percent carbon) and subbituminous coal (77.8 percent carbon) (Fig. 1); the spectra of the coals of higher rank were more diffuse (Fig. 1, 90.5-percent carbon). As was to be expected, the infrared spectra provided no information on the nature of the free radicals produced by irradiation because these radicals apparently do not affect the vibration spectrum other than by possibly producing some electronic background absorption.

Upon preparing specimens for infrared studies it was noted that the hardness of the coals had changed drastically as a result of irradiation; irradiated coals were much more difficult to grind than unirradiated samples. Although no measurements of hardness were made, it would appear that the polymeric structure of the coals was increased by the radiation; this was also indicated by the infrared absorption spectra. Recent investigations of uranium-bearing coals demonstrated that radiochemical dehydrogenation results from α-particle irradiation and that polymerization probably occurs (*5*).

The increase in free-radical content and the apparent polymerization of the irradiated 90-percent-carbon coals suggest the possibility that these coals are highly reactive with chemical agents after irradiation. When unirradiated coals containing about 80 to 90 percent carbon were treated with lithium ethylamine (*6*) and lithium ethylenediamine (*7*), the coals containing 90 percent carbon had the highest concentrations of free radicals and were the most reactive. This high reactivity was paralleled by the largest decrease in concentration of free radicals. Such reactions may be promoted by radiation *in situ* (*8*).

Table 1. Free-radical concentrations and spectral-line widths for coals (*in vacuo*) before and after irradiation. Analytical data given on a moisture- and ash-free basis.

Item	Coal*									
	A		B		C		D		E	
	Orig.	Irrad.	Orig.	Irrad.	Orig.	Irrad.	Orig.	Irrad.	Orig.	Irrad.
Free radicals/gm $\times 10^{18}$	1.2	1.2	3.9	3.7	5.1	4.6	10.1	18.3	14.2	18.7
Line widths (gauss)	6.5	6.6	6.5	6.4	7.8	7.0	1.2	1.2	1.4†	1.1
Ultimate analysis (%)										
Carbon	69.9	69.4	77.8	77.0	82.4	78.4	89.3	86.9	90.5	88.6
Hydrogen	4.5	4.3	4.8	4.5	5.6	5.1	4.9	4.6	4.5	4.4
Nitrogen	1.0		1.7		1.7		1.8		1.3	
Oxygen (difference)	23.9		15.2		9.6		3.4		3.0	
Sulfur	0.7	0.6	0.5	0.6	0.7		0.6	0.6	0.7	0.5
Proximate analysis (%)										
Volatile matter	47.1	43.9	36.1	35.0	39.8	30.3	23.5	22.3	16.6	17.7
Fixed carbon	52.9	56.1	63.9	65.0	60.2	69.7	76.5	77.7	83.4	82.3
Calorific value (Btu/lb)	11,550	11,500	13,320	12,950	14,790	13,770	15,590	15,120	15,620	15,450

*Coals: A, lignite from Dakota Star mine, Mercer County, N.D.; B, subbituminous coal from Washington mine, Weld County, Colo.; C, high-volatile A bituminous coal from No. 3 Elkhorn mine, Breathitt County, Ky.; D, medium-volatile bituminous coal from Garden Ground mine, Fayette County, W. Va.; E, low-volatile bituminous coal from Slab Fork No. 8 mine, Raleigh County, W. Va.
†The line width for this coal in air is 6.0 gauss. No other large changes were observed with irradiation or with the presence of air.

R. A. FRIEDEL
U.S. Bureau of Mines, Pittsburgh, Pennsylvania
IRVING A. BREGER
U.S. Geological Survey, Washington, D.C.

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Selective Phagocytosis of Nucleated Erythrocytes by Cytotoxic Amebae in Cell Culture

Abstract. Strains of *Acanthamoeba,* which produce cellular damage resembling viral cytopathic effect, are known to occur in cultures of monkey kidney cells. Trophozoites of a similar strain were observed to engulf and denucleate chicken erythrocytes. Nonnucleated guinea pig erythrocytes were apparently left unchanged.

Recently two virus research groups (*1*) have reported on the occurrence of amebae of the genus *Acanthamoeba* as natural "contaminants" in monkey kidney cultures. These amebae were cytotoxic, producing cellular damage not unlike viral cytopathic effect. A similar strain of amebae was isolated in our virus laboratory. An accidental observation of the remarkable behavior of these organisms in relation to nucleated and nonnucleated erythrocytes should be recorded as information of possible importance to other investigators.

We isolated the amebae in 1957 from one of the tubes in a large group of rhesus monkey kidney cell cultures which were under observation for visible viral effects. Six days after the replicate monolayer tube cultures were prepared by the usual trypsinization method, this particular tube was inoculated with a routine throat swab specimen from a healthy child who was in contact with a febrile patient under surveillance. Nine days later (the 15th day after trypsinization) early cellular damage was noted near the borders of the sheet. This resembled the cytopathic effect produced by the enteroviruses, although progression of the cellular degeneration was noted to be unusually slow. Serial passages of the culture fluid were made; by the tenth passage the characteristic effect made its appearance on the second day with complete degeneration of the cellular sheet within a week. Although with passage the incubation period was thus markedly shortened, the titer of the culture fluid, using the cytopathic effect endpoint, was maintained at 10^8. Repeated attempts at reisolation from the original human specimen were unsuccessful.

It became apparent that this was not a known enterovirus, and we initiated additional studies. The monkey kidney passaged agent was found to grow well (as evidenced by the appearance of the specific effect) in monolayer cultures of human amnion, chorion, and HeLa cells; the shortest (overnight) incubation period was observed in dog kidney cell cultures. To elucidate further the nature of the presumably viral agent, we attempted the hemadsorption procedure, which at that time was being developed in our laboratory (*2*), on some of the tubes with visible cellular degeneration. Chicken and guinea pig erythrocytes were added to the monkey kidney culture tubes which were examined under low magnification (×150) for the characteristic hemadsorption patterns. We did not find hemadsorption with either type of erythrocyte, but clumping, suggestive of hemagglutination, occurred in the tubes to which chicken erythrocytes were added. The clumping apparently was caused by small round bodies which at first were assumed to be detached renal cells.

The possibility that the agent was a strain of cytotoxic amebae was considered, and we examined the tubes under higher magnification (×795) (*3*). The peripheral portions of the kidney cell sheet were found to contain intracellular and extracellular thick-walled cysts approximately 20 μ in diameter; some of the renal cells contained several cysts crowding the cell nucleus to the side; these bulging cells occasionally ruptured, releasing the cysts into the nutrient fluid which already contained numerous motile trophozoites.

Both types of erythrocytes were then added to hanging drop preparations of culture fluid to study the clumping of erythrocytes. Addition of nonnucleated guinea pig erythrocytes caused little change: the trophozoites showed no visible reaction or merely "palpated" nearby red blood cells with their pseudopodia; an occasional trophozoite engulfed a guinea pig cell but promptly rejected it seemingly unchanged. Addition of nucleated chicken red cells produced the characteristic clumping: the freely moving trophozoites attached to the erythrocytes and engulfed them. After a variable period of time the amebae ejected misshapen, apparently completely *denucleated,* erythrocytes.

Limited attempts at characterization of the pseudo-viral agent disclosed that it was removed completely from the cell culture fluid by filtration (Selas 03), by centrifugation at 2000 rev/min for 30 minutes (International No. 2) and by heating in a water bath at 56°C for 3 hours (although not after 1 or 2 hours). The amebae were preserved in the culture fluid at room temperature for at least 13 weeks; at 37°C for 2 weeks; at 4°C for 7 weeks and at −50°C for 8 weeks. The incubation period, as measured by the appearance of specific cytotoxic changes, was considerably prolonged with storage at lower temperatures.

The amebae were identified by Leon Jacobs as belonging to the genus *Acanthamoeba* (*4*). Our findings provide further evidence that the previous reports (*1*) of the isolation of the *Acanthamoebae* from monkey kidney cultures were more than spurious observations of air contamination of the culture material or of a unique presence of the organisms in certain monkey organs; on the contrary, this might be a common occurrence generally overlooked.

Awareness of these repeated experiences may prompt the virologist who finds unusual cytotoxic effects to consider cytotoxic amebae rather than a bizarre viral agent. The remarkably selective behavior of the trophozoites in denucleating chicken erythrocytes may provide another clue and a tool for investigation of metabolic requirements of artificially propagated amebae.

LOTTA CHI
JOHN E. VOGEL*
ALEXIS SHELOKOV*

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

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3. Oculars (15×) and a special oil immersion lens (53×) by Galileo were used.

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* Present address: Middle America Research Unit, Balboa Heights, Canal Zone.

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