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FREE RADICALS AS A POSSIBLE CAUSE OF MUTATIONS AND CANCER*

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Abstract—The hypothesis set forth in this note is that free radicals produced outside the body may find their way into the body and produce mutations and/or cancer. The evidence for support of this hypothesis is the presence of radicals as detected by microwave paramagnetic resonance in several carcinogenic agents, and the fact that free radicals are now recognized by radiobiologists as being responsible for a large portion of mutagenic and carcinogenic effects of ionizing radiations.

FREE radicals may be loosely defined as molecular fragments which are characterized by a free valence or an unpaired electron. Because of their highly reactive nature they are not thought to exist in any significant quantity within the organic matter about us, although they are often postulated as important, transient intermediaries in organic and biochemical reactions. Within the past few years, however, microwave spectroscopists (1) have shown that free radicals can be readily detected in organic matter which has been subjected to some form of pre-treatment that can break chemical bonds. Such free radicals are produced in the combustion of organic matter—wood, paper, tobacco, coal, oil. They are produced in excessively cooked foods such as charred steak or scorched toast. They are produced in various forms of matter by ultraviolet light, by x-rays, or by atomic radiation.

The radicals are detected through their resonant absorption of microwave or radio-wave energy when they are placed in a magnetic field of the proper strength. This type of absorption spectrum is known as paramagnetic resonance or as electron spin resonance (2). Electrons in normal chemical bonds are paired in such a manner that their spins and magnetic moments cancel, and hence they exhibit no paramagnetic absorption. Paramagnetic resonance occurs only for the unpaired electrons of the disrupted chemical bond. It therefore provides a specific and powerful means of detecting and studying reactive free radicals within organic matter without interfering absorption or confusing signals from the normal stable molecules of the matter.

The surprising new evidence from paramagnetic resonance is not that free radicals can be easily produced but that they become trapped and stabilized and can be transported from place to place, even through the air within tiny particles of solid matter such as those in smoke. The nature of neither the radicals nor their cages is yet known definitely, although some radicals produced

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in amino acids and proteins by x-irradiation have been tentatively identified from the fine structure of their microwave resonance patterns (3). The information pertinent to the present discussion is that organic radicals produced by physical forces such as heat or irradiation outside the body can be taken into the body through the processes of eating, smoking, or normal breathing, or even by diffusion through the skin. Once inside the body, these radicals may themselves penetrate the cells or they may be converted to other radicals which do so. A radical containing an odd number of electrons must, in effect, meet and react with another radical before its free valence or uncancelled electronic moment is nullified. If it reacts with a normal organic molecule (which has an even number of electrons), another radical is produced. In fact, it is just this odd character which suggests that a lone radical might start a significant chain of events within a cell.

Many types of radicals which have been detected by microwave resonance are stabilized mainly within solid particles of matter. Normal chewing and mixing of food with saliva would tend to destroy them. This destruction may not always be complete, however. We have made tests which show that ordinary chewing of charred toast, beef, and other foods does not entirely kill the resonance signal of the radicals. Extremely small solid particles carrying radicals may diffuse into the tissues of the skin, stomach, or lungs where they would gradually dissolve and perhaps bring about damaging reactions as their radicals are released. Furthermore, these radicals are possibly stable in certain organic solvents which dissolve the solid cages and 'float' the individual radicals into the tissue. Such a solvent might assist in the production of cancer without being a primary cause of it. Strong resonances, like that shown in Fig. 1 for tobacco tar, are found for wood tar, coal tar, and other tars. H. Shields and the author have dissolved tars in organic solvents including benzene, acetone, and croton oil, and have found that the resonance of the tar radical remained strong. The role of agents such as croton oil, which are not themselves carcinogenic agents but which augment the effects of certain carcinogenic agents, may be that of facilitating the entrance of carcinogenic radicals into the body.

Radiobiology experiments (4) indicate that much of the effect of ionizing radiations on the cells themselves may be indirect; that is, irradiation produces a free radical in one part of the cell which later migrates to a more vital part of the cell where it may react to bring about a mutation. Alternately, the first radical formed may react to form a second radical, or a third, which finally causes the mutation. In particular, OH and OOH radicals have been postulated as important intermediaries in radiation damage. Of course a mutation might be brought about by a so-called direct hit, but indirect effects also appear to have significant consequences. We are proposing an extension of the indirect effects to include cases where the primary irradiation occurs entirely outside the injured body. In our laboratory, microwave evidence has been obtained to indicate that hydrocarbon radicals, R, produced by irradiation, are often converted to peroxide radicals, ROO, where they come in contact with oxygen. In the tissue such radicals might be further converted to the OH or OOH radicals, already under suspicion by radiobiologists.

The striking evidence which prompted this communication is the abundant paramagnetic resonance data for the existence of free radicals in many agents

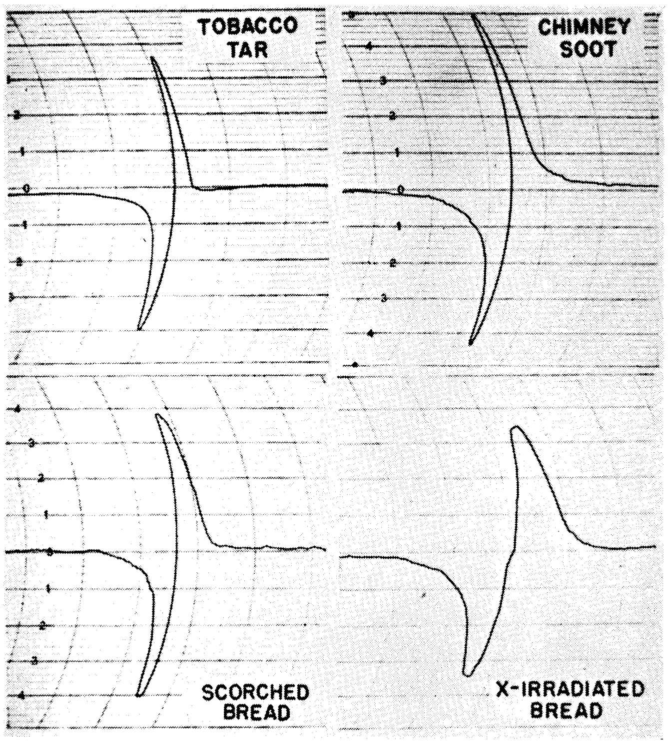

FIG. 1. Microwave electron spin resonances of radicals in some common substances. The tobacco tar was taken from an old pipe stem. Coal, wood, and other tars give similar resonances. The chimney soot was taken from the flue of an oil-burning furnace. Similar resonances were obtained for soot taken from the exhaust pipe of an automobile and from a wood-burning fireplace. Ordinary bread, unscorched and not irradiated, gave no detectable resonance in the same spectrometer.

known or suspected to cause cancer. Among these are cigarette smoke, tobacco tars, various other tars, exhaust fumes from cars, smoke from home furnaces or industrial plants, and charred foods. I shall not attempt to cite the literature references for the various evidences that these are carcinogenic agents. It is well known that x-rays and other ionizing radiations can cause genetic mutations and are likewise carcinogenic agents. It is now well known from electron spin resonance that these ionizing radiations also produce radicals which in many biochemical solids (3) (including various proteins, carbohydrates, and fats) persist for long periods after the irradiation.

The carcinogenic effects of severe chemicals which produce burns of the flesh may possibly result from subsequent diffusion into the healthy cells of free radicals produced in the original, more violent chemical reaction causing the burn. It is known that a burn of the flesh from any source of heat has carcinogenic and mutagenic effects. Since we now know that the charring of any organic matter produces long-lived radicals, it seems probable that some of the carcinogenic and mutagenic effects may result from secondary activity of radicals produced by the burn. Of course chromosome linkages are broken as direct effects of the heat, but it seems probable that most of the cells exposed to the elevated temperatures in the burned area would be killed.

Certainly many known carcinogenic chemicals are not radicals, and I do not suggest that all cancer may be caused by radicals. However, many chemicals recognized as carcinogenic agents, not themselves radicals, may exert their carcinogenic activity indirectly through the production of radicals within the body. This would be analogous to the indirect effects of ionizing radiations already mentioned and might account for the seemingly parallel action of certain chemicals with ionizing radiations which has led to their being called radiomimetic chemicals (5). Many carcinogenic chemicals are large, aromatic, polycyclic hydrocarbons from which it would seem that free radicals might be easily produced.

The radicals are not convicted from 'guilt by association' with carcinogenic agents. Our proposal is not intended to be accepted *per se*, but is offered as a working hypothesis which can be put to rather objective test because of the powerful method of electron spin resonance now available for detection of radicals. That certain radicals are likely to be carcinogenic agents, or that some types can lead to genetic mutations, probably will not be questioned. Others, possibly some or all of those which are sufficiently stable in organic matter to be detected with paramagnetic resonance, may be perfectly harmless. I do not therefore recommend that we become suddenly alarmed about the radicals around us. I do think there is some justification for the careful study of these radicals which can be produced, transported, and taken into the body so easily. This study is made easier by the powerful new method of paramagnetic resonance for detection of such radicals.

If externally produced radicals are indeed dangerous, we can fortunately detect and avoid most of the ones we now are eating, breathing, or rubbing into our skins. INGRAM (1) has shown that the number of radicals produced by heating organic matter is a sensitive function of temperature. Tests in our laboratory on common foods such as meat and bread show that those cooked in a normal manner have no detectable resonances or only very weak resonances, whereas burned food, scorched toast, charred steak, etc., have strong radical resonances. The temperature at which a cigarette is burned should have significant effect upon the number of radicals produced, although it may be impossible to produce smoke without producing radicals. If it proves harmful, we do not have to preserve our food by atomic irradiation.

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REFERENCES

1. See, for example, comments by:
   G. E. PAKE, J. E. INGRAM, J. CAMBRISSON, and J. UEBERSFELD, R. LIVINGSTON, W. GORDY *et al.*: *Disc. Faraday Soc.* **19**, 179, 184 (1955).
2. General treatments of the subject are given in:
   W. GORDY, W. V. SMITH, and R. F. TRAMBARULO: *Microwave Spectroscopy*, Chap. 5, J. Wiley and Sons, New York (1953).
   W. GORDY: Microwave and radio-frequency spectroscopy. In: *Techniques of Organic Chemistry*, Vol. 9, 71–185, ed. by A. Weissberger and W. West, Interscience, New York (1956). D. J. E. INGRAM: *Spectroscopy at Radio and Microwave Frequencies*, Butterworths, London (1956).
3. W. GORDY, W. B. ARD, and H. SHIELDS: Microwave spectroscopy of biological substances. I. Paramagnetic resonance in x-irradiated peptides; and II. Paramagnetic resonance in x-irradiated carboxylic and hydroxy acids. *Proc. Nat. Acad. Sci., Wash.* **41**, 983–1004 (1955).
4. E. C. EVANS: Effects of hydrogen peroxide produced in the medium by radiation on spermatozoa of *Arbocia punctulata*. *Biol. Bull.* **92**, 99–109 (1947).
   O. WYSS *et al.*: Role of peroxide in the biological effects of irradiated broth. *J. Bact.* **56**, 51–57 (1948).
   W. M. DALE: Some aspects of the biochemical effects of ionizing radiations. In: *Symposium on Radiobiology: The Basic Aspects of Radiation Effects on Living Systems*, 177–178, ed. by J. J. Nickson, J. Wiley, New York (1952).
   R. E. ZIRKLE: Speculations on cellular actions of radiations. In: *Symposium on Radiobiology: The Basic Aspects of Radiation Effects on Living Systems*, 333–356, ed. by J. J. Nickson, J. Wiley, New York (1952).
5. A. HADDOW: Comparative studies of the biological effects of ionizing radiation and of radiomimetic chemical agents. *Proc. Int. Conf. Peaceful Uses of Atomic Energy*, **11** 213–218, United Nations, New York (1956).