Attack in the Dark of Night

Melanin fragments formed hours after sun exposure may damage DNA and cause skin cancer

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Skin pigment, known as melanin, can fragment and form highly reactive chemical compounds when impacted by sunlight. According to a study involving Brazilian researchers published in the February 20, 2015, edition of *Science*, these compounds can damage the structure of the DNA molecule, which sits in the cell's nucleus, leading to skin cancer. The study says the attack on DNA can continue for more than three hours after direct exposure to sunlight, a sign of yet another limitation of sunscreens applied to protect the skin against the damaging effects of sunlight's ultraviolet radiation.

"Sunscreen is not going to completely prevent DNA damage, which continues even after sun exposure," according to chemist Etelvino Bechara, a senior professor at the University of São Paulo (USP), one of the authors of the study and the researcher responsible for several related thematic projects funded by FAPESP on the impact of free radicals. This study is also connected to the National Institute of Science and Technology (INCT) for Redox Processes in Biomedicine, coordinated by Ohara Augusto of the USP Chemistry Institute, with the support of FAPESP and the federal government.

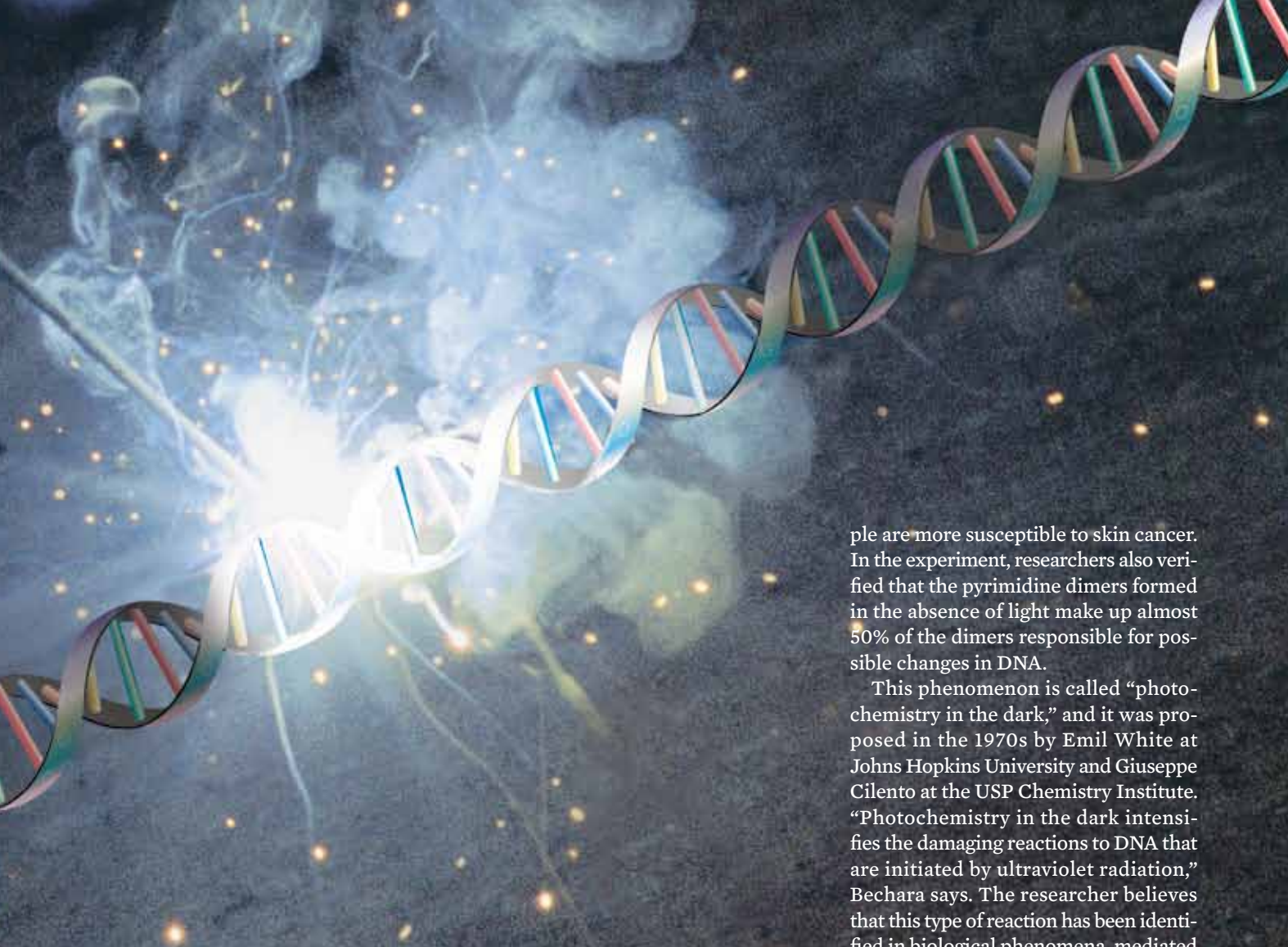
Based on the research findings, Bechara recommends even more caution with artificial tanning and notes the urgent need for lotion formulas that can prevent the formation of compounds harmful to DNA even after sun exposure. The study suggests that one approach to reducing this type of damage is the use of sorbic acid, a food additive. However, its effectiveness, dose and method of application have not yet been determined. Another possible way to minimize sun damage, aside from using ultraviolet radiation filters, would be to use Vitamin E, which is already in some cosmetics.

In early 2012, Bechara received an e-mail from Douglas Brash at Yale University requesting his collaboration in finding a solution to problems related to DNA damage in melanocytes, the cells that produce melanin. The damage was associated with the onset of melanoma, an aggressive form of skin cancer. Because of doubts he had, and the subject itself was related to the work of Camila Mano, who's PhD he was supervising at USP's Chemistry Institute, he asked Mano to participate in the study, and soon after, to travel to Yale. Mano, who co-authored the article published in *Science*, left Brazil in late 2012 and stayed almost six months, returning in February 2013. Her first task was to familiarize herself with the problem they had been unable to solve.

"They saw changes in DNA that appeared to have been caused by the sun's radiation, but which happened after sun exposure," says Mano. After clarifying her understanding of the problem, she learned how to work with mouse cells and began experiments that might lead to an answer. The initial testing was unsuccessful, but soon she concluded that melanin itself might be causing the changes in DNA.

QUALITY CONTROL

Under normal conditions, the sun's ultraviolet radiation forms so-called dimers (chemical compounds consisting of two units) of thymine and cytosine, which are two of DNA's basic components, in melanin-producing cells. The dimers can change how DNA works when the cell multiplies. Luckily, there is a rigorous quality control process that undoes part of the dimers. During DNA replication, some proteins – repair enzymes – verify whether the copy matches the



MONTAGE WITH PHOTO EDUARDO CESAR ILLUSTRATION SÍRIO CANÇADO

original, similar to a spell-checker that replaces the incorrect letters as soon as the words have been written. Other enzymes are also on permanent alert to fix DNA wherever it is broken.

Commenting in the journal *The Scientist*, David Fisher, a biologist and skin cancer specialist at Boston's Massachusetts General Hospital who was not involved in the study, called the study "very interesting and provocative." Fisher continues: "It emphasizes yet again what we knew: the biochemistry of melanin is a double-edged sword." Melanin, the skin's dark pigment, can prevent the formation of dimers. However, as this study showed, it can also cause an opposite effect, promoting the formation of pyrimidine dimers (thymine and cytosine) for at least three hours after direct exposure to the sun's ultraviolet radiation. The resulting impairment of the DNA molecule's repair mechanisms can result in an increase of harmful genetic mutations.

Through experiments conducted at Yale and USP, researchers confirmed that ultraviolet radiation sets off the production of a series of enzymes that will generate reactive oxygen species such as superoxide or nitric oxide. They combine to form peroxynitrite, a compound that is known to be reactive and that degrades the molecules with which it interacts inside the cell. The reaction between peroxynitrite and melanin or its precursors generates high-energy compounds, which are transferred to DNA, forming the dimers.

"Ultraviolet radiation only initiates these reactions, which can continue for hours, even after only 10 minutes of cell exposure to radiation," says Mano. She also says that the formation of reactive compounds is more intense with a precursor of melanin called pheomelanin, which is found in the cells of redheads or blondes, than with eumelanin, which forms the melanin in dark skin. This would explain why light-skinned people are more susceptible to skin cancer. In the experiment, researchers also verified that the pyrimidine dimers formed in the absence of light make up almost 50% of the dimers responsible for possible changes in DNA.

This phenomenon is called "photochemistry in the dark," and it was proposed in the 1970s by Emil White at Johns Hopkins University and Giuseppe Cilento at the USP Chemistry Institute. "Photochemistry in the dark intensifies the damaging reactions to DNA that are initiated by ultraviolet radiation," Bechara says. The researcher believes that this type of reaction has been identified in biological phenomena, mediated by high-energy chemical compounds in plant roots and the internal organs of animals.

Melanin also absorbs visible light and then transfers some of its energy to oxygen molecules, generating a highly reactive form, the so-called singlet oxygen. The excited oxygen can react with molecules such as DNA and the cell's organelles (or functional chambers) and damages them, according to a recent study performed by researchers in São Paulo and Paraná (see *Pesquisa FAPESP* Issue Nº. *227*). ■

Project

Triplet excited species in biological systems (nº 09/02062-8); **Grant Mechanism** Fellowships in Brazil – Doctoral; **Principal Investigator** Etelvino José Henriques Bechara (USP and Unifesp); **Recipient** Camila Marinho Mano (IQ-USP); **Investment** R$156.227,65 (FAPESP).

Scientific Article

PREMI, S. *et al*. Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. **Science**. V. 347, No. 6224, p. 842-47. 2015.