

# This Week's Citation Classic

Miller E C, Miller J A & Hartmann H A. N-hydroxy-2-acetylaminofluorene: a metabolite of 2-acetylaminofluorene with increased carcinogenic activity in the rat. *Cancer Res.* 21:815-24, 1961. [McArdle Memorial Laboratory for Cancer Research & Department of Pathology, Medical School, University of Wisconsin, Madison, WI]

**N-hydroxy -2 -acetylaminofluorene (N-hydroxy-AAF), a major metabolite of 2-acetylaminofluorene (AAF) in the rat, was more carcinogenic than the parent amide in this species. The data provide strong evidence that N-hydroxy-AAF is a proximate carcinogen in the induction of tumors by AAF. [The SCI<sup>®</sup> indicates that this paper has been cited over 240 times since 1961.]**

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"This paper, in which we were fortunate to have the collaboration of Dr. Henrik A. Hartmann of the department of pathology of our medical school, was particularly exciting to us, since it reported the attainment of an intermediate goal of our research, i.e., the finding of a metabolite of a carcinogen that was more active than the parent compound. More importantly, it also provided a focus for future work on the elucidation of the mechanisms involved in the induction of tumors by AAF and other chemicals. On the other hand, this paper was just one of the series of papers from our laboratory and those of other investigators that, bit by bit, have led to a rather comprehensive knowledge of the metabolism, both in activation and deactivation, of chemical carcinogens and the means by which their metabolites can affect the functions of the target cells.

"Our interest in metabolic activation of chemical carcinogens stemmed from our studies in 1947 on the observation that hepatocarcinogenic dyes, though chemically unreactive, became bound covalently *in vivo* to proteins in the liver of the rat. The amount of dye binding produced by different dyes appeared to correlate approximately with their hepatocarcinogenic activities. Subsequent studies in many labora-

tories showed that covalent binding *in vivo* of carcinogen residues to proteins and nucleic acids in target tissues was a common property of chemical carcinogens. These bound forms appear to be a necessary but not sufficient requirement for tumor formation. The facts that most chemical carcinogens are not reactive chemically and are carcinogenic only in certain tissues made it apparent that metabolism of the carcinogens to reactive intermediates must occur *in vivo*.

"Our efforts to find metabolites of the aminoazo dyes with increased carcinogenic activity failed,<sup>1</sup> and we turned to a study of the versatile carcinogenic amide AAF. With Dr. John W. Cramer (now professor of pharmacology, University of Nevada School of Medical Sciences), then a post-doctorate in our group, we observed a new urinary metabolite of AAF in the rat and found that the amounts excreted increased with the continued feeding of AAF for several weeks.<sup>2</sup> Isolation and characterization of this new metabolite showed that it was N-hydroxy-AAF, a new kind of metabolite of an aromatic amide. Once we had learned how to synthesize this compound, we administered it to rats. To our amazement it was more carcinogenic than AAF at the usual sites of tumor formation such as the liver, mammary glands, small intestine, and ear duct glands. In addition, it was carcinogenic in the skin, subcutaneous tissue, forestomach, and peritoneum where AAF was inactive. *In vivo* N-hydroxy-AAF gave rise to higher levels of macromolecule-bound AAF residues than did AAF.

"Subsequent work in many laboratories has shown that N-hydroxylation is an obligatory step in carcinogenesis by aromatic amides and amines. However, N-hydroxy-AAF was not reactive toward proteins and nucleic acids *in vitro*, and it finally became evident that a second step was required in the metabolic activation of AAF to an ultimate carcinogen. The ultimate carcinogenic metabolites of most if not all chemical carcinogens now appear to be strongly reactive electrophilic and mutagenic compounds, but this is the story of another decade in research in chemical carcinogenesis."

1. Miller J A & Miller E C. The carcinogenic aminoazo dyes. *Advan. Cancer Res.* 1:339-96, 1953.
2. Cramer J W, Miller J A & Miller E C. N-hydroxylation—a new metabolic reaction observed in the rat with the carcinogen 2-acetylaminofluorene. *J. Biol. Chem.* 235:885-8, 1960.
3. Miller E C & Miller J A. The metabolism of chemical carcinogens to reactive electrophiles and their possible mechanisms of action in carcinogenesis. *American Chemical Society Monograph No. 173: Chemical Carcinogens.* (Searle C E, ed.) Washington, DC: American Chemical Society. 1976. p. 737-62.