The Concept of Reactive Electrophilic Metabolites in Chemical Carcinogenesis: Recent Results with Aromatic Amines, Safrole, and Aflatoxin B₁

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Chemical carcinogens appear to be the first class of foreign compounds demonstrated to be converted in vivo to reactive metabolites which bind covalently with tissue macromolecules (1,2), and a large literature now exists on this subject and its relevance to carcinogenesis by these agents (3–5). Considering the great structural heterogeneity of chemical carcinogens (5–8) and of drugs in general, it is not surprising that at high dosage levels a few drugs are known to be similarly activated in vivo to reactive metabolites which can exert acute toxic reactions through covalent binding with tissue components, especially proteins (9,10). Thus, although most drugs and their metabolites probably interact only noncovalently and thus reversibly with cellular molecules in their pharmacological actions, closer inspection of the metabolism of various drugs will doubtless reveal various degrees of covalent interactions in vivo. Such interactions may pose carcinogenic, mutagenic, teratogenic, allergenic, necrogenic, and possibly other hazards that must be weighed against the benefits provided by each drug in this category.

METABOLISM OF CHEMICAL CARCINOGENS AND MECHANISMS OF CHEMICAL CARCINOGENESIS

Carcinogenesis appears to involve at least a quasi-permanent change of phenotype of normal cells involving the control of growth. Hence it is axiomatic
that carcinogens must interact directly or indirectly with critical informational macromolecules that control cell replication. The identity of neither the critical macromolecule(s) nor their critical modification(s) has yet been achieved unequivocally in studies on the mechanism of action of any chemical, viral, or physical carcinogen. Approaches to these goals in chemical carcinogenesis have centered primarily about the metabolism of chemical carcinogens, especially to reactive forms, the natures of the interactions of chemical carcinogens and their metabolites with macromolecules, and the relations of these interactions to tumor formation.

In 1947 the authors (1) reported the covalent binding of the hepatocarcinogenic dye 4-dimethylaminoazobenzene in the livers of rats fed this agent. Several observations were made that pointed to a causal role of this binding in the formation of liver tumors by the dye, and subsequent work with related dyes amplified these findings (11). In 1951 (2), fluorescent protein-bound derivatives of 3,4-benzpyrene were noted in the skin epithelium of mice treated topically with this carcinogen. Neither of these carcinogens nor any of their known metabolites reacted with protein in vitro in this manner, so it seemed likely that other metabolites of these carcinogens reacted directly or in enzyme-mediated reactions to yield the protein-bound forms. Hydrolysis of these protein adducts yielded highly polar derivatives of the carcinogens, presumably carcinogen–amino acid adducts. In the early work with 4-dimethylaminoazobenzene, no binding to the hepatic nucleic acids was detected with the colorimetric method employed; the structures of the recently characterized major nucleic acid-bound derivatives of this carcinogen in rat liver now provide an explanation of our failure to detect them. Subsequently, in 1956, the first observation of nucleic acid binding of a carcinogen in vivo was made with a 14C-labeled nitrogen mustard (12). By 1966 numerous observations of the binding in vivo of chemical carcinogens to tissue protein and nucleic acids had been made (3), and today a wide variety of carcinogens are known to bind covalently with macromolecules in vivo (4,5).

The amounts of the bound residues of chemical carcinogens found in proteins and nucleic acids in vivo are of the order of one carcinogen residue per $10^4$ to $10^7$ monomer residues. In some cases, fair to good correlations have been found between the total binding to the total protein and/or nucleic acids of tissues and the carcinogenic activities of a series of similar structures (11,13–15). In other cases the correlations between carcinogenic activities and the levels of bound forms are poor (16–19). Noncarcinogenic compounds closely related to chemical carcinogens are frequently bound at inappreciable or only low levels, but some weakly carcinogenic or apparently noncarcinogenic compounds bind appreciably and some binding of chemical carcinogens occurs in apparently nontarget tissues (11,14,20). However, at present no case is known in which an adequately studied chemical carcinogen has failed to exhibit covalent binding to macromolecules in its target tissue in vivo. Attempts to correlate the gross amount of binding of a chemical carcinogen with carcinogenicity are no longer