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Discussion of Dr. E. Farber's Paper, "Biochemistry of Carcinogenesis"

Walter Troll

Institute of Environmental Medicine, New York University Medical Center, New York, New York

I would like to take this opportunity to record observations which amplify some of the views offered by Dr. Farber. From a hypothesis of a single mechanism for carcinogenesis, the concept has now grown to that of a multiple insult. In the case of skin carcinogenesis two types of reactions are clearly involved: one that is irreversible in its action which has been called initiation, and a second which is reversible and has been called promotion. As pointed out by Dr. Farber, the mechanism of the action of these two types of carcinogens must be clearly different. We have used as our working model, initiation representing somatic mutation and promotion involving phenotypic expression. As support for the somatic mutation theory, we have shown that initiating agents are capable of reacting with DNA, modifying Tm, buoyant density, and priming activity with bacterial RNA polymerase irreversibly (3, and W. Troll, E. Rende, and P. Day, submitted for publication). We have looked, furthermore, for mutations in microbiologic systems, and noted that initiating agents are capable of causing a specific mutation in bacteria (2). Dr. Mukai of our laboratory, demonstrated that β-propiolactone, 2-acetoxyacetylaminofluorene, 2-hydroxylaminofluorene, 1- and 2-hydroxylaminonaphthalenes are all active in this test, while acetylaminofluorene, 2-hydroxyacetylaminofluorene, 1- and 2-naphthylamine, and 2-amino-1-naphthol are inactive. The mutagenicity tests which depend on the frequency of reversion to amino acid independence of a number of nutritional mutants of *Escherichia coli* are also capable of identifying the type of change that has occurred in the DNA. All the compounds listed appear to involve a substitution of adenine thymine for a guanine cytosine pair in the DNA (1).

Dr. Mukai has also tested the naphthylamine-related compounds in skin carcinogenesis and has found complete accordance of initiation and mutagenesis. Thus, 1- and 2-hydroxylaminoaphthalene were active as initiators in mouse skin carcinogenesis with croton resins as promoters, while the naphthylamines and 2-amino-1-naphthol were inactive. This correlation of mutagenesis and initiating activity is gratifying and needs expansion. Another postulate of the somatic mutation theory requires that the DNA in a tumor cell should be different from that of the normal one. Since such differences could be very slight, a negative result would have little meaning.

As indicated by Dr. Farber, a great need persists to develop an experimental hypothesis for the reversible promoting activity, which is clearly demonstrated in skin carcinogenesis. With the availability of fractions of varying promoting activity from croton oil (provided by Dr. Ben VanDuuren), we have begun to look at the *in vitro* correlation of biologic activities with these agents. One property which appeared to precisely correlate with the promoting activity of this agent was the ability to release lysosomal enzymes from rabbit liver lysosomal preparations (4). This correlation was also noted with Tween preparations. There appeared to be some specificity for the lysosomal membrane by these agents, since the fraction did not cause hemolysis or break mitochondrial membranes in relation to the promoting activity. Several hypotheses can be built on this observation of a specific interaction of promoting agents with a membrane. The one we are testing is a release of an enzyme capable of removing a repressor from a DNA site, e.g., by hydrolysis.

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