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Reflections in Mutation Research

Reflections on a career and on the history of genetic toxicity testing in the National Toxicology Program☆,☆☆

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ABSTRACT

One of the highly visible aspects of the U.S. National Toxicology Program (NTP) has been its genetic toxicity testing program, which has been responsible for testing, and making publicly available, in vitro and in vivo test data on thousands of chemicals since 1979. What is less well known, however, is that this NTP program had its origin in two separate testing programs that were initiated independently at the National Cancer Institute (NCI) and the National Institute of Environmental Health Sciences (NIEHS) before the NTP was established. The NCI program was in response to the 1971 National Cancer Act which dramatically increased the NCI budget. In contrast, the NIEHS testing program can be traced back to a publication by Bruce Ames, not the one describing the mutagenicity assay he developed that became known as the Ames test, but because in 1975 he published an article showing that hair dyes were mutagenic. The protocols developed for these NCI contracts became the basis for the NTP Salmonella testing contracts that were awarded a few years later. These protocols, with their supporting NTP data, strongly influenced the initial in vitro OECD Test Guidelines. The background and evolution of the NTP genetic toxicity testing program is described, along with some of the more significant milestone discoveries and accomplishments from this program.

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1. My introduction to genetic toxicology

I had the good fortune to become involved with mutagenicity testing at the time when genetic toxicity was a nascent area of toxicology, and just beginning to gain recognition as a necessary endpoint for human health and safety considerations as part of the evaluation of the toxicity of new chemicals. This involvement predated the Ames test and came about through a number of synchronous, unplanned occurrences. In late 1968 I was finalizing my Masters thesis in microbiology (mycobacterial cell wall chemistry) at George Washington University (GWU) in Washington, DC. I had already taken enough coursework for my Ph.D. with a primary interest in clinical microbiology and infectious diseases. I was looking for a part-time laboratory job because my wife was planning to quit work to remain home after the birth of our second son, but was having trouble finding a job in a clinical microbiology laboratory with a work schedule restricted to evenings, nights, and weekends. I had just been turned down for a night technician job at a local hospital (one of a number of turn-downs) because, although they said I was highly qualified, they would not hire someone with a beard, which I had grown the previous year (at this time in Washington DC, I was told that “only hippies and communists had beards”). When I got back to the school lab I commented to one of the other graduate students that I wasn’t able to find a job. Suddenly, a voice behind me said “I know somebody who is hiring.” It was Rosalie Donnelly, a microbial geneticist who had just begun a part-time appointment in the department and whom I had not yet met. She was also doing research part-time at the US Food and Drug Administration (FDA) for Marvin Legator. I called Marvin for an appointment and met with him the following week. The interview went well and he offered to hire me, not for the part-time position I had requested, but as a full-time FDA employee. In addition, because he had recently gotten an adjunct appointment in the GWU Microbiology Department, any FDA research project that I designed and conducted could be used as the basis for my Ph.D. dissertation, with him serving as my advisor (I added Rosalie Donnelly as my

☆ This article is part of the Reflections in Mutation Research series. To suggest topics and authors for Reflections, readers should contact the series editor, G.R. Hoffmann (ghoffmann@holycross.edu).
☆☆ A number of events described here were described previously [1].

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co-advisor because of her training and experience in microbial genetics). I was so relieved at getting what I thought would be a part-time interim job, which suddenly became a full-time, salaried job that would also allow me to finish my Ph.D., that I never asked for details about what I would be doing.

On my first day on the job in July 1969, Marvin gave me a rack of Salmonella cultures labeled G46, C3076, D3052, and C207, that he had received from Bruce Ames at UC Berkeley, and introduced me to the Host-Mediated Assay (HMA), a procedure that his lab had developed and which he had just published [2]. In this procedure, indicator bacteria are injected intraperitoneally into mice and the test chemical is administered orally or intramuscularly. This allowed for the bacteria to respond to mutagenic metabolites formed from the in vivo metabolism of the test chemical. Three hours after treatment the animals were sacrificed and the bacteria were removed from the peritoneal cavity and plated for determination of survival and mutation so that the mutation frequency could be determined. Now I knew why Marvin had been so enthusiastic about hiring me. During our initial interview, he recognized that the procedures involved in the HMA were identical to procedures I had used 5 years earlier when I was doing peritonitis research in mice at the Walter Reed Army Institute of Research where I was assigned after my Army basic training. The only difference between my Army research procedures and the HMA was that, although I was using enteric bacteria (*E. coli*, Salmonella spp., etc.) at Walter Reed, I was not looking for mutants, only at total cell counts. Mike Gabridge, who was instrumental in developing the HMA, had left the FDA and Marvin was looking for somebody to run the assay just as I walked into his office.

At the time he interviewed me in January 1969, Marvin and others were heavily involved in forming the Environmental Mutagen Society (EMS) and trying to persuade the FDA and other regulatory agencies to test food additives, drugs, and pesticides for mutagenicity [e.g.,3]. The primary impetus for this testing was a concern for heritable (germ cell) mutation and, secondarily, for cancer. Over the next few years, I performed HMA experiments in fulfillment of my dissertation research project on dietary effects on in vivo metabolism of *N*-nitrosamines to mutagens, in addition to in vitro mutagenicity studies with chemicals not requiring metabolic activation.

The first three of the Salmonella strains were engineered by Ames to become TA1535 and TA100 (G46), TA1537 (C3076), and TA98 (D3052). C207 (which produced TA1536) was a frameshift strain that was discontinued because it did not appear to add to the information provided by the other three strains. I became acquainted with Bruce Ames shortly after starting work at FDA, and he would send me the latest versions of his tester strains (e.g., the TA1500 series; TA100; TA98; TA97/97a) as they became available, and I used them for the HMA and in vitro procedures. However, Marvin was less than pleased when, after completing my studies, I concluded that the HMA was a good research tool, but was too insensitive for use in routine screening [4], since Marvin and others had envisioned the HMA as a primary in vivo test [3,at pg. 603]. My conclusion regarding the HMA's lack of sensitivity as a screening assay was confirmed a few years later by the results from an early NCI carcinogen screening project [5].

Entering the field of mutagenicity research at that particular time in those very early years was especially fortuitous because during my first week on the job I was introduced to Heinrich Malling and Fred de Serres from Oak Ridge National Laboratory, and Gary Flamm from the National Cancer Institute (NCI). They had come to FDA at Marvin's invitation to present a mini-symposium on mutagenesis and DNA repair, which served as my introduction to these topics. My involvement with these three individuals who were highly influential in the field turned out to be very important for my career; I worked for each of them at one time or another. Gary succeeded Marvin as FDA Branch Chief and, later, Heinrich and Fred recruited me to work for them in the Environmental Mutagenesis Branch at NIEHS. I was also convinced to join the EMS during that first year of its existence.

2. The start of U.S. government genetic toxicity testing programs

In 1971 Marvin succeeded in persuading FDA to award two contracts for testing direct food additives for mutagenicity in vitro and in vivo. At the time, the Genetic Toxicology Branch that he headed was part of the Bureau of Foods, which later became the Center for Food Safety and Applied Nutrition (CFSAN). I was responsible for the test protocols for the bacterial host-mediated assay and Salmonella plate spot tests and yeast recombination tests, and for reviewing the data, while Sidney Green was responsible for the in vitro cytogenetics and rat dominant lethal studies. This contract turned out to be not very effective because the in vitro tests were without metabolic activation, the protocols used were not very well developed, and the testing laboratories, like other labs at the time, had no experience in mutagenicity testing. As a consequence, the test results were never published or used in any way. About 3 years later, when Gary Flamm was my FDA Branch Chief, I was asked to award a new contract for the in vitro bacterial mutagenicity and host-mediated assays. This was just after the first Ames plate test publications appeared [6], and the plate test and HMA protocols were specified in the contract. This subsequent contract was awarded to Litton Bionetics, which had hired David Brusick for this award as their director of genetic toxicology.

In 1975, the NCI formed an interagency ad hoc advisory committee on which I served with Gary Flamm (FDA), Heinrich Malling, and Fred de Serres (NIEHS). The committee was chaired by Virginia Dunkel (NCI), and its aim was to develop a program to validate in vitro genetic toxicity tests for identifying carcinogens, and to also examine the inter-lab reproducibility of the tests.[1] The tests were being evaluated as adjuncts to support the NCI's cancer bioassay program that was initiated in 1971 in response to the National Cancer Act of 1971 which tripled the NCI budget and got them into the carcinogen bioassay business. At the time NCI formed this gene-tox committee, Ames had not yet published the results of the study that showed that his new plate test procedure was effective for identifying and distinguishing carcinogens and noncarcinogens [7,8], and there were no available studies on the effectiveness of the other genetic toxicity tests we were considering. Data generated under a previous NCI contract program initiated in 1971 to evaluate a number of mutagenicity and DNA damage tests as predictors of carcinogenicity were not useful for a number of reasons, including protocol inconsistencies among labs doing the same procedure, changes in protocols in mid-contract after Ames' publications describing the plate test with metabolic activation, testing too few noncarcinogens, and the fact that the chemicals were not tested blind [10]. I had become involved in that earlier program after the contract awards were made, and I had been asked to monitor the in vitro microbial and yeast studies, and the HMA, provide guidance to the labs, and evaluate the test results.

This NCI interagency ad hoc advisory committee was interesting in several ways. Its organizer and Chair, Virginia Dunkel, was not a geneticist, but a cell biologist with primary interest in cell transformation systems. She saw the potential of genetox testing and enthusiastically advocated its use in the context of carcinogen

[1] The background and genesis of this NCI program, and the reasons for the selection of the Salmonella and mouse lymphoma assays, were described previously [9].

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