

Historical Note

Some Early Stirrings (1950 ff.) of Concern About Environmental Mutagens

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Perhaps it would be of interest to members of the Environmental Mutagen Society (EMS) to cavedrop on my correspondence with H. J. Muller almost 50 years ago. I will, for the most part, let the letters speak for themselves, with a few brief comments and footnotes.

Perhaps most remarkable is the pace of correspondence, which is a match for email today; perhaps the US Post Office was using its fastest ponies between Madison and Bloomington. At any rate, my letter, posted March 15, was responded to by the next day, and my reply again by Monday, March 20, 1950—all for a 3-cent stamp.

The general background of research on chemical mutagens, and particularly on Charlotte Auerbach's wartime discoveries about mustard gas, have been reviewed by Auerbach herself [1973], Sobels [1975], and Beale [1993]. I can recall hearing about Auerbach's work as a student at Columbia, promptly on the publication of her 1944 paper in *Nature*. Although this was ostensibly on mustard oil (sic), allyl isothiocyanate, there was a well-founded rumor that war gases were the real objects of inquiry. Either way, the work put paid to the dogma that genes were somehow outside of chemical metabolism. Now that virtually every compound seems to have an effect on mutation, one way or another, it is hard to recall the idealization of "the gene" as accessible only to the most intrusive insults like those of X-ray, as first discovered by H. J. Muller in 1927, and which won him his Nobel Prize only in 1946. We are glad to learn from Beale [1993] that it was Muller who encouraged Auerbach to begin her studies of mutagenesis even before the war started in 1939.

I cannot resist briefly quoting her recollection of me (from her letter of May 29, 1979, congratulating me on my election to the Royal Society). She had met me at the 1951 Cold Spring Harbor Symposium [Lederberg et al., 1951] and recalls "when you were a very young, very bright, very arrogant, and very likable chap, who talked from 7 PM to 1 AM on transduction." What would it have taken for a 26-year old to appear eager and enthusiastic rather than arrogant? Perhaps deference, if not silence.

By 1950, chemical mutagenesis had been confirmed in a number of systems—reviewed by Auerbach [1973]. Some of the early studies with bacteria did have some methodological lurks [Lederberg, 1948], which got in the way of certitude that the "mutagen" was not merely assuring the selective survival of pre-existing mutants. Beyond these pragmatic obstacles, there was some hope that chemical mutagenesis would give some clue as to the chemistry of the gene—and there was the fillip from UV mutagenesis that DNA might be the target [Hollaender and Emmons, 1941; Stadler, 1997]. However, very little in early experiments with alkylating agents or dye-stuffs could discriminate between DNA and protein.

My own experiments were oriented to understanding bactericide in *E. coli* K-12, specifically the extent to which the killing of bacteria might be explained in terms of genetic lesions. With diploid, compound heterozygote bacteria, I stumbled on a system that seemed to be extraordinarily sensitive: doses of UV that left almost 90% survivors seemed to "haploidize" 50% of the target cells [Lederberg et al., 1951]. But this also became more complex on closer examination. Most surviving "haploidized" clones contained a residuum of parental or recombinant diploids, whose division was, however, delayed. Today we might speculate that one chromosome bore a lesion that interfered with its replication, that SOS was invoked, and the lesion eventually repaired—this was perhaps borne out by cytological correlates of the "residual diploid" as filamentous cells with swollen nucleoids, overtaken by their spawn of rapidly dividing haploids. The system had the features of sensitivity to low doses, on the one hand, and of enabling an observable "mutagenic" response for compounds so toxic as to leave few survivors, on the other.

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Unfortunately, these diploid stocks were intrinsically unstable, and we did not have (or use) the technology of cryopreservation to save the phenomenon. This very discussion is moving me to resurrect the target system in the laboratory.

Briefly, a repertoire of common chemicals had effects similar to radiation, and I inferred that they were mutagens (Table I); as a control, heat, iodine, and streptomycin had killing mechanisms that left no such tags on the survivors. One, dimethyl sulfate, has become a familiar friend in mutagenesis studies, and has its counterpart in chemotherapy with its two-armed analogue busulphan or myleran. Others like formaldehyde have been contentious contenders in the chemical-mutagen/carcinogen sweepstakes. Acetic anhydride is probably a valid entry, but is so quickly hydrolyzed, and is itself so toxic that its mutagenic potential is not a foremost concern upon human exposure.

I reported these findings to Muller (see appendix). I had met him at several scientific meetings, and at informal gatherings of Mid West “phage” workers that Leo Szilard organized in Chicago. He had long taken a friendly interest in my work on genetic recombination in *E. coli*. This letter was my first foray from science to policy; I had very little insight and less experience where to go for that mission, and I was greatly heartened by his prompt response. But nothing came of that flurry in 1950. I must have discussed these matters with my close friend and colleague, James Crow, but I have no correspondence—alas, but he was next door—nor recollection of any immediate consequence there. A few years later he was to take a major part in the national debate.

I did see Muller again at the Genetics Jubilee in Cleveland, in September 1950, where we both participated in a symposium to celebrate the birthday of modern genetics, the rediscovery of Mendel’s laws. “Mutagenesis” has only one entry in the index; it is to my paper [Lederberg, 1951], which contains the following quote.

TABLE I. Effects of Bactericidal Agents on Diploid *Escherichia coli**

Radiomimetic	Non-Radiomimetic
X-ray	High temperature
Ultra-violet	Streptomycin
N-Mustard	Methyl green
Formaldehyde	Urethane
Hydrogen peroxide	Ninhydrin
Acetic anhydride	Iodine
Acetyl chloride	Iodoacetamide
Dimethyl sulfate	Sodium desoxycholate ^a
Ethylene oxide	Acriflavine ^a

*From Lederberg et al., 1951.

^aInconclusive owing to severe clumping or inadequate bactericidal effect.

“[We] must be very cautious in interpreting chemical mutagenesis as a direct chemical reaction with the gene. Cells, including bacteria, react in a very complex pattern to treatment with mutagenic agents. The possibility cannot be excluded that some mutations are produced indirectly as a consequence of accidents during recovery or of non-specific and non-localized disturbances of nuclear structure.”

During 1955, though my base was in the College of Agriculture, I became more and more involved with Dr. John Bowers, the new Dean of the Wisconsin Medical School—I had met him at a dinner at Curt Stern’s at Berkeley in 1953, and knew he had a particular interest in genetics, uncommon for physicians in that era. But he had directed the program in biology and medicine for the Atomic Energy Commission before going into medical education. He warmly supported my proposals for establishing a medical genetics department, which would, i.a., incorporate teaching about environmental hygiene, specifically the “genetic hazards of radiation and other mutagens.” The same ideas were embodied in my prospectus for the Genetics Department that I founded at Stanford Medical School when it inaugurated its new campus in Spring 1959.

In October 1955, I submitted a brief letter to the editor of the Bulletin of the Atomic Scientists (Letter 5), suggesting that chemical mutagens had to be given equal weight with radiation. I also shared this with Dr. Detlev Bronk (Letter 6) (then president of the Rockefeller Institute; I could hardly have guessed I would succeed him).

In 1960, the Macy Foundation initiated a series of conferences on human genetics [Schull, 1962], one of which was devoted to mutagenesis. My pharmacologist colleague Avram Goldstein, who had helped recruit me to Stanford (in 1958), took up the cudgels. Also in attendance, besides myself, were Atwood, Auerbach, and Demerec, and this meeting surely helped to crystallize a scientific consensus and the beginnings of national action.

Other reminiscences have appeared in this journal [Crow, 1989; Wassom, 1989] particularly as they relate to the founding of the Society in 1969. The latter coincides with the emergence and acceptance of the “Ames test” and similar genres in the late 60’s. The turning point was probably the accumulated data and recognition that mutagenesis might have important predictive value for carcinogenesis, an endpoint far more visible to existing generations, and provocative of unending controversy.

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APPENDIX

Letter 1

"Blastophthoric lead poisoning" [Anon., 1948] discusses anecdotes about three mentally retarded children of a plumber, occupationally exposed to lead intoxication. The term "blastophthoria" appears in Dorland's medical dictionary: "degeneration of the germ cells"; but has not appeared recently in the medical literature.

THE UNIVERSITY OF WISCONSIN
College of Agriculture

Madison 6

Department of Genetics

March 15, 1950

Dr. H. J. Muller
Department of Zoology
Indiana University
Bloomington, Indiana

Dear Dr. Muller:

I am seeking your counsel on an issue that is somewhat related to "mutational prophylaxis", and to which, therefore, I suppose you have given some thought.

Lately, we have been studying the mechanism of radiation killing of bacteria, by examining the effects of X-ray and UV on heterozygous diploid *E. coli*. It may not surprise you that recessive lethals do not play a detectable role in killing, but that there is a very striking degree of "haploidization" of the treated diploid cells, which I assume to reflect grosser chromosome damage and loss.

I next thought that this might be a useful method for classifying bactericidal compounds and agents into those with predominantly "nuclear", and predominantly "cellular" modes of killing. Nitrogen mustard, as expected, gave the same results as radiations. However, we were surprised to find that quite a considerable number of other organic reagents gave comparable results too, including: formaldehyde, dimethyl sulfate, acetic anhydride, and hydrogen peroxide. Killing by heat, basic dyes, iodoacetamide or iodine, urethane, and some others, had no detectable genetic correlate.

These results raise a number of questions, some of first theoretical interest. In view of the homologies, I think it is likely that radiogenetic effects are mediated by reactive compounds, free radicals or ions which share the capacity to bring about substitution reactions, like those mediated by alkyl peroxides, cyclic ethan ammonium, formal, alkylating anhydrides, etc. The results do not bear on the problem of the immediacy of the effects on genes. But aside from this important theoretical question, I am led to wonder whether the potential mutagenic effects [speaking very broadly] of such a wide variety of organic reagents does not create a hazard

broader even than those of ionizing radiations. Clearly, we do not know whether such agents are likely to induce mutations in mammals, considering problems of penetration, but it seems to me that this ignorance is potentially dangerous, for the same reason that personal ignorance of X-ray effects is dangerous to the species.

I wonder whether this whole problem should not be brought before some such body as the National Research Council. Ordinarily, I would not be very enthusiastic for programmatic research, but it is obvious that any undertaking to investigate mutagenic effects of industrial chemicals in mammals would have to be organized on a large scale, and receive very broad support, presumably from the Public Health Service or some other governmental agency. I do not know of any existing institution that would be capable of absorbing such a program. But I think that you will agree that no study of the toxicology of industrial compounds would be complete if it left unrelieved any suspicion of potential mutagenic effects.

My own experience with such matters is so limited that I feel that any comments you might make would be very valuable. Perhaps the problem is exaggerated, but I have the feeling that, in our ignorance, chemical mutagenesis poses a problem of the same magnitude as the indiscriminate use of radiations. On the other hand, it would be unfortunate if these notions were improperly publicized -- I should not like to see many repetitions of the "Blastophthoric lead poisoning", which appeared in The Journal of Heredity a year or so ago.

Yours sincerely,

Joshua Lederberg

Letter 2

INDIANA UNIVERSITY
Bloomington, Indiana

Science Hall 101
Department of Zoology

March 16, 1950

Dr. Joshua Lederberg
Department of Genetics
The University of Wisconsin
Madison 6, Wisconsin

Dear Lederberg:

The results you mention are exciting, and I'm glad they're stimulating you to consider the need for a comprehensive attack. I was especially glad to get your letter because I too have felt the need for such an attack, although I have not yet had experience with the chemical production of mutations. I hope that we can get together some time soon and talk over the possibility of getting support for work of a whole group, dealing with different organisms or phases of the work at different institutions. The attack could be carried on at several levels, the first being your attack on bacteria. The cheapest mammals, mice, are so expensive in such work that there ought to be an in-between level or levels as well. I think *Drosophila* should come in as an in-between level and possibly one or more "cellular" plants, such as *Neurospora* and/or *Oenothera*. I am thinking of the latter (*Oenothera*) only because it might lend itself rather readily to the detection of gross structural changes in chromosomes and because Cleland's interest in doing that might perhaps be aroused.

Your letter came this morning and I went right over to Cleland about it because he is the one who would have to officially initiate such a project if it were to be sponsored by the National Research Council. He maintained a cautious attitude but I think he might

be convinced, though it may be that some more publications along these lines will have to appear first, or at least he will have to be shown more definite evidence. He says he does not want the NRC to go on a big money raising campaign and have it turn out to be "a wild goose chase". (Of course I tell you this only confidentially.)

In my opinion the matter should be put on a broader basis than one of only looking for mutagenic chemicals that might be received from outside. It ought to include an attack on biological conditions which are predisposed to or against mutations, of different kinds. And while direct, though long-in-maturing, practical value for humanity in helping us to avoid mutations should be the most important angle in getting people to agree to give funds, the funds ought explicitly to be given for fundamental mutation study in general. One can, to a certain extent, get money (and a few people can get a lot of it) for radiation mutation work, as from the AEC, but that is by no means broad enough as an attack. And while Hollaender is beginning to realize that spontaneous mutations and chemical should be studied too, I do not think all that work should be left to Oak Ridge or that enough of it can be done there. Nevertheless, when mouse work is under consideration in the broader program, the Russells, who are doing radiation mutation work on mice at Oak Ridge, should be asked to join in the planning, and probably Hollaender too. As I am a consultant in that work, I could throw out feelers in that direction when the time seemed propitious.

My own work is dependent on cancer grants and, as I expected, the cancer people are pulling the purse strings tighter when it comes to giving money for genetics. It is not right that mutation work should have to be a tail to the cancer kite. I think the time has come when it ought to be recognized in its own right and that we ought to make an effort to get a movement to support it started by the NRC, unless some more suitable agency can be found. We have to proceed very cautiously, however, so I should like to talk it over with you privately before much more is done. If there were to be another meeting of the phage genetics group soon which we both were to attend, we could do it there, but that might be waiting too long. Are you expecting to have to come east within the next few weeks? If so, you should plan to stop here on the way.

With kindest personal regards,

Yours sincerely,

H.J. Muller

HJM:hs

Letter 3

March 20, 1950

Dr. H. J. Muller
Science Hall 101
Indiana University
Bloomington, Indiana

Dear Dr. Muller:

I was very pleased to note that we are in accord concerning the need for a comprehensive attack on the problems and social implications of chemical mutagenesis.

Although there can be no question of the need for continuing support of fundamental research on the chemical as well as the physical mutagens, in my letter I had in mind a program of a somewhat more applied nature. To my mind, the mutagenic activity of several chemicals is already incontrovertible. At least three of the

compounds which have been shown to possess mutagenic activity for *Drosophila* are already in use as therapeutic agents to some extent. In the form of Urotropine, or hexamethylene tetramine, formaldehyde has been used rather widely in the treatment of cystitis and other urinary infections; nitrogen mustard is, of course, an important chemotherapeutic agent in the management of leukemia; and allyl isothiocyanate is the active ingredient of the familiar mustard plaster. Need we go any further in the study of mutagenic effects at so-called "in between" levels of organisms to justify the necessity for large scale work on mammals, which should provide the necessary information for the possible role of such chemicals in human affairs? It is precisely because the program of genetic research, sufficiently comprehensive to give us detailed information, will be so expensive that I felt the need to call upon the National Research Council to study the problem. I had in mind that the NRC might first set up a study committee which might then be in a position to recommend any further action. Once some concern for this aspect of applied genetics is disseminated it might then be time to push for a large scale support of mutation work in general, but I have the feeling that it might be more important to determine whether or not we are facing a critical problem at the present time.

I do not feel that it would be advisable to attempt to coordinate fundamental research on mutation in the direction of this problem. However, any attempt to do such work with mammals is obviously going to be so expensive that it likely to be beyond the scope of any existing institution, and it is at this level primarily that an organization such as the NRC might most feasibly act.

Unfortunately, I can see nothing in prospect that would make a fuller private discussion with you convenient, but unless there is something about this matter with which I am not familiar, I can't see that a few weeks delay will be very critical. It might be worth while to use this interval to let the notion "simmer" in Dr. Cleland's mind, and I'm looking forward to seeing you at the next Virus Seminar which will probably be held sometime early in May, possibly on this campus. If the seminar is held here, would you be able to attend? If not, that might be sufficient reason to withdraw that proposal.

Yours sincerely,

Joshua Lederberg
Assist. Prof. of Genetics

Letter 4

"Letter to Rabinowitch" is a reference to my letter to the Bulletin of the Atomic Scientists, reproduced as Letter 5. "Princeton meeting" is likely the committee chaired by Bronk, see Letter 6.

INDIANA UNIVERSITY
Bloomington, Indiana

Department of Zoology

November 16, 1955

Dr. Joshua Lederberg
Department of Genetics
The University of Wisconsin
Madison 6, Wis.

Dear Lederberg:

I am delighted to have your letter of Nov. 15, to myself, together with the one that you wrote to Rabinowitch. In two or three days I am leaving to go to Princeton to attend a conference on the genetic effects of radiation that the National Academy has called. I am very doubtful about what they will accomplish, particularly since there will be men on the panel who are antagonistic to the idea of there being a genetic danger, and others who may want to muddy up the issue by claiming that an increase in heterozygosity is beneficial for mankind. However, I have been thinking of proposing to them that the whole inquiry should be on a much broader basis, and concern

itself not merely with radiation. Your letter to Rabinowitch comes just in time to give me valuable support in this, and I hope you will not mind my reading it to them. It would, however, have been much better if you had been one of the members. However, the talks are only beginning and if I can work it, I will have you called in later, provided you do not object.

I quite agree with you that this matter should not be left to the AEC or even to the American Cancer Society or the National Cancer Institute as it has been in the past for with these organizations the whole problem is just a rather obnoxious and expensive side issue. Something like the Public Health Service ought to take it up. Certainly the World Health Organization would be and is interested, but that would not have the funds as a national organization would. I intend to speak to Dr. Chisholm who is on the campus giving lectures to see if he has any suggestions about the matter since he was for some years head of the World Health Organization and is very forward looking in such matters. Your letter ought to help me with him, too.

With best wishes,

Yours sincerely,

H. J. Muller

HJM:slh

Letter 5

Lederberg, J. . 1955 Letter to editor, Bull. Atom. Sci., 11(10):365, Dec.

To The Editor:

Several writers have emphasized that any use of atomic energy entails a calculable risk, no less than those features of modern technology that lead to auto accidents and gastric ulcers. Nuclear warfare poses such an immediate and overwhelming peril to simple survival that concern for the ultimate genetic hazards of atomic energy betokens an almost unwarrantable optimism for the maintenance of world peace, but an optimism that is our only constructive recourse. However, if we postulate survival, we cannot overlook the long-run genetic problems entirely for preoccupation with the narrower issues of public affairs.

As the Bulletin shows, the attention of the informed public is rightly focussed on the production of deleterious mutations by penetrating radiations, but this emphasis may have obscured the possibly wider contact of genetic hygiene with industrial civilization. Radiobiological discussions have often taken the spontaneous mutation rate as a reference base, as an unavoidable evil which could not be averted and ought not be aggravated. However, recent studies have established two relevant facts: 1. A variety of chemical reagents can also induce mutations. Many of these compounds are special drugs, but the list also includes such common substances and natural metabolites as formaldehyde, hydrogen peroxide, and caffeine. 2. Still other chemicals can reverse these mutagenic effects and can also reduce the "spontaneous" mutation rate. Much (but by no means all) of this research has been conducted with microorganisms and more extensive studies are needed to establish, for example, whether the germ cells of man are physiologically insulated against such chemical insults from the environment. On the other hand, it may be possible to ameliorate the intracellular biochemical accidents that can now plausibly be considered as one source of "spontaneous" mutations.

From this perspective, the genetic hazards of atomic energy are but one facet of a much broader and correspondingly more urgent problem of chronic toxicity and the health of the public (and its future generations).

Joshua Lederberg University of Wisconsin

October 1955.

Letter 6

I can locate no response on the part of Dr. Bronk or NAS. As far as I can determine, National Research Council [1983] marks the first formal involvement of NAS with chemical mutagenesis. Dr. J. F. Crow chaired that group, and its historical chapter does review earlier efforts to articulate public policy on chemical mutagens.

THE UNIVERSITY OF WISCONSIN
College of Agriculture

Madison 6

Department of Genetics

October 26, 1955

Dr. Detlev Bronk
Chairman, N.A.S. Committee on Genetic Hazards of Atomic Energy
Rockefeller Institute
York and 66th
New York 21, N.Y.

Dear Dr. Bronk:

I am asking Professor Sewall Wright to transmit the enclosed comment to you, as chairman of the committee, together with any comments he may wish to add. The enclosure was prepared as a Letter to the Editor, intended for the December issue of the Bulletin of the Atomic Scientists. It argues for a consideration of genetic hazards of atomic energy from a perspective which includes similar hazards from many other sources, in the light of evidence of mutagenic activity of many chemical substances which may be found in the environment.

If this argument is accepted, I suppose it will have to be considered whether responsibility for protection against genetic hazards ought to rest principally with the AEC, or should be shared with other agencies whose primary mission is the public health. However, I felt that to bring in these corollaries might dilute the technical argument, on the one hand, and that it certainly would overreach the zone of my own competence, on the other.

I am sure that Professor Wright, or other professional geneticists on the committee, will be well acquainted with the documentation for my remarks on chemical mutagenesis, but I will be happy to amplify them if that should appear convenient or desirable for your committee.

Yours sincerely,

Joshua Lederberg
Professor of Genetics

CC: Prof. Wright

[Enc: Letter to editor, Bull. Atom. Sci., published 11(10):365, Dec. 1955]