NATIONAL CYSTIC FIBROSIS RESEARCH FOUNDATION
202 East 44th Street
New York, New York 10017

APPLICATION FOR RESEARCH SUPPORT

SECTION 1

TO BE COMPLETED BY PRINCIPAL INVESTIGATOR:

1. Abbreviated Title of Research Proposal: Biochemical Alteration of Cellular DNA

2. Type of Application: ____ New Project or X Reapplication

3. Dates of Entire Proposed Project Period: From: March 1, 1971 Through: February 28, 1973

4. Total Amount Requested for Entire Period: $30,000

   a) Amount Requested for First 12 month period: $15,000

5. Name of Principal Investigator: Joshua Lederberg

   a) Degree: Ph. D. (Sc. D, h.c.; M.D., h.c.)

   b) Title of Position: Professor and Chairman

   c) Department, Service, Laboratory or Equivalent: Department of Genetics

   d) Major Subdivision: School of Medicine

   e) Mailing Address of Principal Investigator: Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

   f) Telephone Data: Area Code: 415 & Telephone # 321-1200,Ext.5801

6. Identify Organizational Component Responsible for Conduct of Scientific Aspects of Project: School of Medicine

   a) Department: Genetics

   b) Address Where Research Will be Conducted: Stanford University School of Medicine, Stanford, California 94305

3A. Research plan.

I. The first year's application was intended to bolster our ongoing studies on the intracellular modification of DNA introduced in the transforming system of Bacillus subtilis. We were particularly intrigued by the possible occurrence of enzyme systems for the repair and restitution of arbitrarily broken fragments of DNA, as may occur in the formation of chromosome translocations in higher organisms.

This particular line is understood to be a long gamble, but we intend to continue to work on it. We have, meanwhile, differentiated another subproject, not part of our original application to NIH or to NCFRF, and submit this now as the basis of this request for continued NCFRF support.

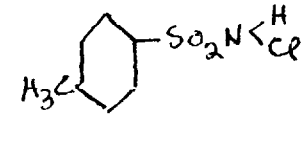
This concerns the modification of bacterial (and, in prospect, mammalian) DNA by chlorine, which is a pervasive environmental chemical, not previously suspected of potential mutagenic effect. Large quantities of chlorine are, of course, consumed as dilute solutions of residual hypochlorite and chloramines (usually about 0.5 mg/liter) in municipal drinking water supplies. Much larger concentrations are imbibed in smaller volume from swimming pools, and through incidental use of chlorine as a bleach and a disinfectant.

Although chlorine has been used for 60 years in water treatment, it has never been systematically studied from this point of view. The traditional teaching that chlorine disinfects by the oxidation of bacterial sulfhydryl groups is probably false. At least, we already have considerable evidence for the interaction of chlorine with bacterial DNA. It remains for further work to establish whether body fluids are in fact perfectly efficacious in neutralizing chlorine (esp. chloramines) to prevent its transport to germinal and significant somatic cell DNA.

Suprisingly little is known about the chemistry of chlorine * interactions with body constituents. The most common reaction may be with ammonia, amines and amine-derivatives to form substituted chloramines:

1) $NH_3 + OCl^- \rightarrow NH_2Cl + Cl^-$
2) $RNH_2 \ \ldots \rightarrow RNHCl \ \ldots\ldots$
3) $R_2NH \ \ldots \rightarrow R_2NCl$

Although far less reactive than hypochlorite, these derivatives are still efficacious chlorine-donors. Chloramine-T



* interchangeable with hypochlorite in aqueous solution: $Cl_2 + H_2O \rightleftarrows 2H^+ + Cl^- + OCl^-$

is for example widely used as a disinfectant, precisely because it acts as a "chlorine-buffer" which persists much longer than hypochlorite in the presence of reducing compounds.

Analogous chloramines are readily formed with amino acids, peptides and proteins. This is in fact the basis of widely used tests for the location of --NH groups in paper chromatography. The chemical behavior of various classes of chloramines is, however, poorly known.

We have already established that DNA, and its constituent bases (especially cytosine) also react readily with hypochlorite to produce two main classes of products; 1) N-chloramines, the Cl substituting on free $-NH_2$ groups; 2) 5-chloropyrimidines, presumably by subsequent migration of the Cl to a stable C-Cl substitution on the ring.
See progress report.

We also find that the chlorination of cell-free DNA, a reaction that will occur at a significant rate in a medium analogous to the contents of a swimming pool, inactivates its genetic activity in the transforming system assay. The target size has not yet been precisely measured, but is consistent with the concept that DNA-inactivation is the principal disinfecting process by which chlorine works.

We have also found that chlorinated bases can be identified in the DNA of bacteria that have been killed by hypochlorite. However, if treated bacteria are incubated without growth, a repair process is observed that involves extensive excision of chlorinated bases from the cellular DNA. The DNA extracted from cells during this stage has a low viscosity, and behaves as if it is altered by numerous single-strand gaps, some of which can be repaired anabolically in the cell. The extent of this repair has been highly variable, and we propose to dissect the factors that encourage or inhibit it.

Gap-ridden DNA is a rather general index of DNA damage, and our studies on its physical and biochemical properties are also directed at calibrating a general-purpose assay of environmental injury to DNA.

Finally we also have evidence of considerable intra-molecular cross-linkage after extensive chlorination, the chemical basis of which is quite obscure.

We have some evidence that the cross-links are potentially reversible. This would have interesting ramifications for various problems in the manipulation of DNA, where protecting selected segments from thermal

denaturation would be a useful artifice.

New lines of work. We have in mind

a) tying together the loose ends of our work now in progress in the B. subtilis system, and

b) exploring the implications of these effects for "genetic hygiene" in man. To the latter end we envisage the following experiments.

1. Exposure of mammalian cells (e.g. organ perfusions and cell cultures and suspensions) to hypochlorite solutions, followed by DNA extraction and analysis, similar to the B. subtilis studies. Different behavior may (or may not) be expected in view of the potential chlorine-neutralizing capacity of the cytoplasm of larger cells.

2. Direct studies on mutagenesis and chromosome breakage by sub-lethal levels of hypochlorite applied to cell cultures. Dr. Margery Shaw of the University of Texas has already done some preliminary trials for us with provocative results.)

3. Chemical studies of the reaction of hypochlorite with plasma proteins and other body fluids. The level of chemical reactivity of N-chloropeptides, to donate Cl to nucleic acid base, is the central issue here. We must also look at competing reactions, e.g. with -SH groups and possibly also sugars and mucopolysaccharides, that would in fact reduce the hypochlorite to innocuous chloride.

4. Absorption and transport studies on radioactively labelled hypochlorite fed to rats. Druckrey had reported raising several generations of animals fed water with 10 mg. per liter of hypochlorite, and noted no obvious pathology. However, these results still tell us nothing of the actual disposition of this potential mutagen.

II. Methods of procedure

Our basic techniques are those current in contemporary molecular-biology research, which we share with many other workers. Our listed publications will authenticate our mastery of them.

III. Significance

Although chlorine is a commonplace chemical, and has been widely used for decades, very little is known about its mechanism of action, nor its hazards as a potential mutagen. A pessimistic extrapolation of what we have

already learned would point to chlorine as a serious threat to genetic hygiene, i.e., as a significant source of genetic damage. This is not in any case to contemplate the rescission of water-chlorination as a public health measure, which might impose dreadful costs in water-borne epidemics. It may speak to the need for much more careful analysis and monitoring of the actual use of chlorine, to ensure that free hypochlorite and residual (but still reactive) chloramines are not present in the water actually consumed. Before we can evaluate whether this is a real genetic hazard, and further design necessary remedial and reparative measures, we simply must learn more about every aspect of the problem. It might be thought that this belongs entirely in the province of environmental health; however, genetic damage is still not taken seriously as an aspect of environmental pollution, and we have not fared well in eliciting support for this research from environmental control agencies.

The enormous range of variation in the way that chlorine is used, the concomitant intake of nutrients, and the health, age, and genetic status of the human consumer population, make a direct population study of chlorine effects almost certainly futile at this time. When more basic information is consolidated, we may be able to frame more specific models, e.g., of who would be most vulnerable, and what to measure, that would then justify a population approach. For example, one could speculate that gastric mucins would be the impenetrable barrier that protects normal people from any injury. This would have to be justified by direct chemical studies of chlorine interactions with this group of materials; and we might then also focus on people with genetic idiosyncrasies in mucin production.

In any event, this project has both daces -- a fundamental study of the chemistry of the chlorination of DNA, and its applications in relating these findings to public health and genetic hygiene.

Bibliography. Studies on --

CHLORINE

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