THE BIOCHEMICAL APPROACH TO MUTATION MONITORING IN MAN

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1. Introduction

At present, there is no simple, inexpensive, direct way to determine whether an increase in the mutation rate in human populations has occurred or is occurring. The problem is increasingly serious with the ever more widespread use in our society of many potentially mutagenic agents. A widespread, highly mutagenic agent could have serious long term effects on the overall viability and fertility of a population, as well as increasing the incidence of specific genetic defects and diseases.

In order to protect human populations from the effects of potential mutagens, we must be able to make two kinds of measurements. First, we must determine the mutagenicity of specific environmental agents, preferably prior to their wide scale use, on a variety of experimental organisms. Second, we must be able to measure accurately the overall mutation rate in human populations, as a last check against a genetic emergency caused by previously undetected mutagens or mixtures of individually innocuous substances.

2. Tests on organisms other than man

Most work on the measurement of mutation rates has been done with nonhuman systems, both because of the greater number of organisms that can conveniently be studied, and because obviously it would be unethical to use humans as laboratory animals for the preliminary screening of potentially very dangerous materials. It is clear that the results of mutagenicity determinations on nonhuman systems cannot be blindly extrapolated to man. Organisms differ both in their inherent sensitivity to mutational damage, in their ability to repair mutation effects, and the ability to detoxify mutagens or to convert innocuous material into mutagens. However, from the standpoint of safety it would seem obvious that no material significantly mutagenic in a mammal should even be tested on man much less used in the environment, and that no material significantly mutagenic in *any* biosystem should be used in the environ-

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ment as a whole unless it can be clearly shown to be nonmutagenic on other mammals.

With this basic limitation in mind, we will now consider briefly some of the most widely used methods for detecting specific mutagenic effects in lower organisms. Probably the most sensitive and economical test is the bacterial one developed by Ames [1]. Specific strains of bacteria are exposed to a suspected compound under conditions such that only bacteria mutagenized by the compound will form colonies. Various bacterial strains can be used to test for different kinds of mutations: base pair substitutions, insertions, or deletions. This method is simple and extremely sensitive, since 10^9 cells can be tested on a single petri dish; but it suffers from the limitation that both the metabolism and the genetics of the organism tested are unlike those of people.

A related method, the host-mediated assay (Legator and Malling, [6]) involves the injection of test bacteria into a mammal. The mammal is then treated with the potential mutagen, and the bacteria subsequently removed and tested for mutations. This test indicates whether the mammal can detoxify the examined compound, or metabolize it to form mutagenic products. This system is extremely useful in that it combines the sensitivity and range of the bacterial test with the metabolic (although not the genetic) functions of the host mammal.

The choice of a test mammal for any mutation detecting system depends on three factors: ability to extrapolate experimental results to man, generation time, and maintenance cost. Mice are frequently used because their short generation time allows the effects of a potential mutagen to be measured for several generations in a relatively brief period. Also, mice are perhaps the least expensive mammals to maintain in a laboratory colony. These advantages have led investigators to question the efficiency of using larger mammals, such as monkeys and chimpanzees, for mutation detection tests. Just how much better can we predict human response using these primates? Comparative studies with proteins and nucleic acids (Sarich and Wilson, unpublished) indicate that the genetic difference between chimpanzees and man is 10 to 20 times less than the mouse-man difference, and the rhesus monkey-man difference is 3 to 4 times less than the mouse-man difference. The quantitative estimates of genetic relatedness may enable investigators to more effectively balance generation time, cost, and predictive value.

There are a number of ways to detect directly an increase in extremely deleterious mutations in mammals. We will mention just one, the dominant lethal test (Bateman and Epstein, [2]). Generally, a male mammal is treated with a potential mutagen, and mated with an untreated female. The pregnant female is dissected and the embryos examined for morphological or chromosomal aberrations which would have caused abortion. This test has the disadvantage of being much more laborious and expensive than the bacterial methods, and of only detecting one class of mutants, namely, those with a dominant effect strong enough to cause visible defects in the embryo. These mutants are probably only a small class of the deleterious mutations possible.

3. Monitoring human populations

Let us now turn to the main point, the methods available for the detection of increased mutation rates in human populations. The classical method is to determine the frequency of certain dominant mutant phenotypes in the population, Crow [4]. To be useful in such a system, a mutant phenotype must meet the following criteria: (1) dominant, so as to appear in the first generation after a mutation occurs, (2) expressed at birth or early childhood, (3) serious enough to cause death before reproduction or reproductive failure so that all cases are due to new mutations, (4) not mimicked by phenocopies, and (5) so easily diagnosed that all such individuals will be recognized and reported. There are no mutant phenotypes now known that meet all these criteria. At best, this system is expensive and somewhat ambiguous, since a rise in congenital defects may have many causes other than mutation. Of course, the interaction of mutational events with other environmental insults is also of interest, so such an ambiguity is not necessarily a disadvantage. A more serious difficulty is that we are measuring only one class of mutants, as was the case with the dominant lethal test. In addition, of course, the detection of all individuals in a large population with a particular serious genetic abnormality is a task of tremendous scope.

A method of detecting a greater variety of mutations, including point mutations, is to examine a number of proteins electrophoretically. There are at least ten proteins which seem suitable (see Neel and Bloom [9]). These proteins show little or no polymorphism in the population and are detectable by electrophoresis with sufficient sensitivity and precision. The frequency of such mutants in man is not well known. Crow [4] suggests that the frequency of such mutations is on the order of 10^{-5} per gamete. In that case, since there are about 3×10^6 individuals born per year in the U.S., about 60 new mutants would be detected at each locus in a year, assuming complete detection. As the standard deviation is about 8, only an increase of one-third in the mutation rate, to 80 new mutants in the population, would be significant at the five per cent level. If we examine ten proteins, we would need to screen 300,000 infants a year to detect an increase of one-third in the mutation rate.

The frequency of electrophoretically detectable mutants, however, may be an order of magnitude higher than Crow estimates. Neel [8] estimated that certain rare, highly deleterious mutant phenotypes appeared at a frequency of about 10^{-5} in human populations. In *Drosophila*, there are about 30 times more mutations showing only a small decrease in viability than there are lethals (Mukai, [7]), and in *Salmonella*, only 10^{-1} of the mutants are lethals (Whitfield, Martin, and Ames, [10]). Thus, it may be reasonable to guess that the overall mutation rate in man could be as high as $10^{-5} \times 10 = 10^{-4}$. In this case, a one-third increase in the mutation rate could be detected by screening 30,000 infants a year for ten proteins. This is probably within current capabilities, as it involves examining 100 to 150 samples a day. This implies that, although electrophoresis

is probably not capable of detecting small increases in the mutation rate, it might be useful for detecting large increases. Obviously, the more individuals or the more proteins examined, the smaller the detectable mutation rate increase. If the mutation rate in human populations is as high as 10^{-4}, the problems with electrophoresis are probably financial rather than technical.

Electrophoretic methods detect only those mutations causing a change in the charge of the protein. On the basis of the genetic code, these are about one-third of all new point mutations. The immunochemical method of microcomplement fixation can detect a substantial proportion of these electrophoretically "silent" mutations. Microcomplement fixation can detect single amino acid substitutions in proteins (Cocks and Wilson, [3]) and can also distinguish between heterozygotes and homozygotes of protein variants. For example, blood from wild type individuals and blood from individuals heterozygous for the sickle cell trait react differently when tested against antihemoglobin serum, as shown in Figure 1. Since most point mutations will appear in heterozygous form this is a particularly valuable asset. This method probably measures the true mutation rate most realistically, since it is sensitive to all classes of mutations. The main difficulty is that in its present manual form it is by far too laborious—much more so than electrophoresis. Therefore, the only hope of using microcomplement fixation for the detection of mutation rate increases is through automation, which is now under investigation in our laboratory but has not yet been accomplished.

4. Tests for somatic mutations in man

A major problem in screening human populations is that each person tested provides only one item of data, thus requiring large sample sizes and the attendant problems of sample handling. Probably in large scale electrophoretic screening, the expense of sample procurement and preparation would considerably exceed that of the actual biochemical testing. One way of greatly amplifying the mutation detecting power of any monitoring method is by measuring the rate of somatic mutations, rather than of those occurring only in the germ line. Of course we are basically concerned with the effect of mutagens on the germinal mutation rate, but since the two sorts of mutations probably occur by the same underlying mechanisms, and are subject to similar physiological factors, the correlation between the two types of mutations is undoubtedly very high.

Two approaches have been proposed to make use of somatic mutations. The first involves the cytological examination of blood cells, usually from cord blood. Unfortunately this method detects only chromosomal abnormalities and not point mutations, which are probably of much greater importance. In addition, the effort necessary to examine cytologically large numbers of cells is prohibitive. Much effort has been spent on the automation of this procedure, however, and it is possible that it may eventually be useful as a screening method.

Another somatic cell procedure involves the detection of biochemical mutants

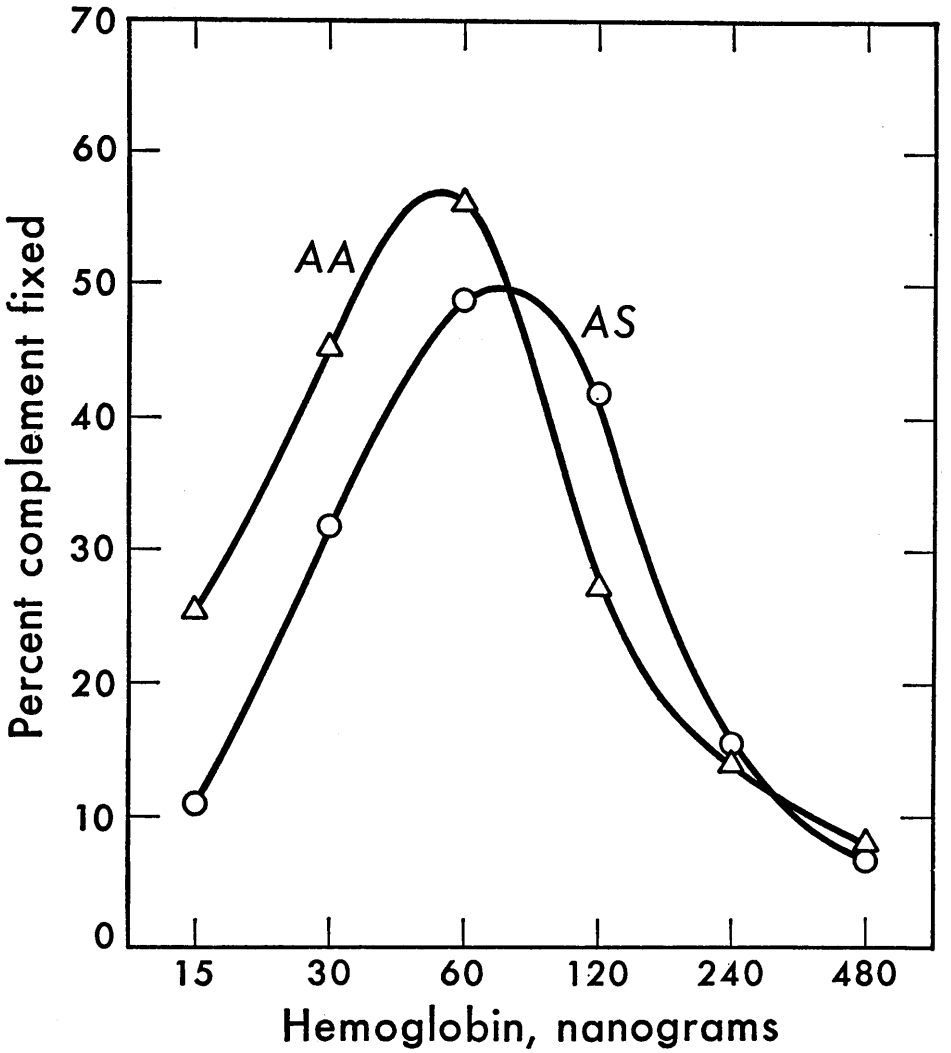

FIGURE 1

Microcomplement fixation test conducted with an antiserum prepared by immunizing a rabbit with pure hemoglobin A. The antiserum was reacted with hemolysates from a homozygous (AA) individual and from a heterozygous (AS) individual. (Goodman and Wilson, unpublished data.)

in human leukocytes using cytochemical techniques. Sutton has suggested testing for point mutations at the glucose-6-phosphate dehydrogenase locus in leukocytes from peripheral human blood (Hook, [5]). Over 70 variants of this enzyme are known in human populations. A substantial number of the variant enzymes can use 2-deoxyglucose-6-phosphate as a substrate in place of glucose-

6-phosphate. Sutton suggests developing a staining technique which will detect an enzyme at this locus with an altered substrate specificity. Such alterations might be shown to be due to somatic mutations in the stem cells of the leukocytes.

A cytochemical approach, using this or other enzymes, offers several unique possibilities. For one thing, meaningful data can be obtained from the cells of a single individual. This would permit us to correlate exposure to a particular environmental factor with its mutagenicity, and would enable us to determine the actual mutational damage to any particular person exposed to high risks. A further advantage lies in the possible use of this method as a final screening procedure for compounds that have been found to be not appreciably mutagenic in animals. Since humans will be exposed to such compounds anyway, it is surely better to test them on one or two individuals, measuring any increase in the somatic mutation rate in their leukocytes, than on the population at large, which is the only alternative and the one currently practiced.

To summarize, the technically possible methods of determining meaningful mutation rates in man are at present limited to monitoring for rare dominant deleterious phenotypes, and to electrophoretic screening. In each case the problems are those of efficient sampling and finance, not of genetics or biochemistry. The more sensitive methods of microcomplement fixation and detection of somatic cell mutations, in spite of their great potential, do not yet seem technologically feasible for large scale use. Because of this potential, though, we urge, along with Crow and others, that major effort be given to their development.

Note added in proof. We have tried various modifications of Sutton's method for staining white blood cells for glucose-6-phosphate dehydrogenase activity, with glucose-6-phosphate and 2-deoxyglucose-6-phosphate as substrates. In our hands this method does not give reproducible results (Clark and Wilson, unpublished work).

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Discussion

Question: John R. Goldsmith, Environmental Epidemiology, California Department of Public Health

Bateman has emphasized the value of spontaneous abortion rate and of cytogenetic abnormalities as an index of mutagenesis (and to a different extent teratogenesis). As therapeutic abortion becomes more frequent, cannot the cytogenetic analysis and also biochemical analysis of abortuses yield a greater amount of data on prevalence of mutations, since by definition some of them would not yield a live birth? Do you know of any laboratories studying biochemical mutagenesis in abortuses?

Reply: M. C. Clark, D. Goodman, and A. C. Wilson

We believe this question deserves serious attention, but we suspect that the mutation rate in fetuses from therapeutic abortion will not be as high as the rate in fetuses from spontaneous abortions. In addition, it would be difficult to obtain a representative sample of either spontaneous or induced abortions. We know of no laboratories studying biochemical mutagenesis in abortuses; several groups, including T. Shepard at the University of Washington and J. Miller at the University of British Columbia, are studying the frequency of gross abnormalities in abortuses.

Warren Winkelstein, Jr., Epidemiology, University of California, Berkeley

Since potential mutagens are probably unequally distributed between urban, rural, and regional areas, the number of samples required may be very substantial to provide adequate nationwide surveillance.