The overall objectives of this work are threefold:

1) To develop and improve methodology for measurement of mutation and repair in mammalian cells generally and particularly in human cells and to apply it to measurement of the effectiveness of mutagens, antimutagens, and other molecules which may affect the system, so as to achieve greater power in the prevention of cancer and genetic disease.

2) To analyze theoretically and experimentally the action of specific mutagens and antimutagens.

3) To investigate the role of genome exposure reaction in cancer and other disease conditions so as to secure improved preventive and treatment modalities.

Application of conventional in vitro mutagenesis testing has so far failed to result in marked reduction of the total incidence of cancer. At least part of the reason appears to lie in the frequent use of a cell target too small to yield adequate sensitivity and in failure to take into account the effects of cells killing and repair in the assessment of mutagenic action. We have developed a theoretical analysis which fits the results of experimental data on gamma radiation applied to single marker gene testing in bacteria and also to cytogenetic analysis of radiated mammalian cells. It permits the determination of the mean lethal dose, the mean mutagenetic dose, and the dose which can be repaired by the cell population under test. Cytogenetic analysis of human lymphocytes has been carried out by this procedure which permits detection of mutation by doses of gamma radiation as low as two rads (i.e. two CGY). This approach appears to offer the possibility of detecting (and therefore, preventing) exposures that may produce cancer and genetic disease. A variety of chemical mutagens have been examined and their range of effectiveness determined.

A new version of the mutagenesis test has been developed as follows:

1) A single cell survival curve is carried out on the agent to be tested a) in the presence and b) in the absence of caffeine. This furnishes a measure of the mean lethal dose for the agent, and an immediate indication as to whether or not it is a mutagen since, if it is, the survival curve in the presence of caffeine should fail to exhibit the initial lag due to repair. For agents which behave like X-rays, the limiting slope of the survival curve in the absence of caffeine should be the same as the slope in the presence of caffeine. Agents which fail to display this behavior can then be analyzed for the cause of their deviation from the standard curves which may be due to processes like cell damage to structures other than the genome.
DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.
2) The standard mutagenesis assay is applied to the test compound both in the presence and absence of caffeine. These two procedures will provide a direct measure of $D_{L}^{0}$, the mean lethal dose, $D_{M}^{0}$, the mean mutagenic dose and afford confirmation of the value for $D_{R}^{0}$, the amount of the mutagenic dose that can be neutralized by cellular repair processes.

3) The mitotic index on each of the mutagenesis slides will be counted both in the presence and absence of caffeine. This will afford confirming evidence for the repair capacity of the test cell and will also indicate whether the agent involved is exerting complex actions on the test cell beyond simple mutagenesis.

Study of the genome exposure reaction has been carried out by measurement of the amount of DNA liberated from isolated mammalian cell nuclei by carefully controlled exposures to DNase 1. Under these controlled conditions it was found that most cancer cells liberated less DNA than normal cells. This was found to apply to CHO as compared to its reversed transformed state, and the RAS transformed 3T3 cell as compared to the untransformed 3T3. These results agree with those found earlier on a series of other cancers using a more elaborate technique with whole cells.

It was found that calcium ion is required in the medium for this reaction.

4) An experimental study has been initiated to test whether differential inactivation of the X chromosome in normal human female cells follows the pattern predicted by the theory of genome exposure. In FISH experiments carried out so far, the behavior of the X chromosomes follows that predicted by the theory of genome exposure with respect to location of the two X's, their degree of condensation or diffuseness, and the total amount of fluorescent light emitted by the two sister chromosome.

List of Publications

Submitted by:

Theodore T. Puck, Ph.D.
Senior Fellow
Principal Investigator

Date 5/9/96