MASTER

DOE/ER/60016-1

TO: Dept. of Energy,
George E. Stapleton, Ph.D.
Health Effects Research Div.
Office of Health and Environmental Health

DOE/ER/60016--1

DE82 013352

RE: Progress report and application for continuation of DOE contract No. DE-A505-81ER60016. "In Vitro and In Vivo Carcinogenesis of Metals and Their Compounds Produced by Coal Combustion".

FROM: Max Costa, Ph.D.
Contract Project Director

DATE April 8, 1982

OF

REPORT:

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I. Progress report. (Sept. 1, 1981 - August 30, 1982)

Attached to this report are 6 copies of seven publications which list the present DOE contract number as providing partial support for the research results presented in these papers. It should be noted that DOE funds provided a small portion of the funds required to produce these results and primary support was provided by U.S. EPA funds. The reader is referred to these papers for detailed descriptions of results and methods. Paper 1 describes the findings of various experiments which examined the phagocytosis activity and induction of cell transformation by a number of particulate metal compounds in tissue cultures systems (crystalline or amorphous NiS, CoS, CuS and CdS). These results demonstrate that crystalline metal sulfide particles are actively phagocytosed by cultured cells and cause a high incidence of morphological transformation. In contrast, the amorphous structure of these same elements are not phagocytosed and do not induce as high of an incidence of transformation as the crystalline compounds. Also shown in this paper is the observation that inert particles such as latex beads which are actively phagocytosed by cultured cells do not induce transformation. Paper #2 and 3 extends these results by demonstrating the induction of DNA strand breaks by various water soluble and insoluble metal compounds. Crystalline NiS, AgS, CuS and Ni₃S₂ induce DNA strand breaks while amorphous NiS was inactive. Detailed analysis of the alkaline sucrose gradients allowed us to determine the change in DNA molecular weight induced by these metal compounds. Activated charcoal and latex beads which are both actively phagocytosed did not induce DNA strand breakage, suggesting that it is the ionic metal component rather than the intracellular particulate material contributing to the transformation and DNA strand breaks. A number of water soluble metal compounds were also examined for their ability to induce DNA strand breaks.

A critical finding with regard to the mechanisms by which particulate metal compounds induce cell transformation involves the surface charge of the particles (4). The actively phagocytosed crystalline NiS particles have a negative surface charge while particles consisting of amorphous NiS which are not phagocytosed have a positive surface charge. The findings presented in paper #4 demonstrates that by rendering the surface of amorphous

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NiS more negative using LiAlH_4 reduction, the particles are phagocytosed and induce a much higher incidence of transformation. Thus a negative surface charge seems to determine whether a particle is phagocytosed by a cell and this uptake ultimately relates to the ability of a particle to exert its carcinogenic effects.

The ability of nickel compounds to interact and bind with DNA, RNA and protein was also investigated (#5). Crystalline NiS was compared with NiCl_2 in terms of nickel binding to RNA, DNA and protein isolated from cells treated with these compounds. Treatment of cells with crystalline NiS resulted in several orders of magnitude greater binding of nickel to DNA, RNA and protein compared with NiCl_2 treatment. The nickel bound to these macromolecules represented dissolution products derived from phagocytized NiS particles.

Video intensification microscopy was utilized to study the phagocytosis of the nickel sulfide particles (#6). DOE funds provided only a minor portion of the support for this work. Similarly, the extracellular conditions required for the phagocytosis of crystalline NiS particles was also investigated (#7). Again DOE funds provided only a small percentage (<5%) of the support for this project. These papers are primarily included only for completeness and to illustrate further the direction in which the laboratory is headed.

DOE funds were used primarily for the work described in papers #1, #2, #3 and #4. These studies are consistent with the thrust of the initial contract where a number of metal compounds would be examined for their ability to induce cell transformation and cause DNA strand breaks. These metal compounds are important environmental contaminants derived from coal combustion.

List of papers cited in progress report:

1. Max Costa, J. Daniel Heck and Steven H. Robison, "Selective Phagocytosis of Crystalline Metal Sulfide Particles and DNA Strand Breaks as a Mechanism for the Induction of Cellular Transformation", Cancer Research, (in press) July, 1982.
2. Steven H. Robison, Orazio Cantoni and Max Costa, "Strand Breakage and Decreased Molecular Weight of DNA Induced by Specific Metal Compound", Carcinogenesis (in press) 1982.
3. Steven H. Robison and Max Costa, "The Induction of DNA Strand Breakage by Nickel Compounds in Cultured Chinese Hamster Ovary Cells", Cancer Letters, 15 (1982) 35-40.
4. Maria P. Abbracchio, J. Daniel Heck and Max Costa, "The Phagocytosis and Transforming Activity of Crystalline Metal Sulfide Particles are Related to their Negative Surface Charge", Carcinogenesis, Vol. 2, No. 2, pp. 175-180, 1982.
5. Peter B. Harnett, Steven H. Robison, Douglas E. Swartzendruber, and Max Costa, "Comparison of Protein, RNA, and DNA Binding Cell-Cycle-Specific Growth Inhibitory Effects of Nickel Compounds in Cultured Cells", Toxicol. Appl. Pharmacol. 63, 000-000 (1982).

6. R. Mark Evans, Peter J.A. Davies and Max Costa, "Video Time Microscopy Study of the Phagocytosis and Intracellular Fate of Crystalline Nickel Sulfide Particles in Cultured Mammalian Cells", Cancer Res. (in press) July, 1982.
7. J. Daniel Heck and Max Costa, "Extracellular Requirements for the Endocytosis of Carcinogenic Crystalline Nickel Sulfide Particles by Facultative Phagocytes", Toxicology Letters (in press).

II. Work proposed within the constraints of the designated budget for the second year of the contract.

Our laboratory is moving in the direction of trying to understand how metal compounds interact with DNA, to cause mutations and cell transformation. The techniques we are currently experimenting with to study these effects include alkaline sucrose gradients (to analyze DNA strand breaks), alkaline and neutral elution techniques (to study strand breaks and DNA-DNA or DNA-protein crosslinks) and Cesium chloride to study DNA repair. Using these techniques we would study 2-3 metal compounds which are potent inducers of morphological transformation (crystalline NiS, CaCrO₄ and NiCl₂) to understand the effect these compounds have on DNA in intact cells. Preliminary results indicate that nickel compounds damage DNA differently than Cr⁺⁶. Both compounds induce strand breaks in intact cells but the nickel compounds appear to induce more crosslinks than strand breaks and an examination of the time course of strand breakage and dose response relationships indicate that the breakage of DNA is directly related to dose and time. Chromium differs in that there appears to be a threshold dosage which results in 30 times the damage to DNA compared with nickel and there seems to be no direct relationship between concentration and degree of strand breakage. With the proposed funds we could study these compounds further and determine the degree of crosslinking, strand breakage and DNA repair. Our repair assay using dual isotopes of thymidine bromo-deoxyuridine and 2 Cesium chloride gradients is exquisitely sensitive and specific for repair. Additionally, using neutral sucrose gradients and neutral alkaline elution techniques we could study the induction of double stranded breaks in the DNA. With the funds provided, we would be limited with respect to the number of metal compounds we could study. However, we would hope to include several other metal compounds in addition to the ones mentioned above which are important environmental contaminants resulting from coal combustion.

III. Additional studies which could be conducted with the newly proposed budget.

The proposed increase in funds would give us the opportunity to examine the effects of a number of other metal compounds on DNA strand breaks, crosslinks and DNA repair. This may lead to identifying those metal compounds which alter DNA structure differently as appears to be the case in comparing the effects of nickel and chromium. Additionally, we would be able to study the interactions between two or more metal compounds in terms of their effects on DNA structure and function. Preliminary findings indicate that nickel and chromium greatly potentiate each other in terms of inducing strand breaks. For example, at a concentration well below that required for either metal ion to induce breaks in the DNA their addition together results in severe breakage in intact cells. A more detailed

study of this potentiation effect may provide evidence that these two metal ions act at different sites within the DNA. A even more interesting approach may entail an examination of where within the DNA structure these metal ions cause breakage. Is it random or confined to a specific region? Does damage occur at unique DNA sequences or reiterated sequences? Our approach to this problem would be to utilize the repair of DNA to selectively radiolabel the portion damaged by the metal. Subsequent nuclease treatment, hybridization studies and DNA gel studies will yield this type of information. These results will be particularly interesting following treatment with two metal ions that act synergistically to induce strand breaks.

It is becoming increasingly evident that effects upon DNA structure and function are an early and sensitive manifestation of metal toxicity at the cellular level. This is evident by examining the relatively non-toxic levels of metal in which we can detect strand breaks or crosslinks. Additionally, we have determined that a number of metal compounds selectively block cells in the S phase of the cell cycle, further suggesting that DNA structure and function is a primary target of metal toxicity at the cellular level. Elucidating the mechanism(s) involved in metal induced genotoxicity and attempting to understand if different metals which are environmental contaminants from coal combustion cause distinct effects upon DNA is a worthwhile goal. The proposed increase in funds would enable us to examine additional metal compounds Cd^{2+} , Hg^{2+} , Pb^{2+} , Cu^{2+} and Co^{2+} in terms of their interaction with cellular DNA alone and in combination. With the additional funds we could also anticipate studying whether cells that become resistant to metal ions (i.e. Cd resistant cells) are also resistant to other metals in terms of cytotoxicity and effects upon DNA. Since we have also found that Zn^{2+} is capable of protecting cells against Ni^{2+} induced breakage of DNA a study of the interaction of the various metals could prove interesting.

Under conditions not involving the protective effects of metallothionein induction, Zn clearly effects the distribution of ionic nickel in the cell. Is this effect specific for Zn^{2+} or do other metals exhibit similar activity in altering subcellular distribution patterns? The results indicate substantial displacement of nickel bound to specific subcellular fractions by Zn, perhaps by occupying binding sites for this toxic metal ion.