SECTION H, MICROBIOLOGY

The Use of Phytohemagglutinin as an Aid to Cellular Repair During Constant X-Irradiation[1]

CLIFTON ALDRIDGE, JOHANNA G. GRAY, and J. B. CLARK,

Avco Biophysics Laboratory and University of Oklahoma, Norman

The hemagglutinating properties of certain extracts from bean plants have been known for years. Such phytohemagglutinins have been useful in the separation of the formed elements from the plasma of mammalian blood, and if proper conecentrations of phytohemagglutinins are employed, it is possible to agglutinate only the erythrocytes, the leukocytes remaining in suspension even after slight centrifugation. It was reported by Moorhead (1960) that phytohemagglutinin had the ability to initiate mitosis in suspensions of leukocytes isolated from peripheral blood by this technique.

Since it is well known that radiation inhibits or delays mitosis, it is reasonable to suspect that a chemical agent which stimulates mitotic activity may alter the damaging effect of radiation. Workers at this laboratory decided to test this possibility.

Cell suspensions of *Escherichia coli* were irradiated with 200 KVCP X-ray with and without the addition of 50 μg of phytohemagglutinin P (Difco) per ml. Growing cultures of *E. coli* in nutrient broth (Difco) were similarly treated and irradiated to total doses ranging from 7,000 to 28,000 R. Growth rates were checked hourly by measuring changes in turbidity with a colorimeter.

In growing cultures, it was consistently observed that even a trace amount of phytohemagglutinin allowed growth of cells to proceed while being irradiated. Control cultures without phytohemagglutinin failed to grow, and usually began to lyse. Plate counts and direct micrscopic examination confirmed that growth was actually occuring, and was not an effect measured only by the colorimeter. In no experiment was any significant degree of protection observed in plate counts made from the nongrowing cells.

Subsequent investigations demonstrated that phytohemagglutinin was specific, and tests substituting gelatin, porcine beta globulin, porcine albumin, human serum, calf serum, glucose, and ATP failed to reproduce the effect. Attempts to demonstrate an effect on the rate of cell division in *E. coli* by the addition of phytohemagglutinin have been unsuccessful. Growth curves of cultures grown in the presence of phytohemagglutinin have not differed from controls. Attempts to demonstrate a mutagenic effect of phytohemagglutinin have also been unsuccessful.

The addition of phytohemagglutinin to cells before or after exposure to radiation, or the addition of phytohemagglutinin to the plating medium had no significant effect upon the recovery of irradiated cells.

Accumulated evidence to date suggests that the effect of phytohemagglutinin on the damaging effect of X-rays in *E. coli* is unique among agents affording radiation protection in that pre- or post-irradiation treatment with the substance is without effect. However, its presence in a growing culture of *E. coli* during constant X-irradiation will prevent radio-lysis and even permit growth to proceed. This is true in spite of X-ray dose rates high enough to consistently produce lysis in control cultures.

[1]This work was supported by NASA Contract No. NAS2-1545.

This study has recently been extended at this laboratory to include mice. Preliminary results have been encouraging and merit further investigations.

Literature Cited

Moorhead, P.S., P.C. Nowell, W.J. Mellman, D.M. Mattips, and D.A. Hungerford. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res., 20: 613.