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ENZYME THAT CONTROLS RADIATION DAMAGE DETECTED IN HIGHER PLANTS
BY DR. HAROLD WERBIN, SOUTHWEST CENTER RADIOBIOLOGIST

Tender, young pinto bean sprouts survive in the Texas sunlight, which brings with it enough invisible far-ultraviolet radiation to damage the long molecules of genetic material that dictate the pattern of life.

But the Sun's visible light also triggers a "repairman" to mend the radiation damage at high speed until the young plants develop other protective agents, like leaves, pigments and chlorophyll.

The repairman that keeps the genetic system running at a critical time is a photoreactivating enzyme. Its presence in higher plants has now been detected by Dr. Harold Werbin, Associate Professor in the Biology Division at Dallas' Southwest Center for Advanced Studies.

The PhR enzyme is a protein already known to exist in bacteria, phages and animal cells which was discovered by Prof. Claud S. Rupert of the SCAS' radiobiology faculty.

Professor Werbin reports the successful search in plants in the current issue of Photochemistry and Photobiology (April, 9, 389, 1969). Dr. Narimasa Saito, a post-doctoral research associate on the SCAS' staff, is co-author of the report.

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Natural recovery of tobacco plants from leaf damage caused by radiation was demonstrated by the early 1950's, but the mechanism remained unknown until now. Presence of recovery systems in micro-organisms was also shown in the 1950's. One of the principal investigators was Prof. John Jagger, who now is a member of the SCAS' radiobiology group.

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Associated studies of radiation damage and natural repair are being conducted at the SCAS by Prof. Walter Harm, Assoc. Prof. Hans Bremer, and Asst. Prof. Michael H. Patrick, with Professors Jagger and Werbin. The radiobiology faculty, ranked as one of the nation's leading research and teaching groups in the field, has obtained more than $500,000 in research support funding from major granting agencies.

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PhR enzyme activity is assayed by a transformation procedure which measures the enhanced capacity of PhR enzyme-treated, UV-irradiated DNA to impart streptomycin resistance to H. influenzae cells.

Professor Werbin found the enzyme difficult to detect in older bean leaves as he began the study. Nucleases in the older leaves' juice interfered with the assay by degrading the substrate, transforming DNA. This difficulty did not appear in assays of younger leaves because of their lower nuclease content.

The clue led to assays of extracts of five-day-old plants (sprouts) grown in the dark on water-soaked cotton. PhR enzyme activity was concentrated in the plumules (primary buds) and hypocotls (first stem growth appearing above ground) of the young bean plants.

Far-UV radiation induces lesions in the genetic material (DNA, dioxyribonucleic acid) of most cells. Principal damage, which interferes with the replication of the DNA, is the formation of cyclobutane dimers between adjacent pyrimidine molecules in the DNA.

The PhR enzyme's unique functions are the ability to complex the damaged DNA, and also to make use of visible light energy to break the two carbon-carbon bonds of the cyclobutane ring, thereby restoring the DNA to its original state and function.

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Bean leaves for the early experiments were supplied to the SCAS' scientist by Dr. W. Derby Laws of the neighboring Texas Research Foundation, the leading center of crops and soils research in North Texas.

The young plants were sprouted from beans bought at a local supermarket. PhR enzyme assays were also conducted on Great Northern, large Lima, white Navy, Mung, and soybeans. Enzyme activity was high in Pinto, Great Northern, and Lima bean sprouts; lower in Navy beans, and lowest in Mung and soybeans.

The SCAS' experiments were supported by research funding from the Atomic Energy Commission and the National Institutes of General Medical Sciences; both agencies recently extended their contract and grant.

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