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CONTRACT AT(30-1)1286 - 18th Renewal

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SUMMARY: M. L. Minthorn, Jr.

This contract has supported a variety of studies aimed at assessing the effects of ionizing radiation on living systems and their components. During the first eleven years of the contract, the research effort consisted of two unrelated activities: (a) an epidemiological study (by Dr. Hempelmann) on the incidence of leukemia in a population of humans who had been exposed in childhood to ionizing radiation for medical reasons and (b) a series of biochemical studies on radiation effects at the molecular level, conducted by associates of Dr. Hempelmann. About seven years ago, support of the epidemiologic study was transferred to Rad Health (USPHS) and, since that time, the contract has supported only a basic program in molecular radiation biology directed by Dr. S. Okada. However, Dr. Hempelmann has retained the title of Principal Investigator and has continued to contribute to the program (administratively).

After completing ongoing studies on radiation inactivation of DNase I and effects of radiation on the biosynthesis of DNA in regenerating rat liver, Dr. Okada oriented his entire research enterprise around the radiation biology of cultured mouse leukemia cells (Fischer L 5178 Y cells). Considerable time and effort was spent in preliminary work that was required to become familiar with the characteristics of these mammalian cells, to work out experimental procedures, and to obtain baseline data. Thereafter, Dr. Okada established a vigorous, varied, and productive program aimed at elucidating mechanisms whereby various doses of ionizing radiation damage or kill the L 5178 Y cells in vitro. Among other things, this effort entailed the study of the following: the nature and cause of radiation-induced reproductive death and radiation-induced interphase death; radiosensitivity of the cells at various stages of the cell cycle; the relative killing efficiency of intracellular tritium incorporated into DNA, RNA, or proteins (cell suicide experiments); DNA replication and characteristics of the replicating unit or replicon; effects of radiation on DNA biosynthesis and DNA structure; repair of radiation-damaged DNA molecules; effects of radiation on RNA biosynthesis and RNA structure; enzyme biosynthesis at different times in the cell cycle and effects thereon of radiation; and radiation effects on cell membranes. The main emphasis, however, has always centered on DNA structure, function, and biosynthesis as affected by ionizing radiation.

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During the past year, Dr. Okada's research team completed work on the following studies, all of which involved use of cultured L 5178 Y cells: (1) effects of cysteamine and cystamine on normal DNA synthesis and on radiation-induced breakage and rejoining of DNA (Dr. Sawada), (2) quantitative aspects of the process leading to the rejoining of single-strand breaks in DNA (Dr. Okada), (3) synthesis of DNA-hybridizable RNA in normal and x-irradiated cells (Mr. Meltz), and (4) interphase death of irradiated cells and death of cells (both irradiated and unirradiated) in the stationary phase of growth (Mr. Goldstein).

The work with cysteamine and cystamine established that the former is toxic at low concentrations (0.5mM). At this concentration, cysteamine induces single-strand breaks in DNA (in both irradiated and unirradiated cells), depresses DNA biosynthesis, and reduces the rate of rejoining of radiation-induced single-strand breaks in DNA. At higher concentrations, cysteamine protects DNA against radiation-induced single and double-strand breaks. Cystamine was found to be devoid of toxicity and to afford little protection against DNA damage.

In the quantitative work on the DNA rejoining process, it was demonstrated that the number of breaks increase linearly with total dose (up to a dose of 50 Krads), but exponentially with respect to dose rate. Because of rejoining activity the number of single-strand breaks in DNA decreases exponentially with incubation time after irradiation. Like DNA synthesis, rejoining activity is temperature sensitive and, therefore, probably enzymatic in nature. Since the two processes differ quantitatively in their response to temperature, they are presumably catalyzed by different enzymes.

The work by Mr. Meltz yielded useful information on the rate of biosynthesis throughout the cell cycle, radiation effects on biosynthesis, and turnover rate of DNA hybridizable RNA. Three groups of hybridizable RNA differing in turnover rates were identified (t 1/2 = 10 minutes, 1-1 1/2 hours and 20 hours). X-Irradiation (of cells) produced no detectable changes in base sequence or turnover rate of hybridizable RNA. However, irradiation did reduce the overall rate of RNA biosynthesis somewhat, with hybridizable RNA less affected than other species of RNA. On the basis of Mr. Goldstein's work, it is concluded that membrane leakage is a general phenomenon associated with interphase death and is, therefore, not uniquely associated with radiation-induced cell death. Evidence was also obtained that the leakage phenomenon may relate to cellular enzyme activity.

Work in progress at the end of the contract year was concerned with studies of (1) the rejoining of single-strand breaks in DNA produced by small radiation doses (Dr. Sawada), (2) the role of rejoining processes in the joining of newly synthesized replicons in normal cells (Dr. Sawada), (3) the action of radiosensitizers (BUdR and NEM) on the rejoining process in irradiated cells (Dr. Sawada), (4) the role of proteins and protein synthesis in the initiation of DNA synthesis and mitosis (Mr. Roti Roti), (5) enzyme synthesis at different stages of the cell cycle (Mr. Kapp), and (6) the relation of single-strand DNA breaks to radiation induced cell killing (Mrs. Roots). Dr. Sawada's work on the rejoining of DNA breaks is particularly worthy of note. He has developed an improved method of cell lysis which permits DNA breaks to be quantitated with enhanced sensitivity. This improvement, in turn, has made it feasible to perform quantitative studies on single-strand DNA breaks in cells exposed to biologically meaningful doses of radiation (100-1000 rads). Data recently obtained by Dr. Sawada indicate that the rejoining process is operative in unirradiated cells and that it is delayed by BUdR and totally inhibited by NEM. In addition, Dr. Sawada has also found it possible to demonstrate the rejoining of double-strand DNA breaks in cells exposed to very high doses of radiation (10,000-20,000 rads).

During the past year, Dr. Okada accepted a permanent position in Japan and all members of his research team, except two graduate students (Mr. Roti Roti and Mrs. Roots), have left the program. A Dr. Christopher S. Lange has recently joined Dr. Hempelmann's staff to replace Dr. Okada. Dr. Lange will direct the activities of the two remaining graduate students, extend certain aspects of Dr. Sawada's work on the rejoining process, and initiate new studies of his own design. As regards the continuation of Dr. Sawada's work, Dr. Lange has noted that the rejoining of double-strand breaks in L 4178 Y DNA occurs at the same time during the cell cycle that Elkind type repair occurs in He La cells. Dr. Lange, who has done extensive work on repair activity in He La cells, plans to repeat in He La cells work of the type done by Dr. Sawada in the Fischer cells. This should shed light on the interesting possibility that Elkind type repair may be explainable in terms of the rejoining of double-strand DNA breaks. The new work that Dr. Lange has in mind involves (1) a systematic study of stem cell kinetics and cell differentiation, using Planaria as a simple model system and (2) study of details of the repair system in mammalian cells (probably He La cells).

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PROJECT EVALUATION:

The research effort supported by the contract, which has been highly productive and programmatic, is being rapidly phased out to be replaced by a new program under new leadership. In view of these extensive changes plus the fact that Dr. Lange is completely new to the program, it seems to me that termination of the contract is appropriate. Dr. Hempelmann has expressed agreement with this course of action and Dr. Lange is now preparing a new proposal for submission. The main consideration in regard to the terminal contract period is that of providing adequate support for the orderly termination of work initiated under Dr. Okada. Dr. Hempelmann has stated that support at the 60 percent level would be adequate for this purpose.

RECOMMENDATION:

Terminal year at 60 percent of the previous year or $25,200. Since $6,000 has already been provided during a 3-month extension, this would amount to $19,200 for the balance of the terminal period.

MIFM
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