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SEMI-ANNUAL STATUS REPORT

NASA GRANT NsG 262-63

January 15, 1964-July 15, 1964

This research grant supports multidisciplinary studies of effects of high energy protons on biologic processes and living systems. The proton source is the Harvard University 160 Mev Synchrocyclotron.

During the period cited 32 days Cyclotron time were devoted to biomedical studies and 7 days to radiation damage studies.

Individual project reports are attached.

Submitted: *

OTS PRICE

XEROX $ 1.00
MICROFILM $ .50

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*Dr. Preston, Co-principal Investigator is on summer leave.

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BIOMEDICAL

Use of the Bragg Peak of a Proton Beam in Producing Intracranial Lesions

During the past six months 32 patients underwent intracranial Bragg Peak proton irradiation for various disorders.

A breakdown by type of case is as follows:

Pituitary irradiation for diabetic retinopathy. . . .	23
" " " breast cancer.	3
Irradiation of malignant brain tumor.	4
Irradiation of benign pituitary tumor.	2

The stereotactic method is still used and doses and dose rates remain approximately the same as those reported previously.

Blood pressure, pulse rate, respiratory rate, EEG and EKG are now being recorded on all patients undergoing proton irradiation. Readings are taken before, during and after treatment.

Comparing average values of respiratory rates in eleven patients we found that rates in nine patients remained constant (showed no change during or after treatment) and two patients indicated a decreased rate post-irradiation.

Pulse rates were recorded in 18 patients. Eight remained constant; eight showed a decrease and two an increase.

Blood pressure recordings were made in 17 patients. Eight remained constant; six showed a decrease and three an increase.

Both EEG and EKG did not change as a result of irradiation.

We should like to monitor additional patients before reporting conclusive results. However, the data accumulated so far suggest no true basis of change as a result of irradiation. We attribute some degree of change to the

patient's emotional state, outside stimuli and in diabetics, the state of the disease.

Examination of post-operative brain specimens continues; we obtained two pituitary-irradiated specimens recently. Lesion evaluation and comparison of biologic effects with precise dose measurement should prove useful in further developing the procedure.

A history of transient oculomotor weakness developed in several pituitary-irradiated patients. An experiment irradiating the optic and oculomoto nerves in cebus monkeys may help to solve the problem.*

The first Cyclotron patient was treated 3 1/2 years ago; a total of 114 patients have been treated to date. We seek to avoid long term adverse effects or delayed responses to radiation, nevertheless, we follow-up all patients to asses this contingency. Results will be reported at a later date.

R. N. Kjellberg

*See page 3

Effect of Proton Beam Irradiation of the Pituitary on Optic and Oculomotor Nerves

To determine a biological end point for proton beam tolerance of the II-VI cranial nerves, a neuro-opthalmological test was devised. Thirteen *cebus albifrons* monkeys were tested.

Eight of these monkeys were lightly anesthetized and then subjected to proton beam irradiation via two 7 mm portals. The beams were focussed on the pituitary, which was located by skull x-ray. Rate was approximately 1.6 K rads/min.

Doses range from 4 to 15 K rads/monkey. Monkeys receiving 12.5 and 15 K rad were tested until their deaths, 24 and 10 days respectively after irradiation. At that time defects were primarily extra-oculomotor paresis. On the twelfth post-operative day a monkey which received 10 K rad had developed unilateral paralysis of muscles innervated by the third left cranial nerve. No deficit has been detected thirteen days post-operatively in monkeys which received less than 7.5 K rads.

Specimens of cranial nerves and brains of monkeys dying after irradiation have been preserved and are undergoing preparation for histological study.

S. P. Cobble
R. N. Kjellberg
H. T. J. Mount

Anatomic Changes with Beams of Small Dimension

Seven *Cebus* monkeys were spine-irradiated in the lower thoracic region at the mid-dorsal level using a knife-like proton beam (.75 x 28 mm) for determination of anatomic change. Doses ranged from 6 to 15 K rads.

The animals were anesthetized with Diabutol, intubated and the exposed spinal cord clamped to the beam pipe.

Following radiation the animals were observed at regular intervals with the following results:

Monkey	Dose:K rads	Impairment of Legs	Paralysis of Legs
1	6	69 days	Incomplete at 118 days
2	8	69 days	76 days
3	10	69 days	74 days
4	11.5	18 days	28 days
5	12	18 days	25 days
6	13	8 days	15 days
7	15	7 days	10 days

Our future plans include 1) the establishment of a critical dose for horizontal spine-splits and 2) development of a technique for splitting the cord on a vertical axis.

R. N. Kjellberg

Measurements of Brain Lesions

The technique of making two focal lesions in the two hemispheres of the monkey's brain and then comparing their size as a measure of biological effect has been extended to measure the effect of dose rate. Ten monkeys were irradiated to a total dose of about 9 K rad, of dose rates of 0.8 K rad/minute and 8.2 K rad/minute. A preliminary examination of the extent of lesion 30 days after irradiation indicates a greater effectiveness at higher dose rates. We hope to derive more quantitative results presently.

A. M. Koehler
C. Hajek

The Effect of Low Dosage Long-Term Total Body Proton Radiation on the Defense Against Bacterial Invasion

In the past six months, we have concentrated on two problems: first that of continuing the study of the animals radiated in July, 1963 over a 59 hour period and second: plan of a second experiment designed to irradiate animals over a ten day period taking advantage of the biologic information gained during the first experiment.

All of the animals radiated in 1963 that survived have returned to normal as judged by our studies. The initial impression of a substantial drop in the lymphocytic count following as little as 50 rads over the 60 hour period of radiation continues to hold. Complete evaluation of the histology, both light and ultra structure, has not been completed yet.

Beginning in July, a second long-term experiment is planned. This will include approximately 120 to 160 animals and will include studies not only of white cell activity, but turn-over studies of jejunal mucosa, lymph nodal lymphoid cells, spleen and bone marrow using tritiated thymidine.

All cell absorption experiments and parts of the project relating to actual radiation were carried out by A. Koehler.

J. F. Burke

Solid Chemical Radiation Dosimetry

It was decided that more precise information was needed about the G value (number of molecules of acid produced by 100 ev of energy) of the paraffin base dosimeter. A clearer understanding of some of the basic mechanisms was necessary to plan properly the future course of the research toward improvements in sensitivity and stability. During the past six months a concerted effort has been made to develop an accurate technique of measuring the amount of halogen acid produced in the HAP dosimeter by ionizing radiation. Hot water treatment of melted halogen compound - azo dye - paraffin formulations has resulted in a quantitative extraction method which permits pH determinations. A meter with a high sensitivity to chlorides and fluorides has been found satisfactory. A useful technique has been elaborated but improvements are desirable.

The method is being used to study HAP dosimeters of different composition exposed under various conditions to ionizing electromagnetic and particulate radiation. The studies have shown that for the same formulation increased dose results in a linear or almost linear production of acid, and that photoelectric cell measurements of color change, as per cent light transmittance through the solid dosimeter, parallels acid production under these conditions.

There are indications that exposing different concentrations of halogen compound in paraffin to the same radiation dose shows an increase in acid

production proportional to halogen compound concentration. Color changes in the azo dye, however, do not parallel this pH shift. As has been previously reported, a peak color response to x-rays is reached with 1-2 molal chloroform and 4-6 molal bromoform. Higher concentrations of these agents evidently produce more acid but do not exhibit a corresponding color change in the indicator dye. Whether this phenomenon will hold for proton irradiation has to be determined.

Two articles "Proton Dosimetry Using the HAP System" and "Beta Dosimetry Using the HAP System" are being prepared for publication.

M. S. Potsaid

Effects of Proton Irradiation on Mitosing Systems

During the last six month period further series of C3H mice carrying the ependymoblastoma No. 48 have been irradiated and the tumors investigated according to methods described earlier reports. The structures observed in electron microscopy seemed to correlate with earlier mentioned light microscopic findings. Experiments will be carried out to further analyze the effect of the proton beam on the ependymoblastoma No. 48 in C3H mice.

Experiments have been done also with a completely different tumor (Zimmerman tumor) transplanted into another species of mouse.

S. Nystrom
A. M. Koehler

Basic Studies on Biologic Protection Against Proton Radiation

During the six month period work has continued on the comparison of the protons with x and γ radiations, using the methods outlined in the previous report. The studies have concentrated on two aspects which are described briefly below.

A. Damage at the Molecular Level

Both initial damage to dry enzymes as indicated by the radical production and inactivation as determined by biochemical assay has been further investigated in the enzymes ribonuclease and lysozyme. Three series of irradiations have been carried out at various dose levels, and the influence of temperature and the presence of oxygen compared for high energy protons, "Bragg peak" protons and Co^{60} γ rays. A few experiments have also been attempted using fast neutron irradiation from a proton Van de Graaf accelerator. These results are not yet complete, but there are indications that with such high LET radiation, some qualitatively different effects are produced, in contrast to the difference between the effects of higher energy protons and γ rays which appear to be only quantitative.

Our experiments have also indicated that dose rate can influence both the radical yields and the enzyme inactivation, the damage being greater for higher dose rates. The irradiations which we carried out at the beginning of July were planned to investigate this aspect and these results are currently being analyzed.

B. The Effect of Radiation Protecting Agents

We have continued our studies on tissue response to proton irradiation and the modifying effects of the protecting agent AET. The same system, a subcutaneously transplanted rapidly growing murine tumor, has been

used and we have irradiated and then transplanted a total of 114 tumors, each into four or five unirradiated recipient mice, in the last six month period. A smaller series of 40 tumor bearing mice have been irradiated with high energy x-rays. We are continuing these experiments using the 6 Mev linear accelerator at the Peter Bent Brigham Hospital for the x-irradiations. This machine enables us to use the same average dose rate as we use with the Cyclotron proton beam. We thus hope to obtain a comparison of the effects of x-rays and protons without the complicating factor of possible dose rate dependence. The quantal response indicates that for proton irradiation at the 4,000 rad level, AET protects the tumor to the extent of increasing from 1/46 to 15/46 the number of irradiated tumors which grow after transplantation. At levels of 5,000 rad and above, we have observed no growth in periods up to six months in either group of mice.

Retardation of tumor growth due to irradiation at intermediate dose levels, is also being studied and compared with the time pattern of growth of different sized innoculums of unirradiated tumor cells. The application of Hewitt's methods (N. Y. Academy of Sciences 95. 818 (1961)) to this problem is under active investigation.

A. H. Soloway
K. Stratton

Histopathologic, Electrophysiologic and Behavioral Studies

Work in the Stanley Cobb Laboratories during this period has covered three major areas of activity. The work on quantitative histopathology of radiation damage has continued with the development of technqiues for quantitatively measuring cell loss, cell death and cell damage in various neural groups in relation to superimposed plots of the isodose curve of the incident radiation. We hope to have this data substantially complete by early fall for two dose rates, several levels of intensity, and with a comparison of similar doses along the shank and at the Bragg peak of the proton beam. Of particular interest is the attempt to compare intensity of damage at the peak with that elsewhere along the penetrating path to supplement Koehler's careful comparison of the damage in gross lesions which is suggested in increased RBE at the peak. As a supplement to the already available data from our monkey irradiations, we have, in addition, utilized the retina of the rabbit eye. This sheet of complexly oranized central nervous system elements is particularly advantageous for this purpose because in the rabbit it is an avascular structure receiving its nutrient supply by diffusion rather than by direct capillary flow as is common in other central nervous system structures. This anatomic peculiarity gives us the privilege of examining effects on neural elements independent of impairment of the vascular supply which is such a prominent part of radiation pathology in other structures. This technique should allow us to confirm or disprove our impression from the brain pathology that damage occurs in neural elements both earlier than and independent of visual endothelial damage in the vascular supply.

The electrophysiologic studies were not continued during this period but were resumed on July 1st with the addition of an assistant for the summer, Mr. Paul Chervin, who has done previous work on radiation effects on electrophysiologic test systems. His work this summer should complete our earlier series measuring changes in functional activity of the brain in response to radiation independent of gross structural damage. These studies again should be complete by early fall, and with the completion of a comparison of the anatomic with the electrophysiologic changes, this phase of the work will be probably discontinued.

A major activity during the past three months has been the construction and equipping of a behavior laboratory as outlined in our budget proposal for the current fiscal year. We have designed and built a set of screened sound-attenuated monkey operant conditioning chambers and procured equipment for one of them, to test the effects of whole head irradiation on a variety of performance indices designed to test various aspects of complex performances analogous to those presumed important to a man functioning in space. The dose levels to be tested will be at first those expected to be maximal from solar flares as received at the moon's surface. The first monkeys have arrived, and a full time person has been employed to assist with the initial training. Due to the unavailability of Cyclotron time during July and August, the first irradiated animals will not be tested until early fall. By that time, it is hoped that funds for the current fiscal year will become available and equipment can be procured for two further test chambers. During this time, an experienced experimental

psychologist who has worked for the past five years primarily in the effects of radiation on behavior has been interviewed and agreed to accept a position on this program. Conclusion of negotiations with him also awaits notification of budgeting for the fiscal year.

F. R. Ervin

Radiobiology of Protons of Varying Energies in Mammalian Proliferative Tissues and Cells

A. Relative Biological Effectiveness of Peak and Shank Protons in the Production of Chromosomal Breaks in Mouse Ehrlich Ascites Cells

The radiobiology of the Ehrlich ascites cell system has been extensively studied by other investigators. Hence, it provides a workable tool for studies with high energy protons. This system is ideal for use with the Cyclotron since large numbers of cells can be irradiated in-vitro in a small chamber with dimensions that fit within an isodose field. Dose-response curves were obtained with 250 Kvp x-rays and the production of abnormal anaphases (chromosomal bridges or deletions) proved to be linear between zero and 400 rads. Counts were made on Feulgen stained smears. Subsequently cells were irradiated in cooled chambers (2 mm x 18 mm) at "shank" and 2.5-5 mm beyond Bragg peak. The latter was due to an error in peak calculation. Twelve hundred anaphases were counted for cells irradiated with the shank of the proton beam($\sim$55 Mev) and 51% of the anaphases were abnormal at 198 rads. The same dose of 250 Kvp x-rays produced 50-55% abnormal anaphases, thus the RBE for shank protons (about 120 Mev) appears to be one. At 2.5-5 mm beyond physical Bragg peak a dose of 77 rads produced 52% abnormal anaphases (600 anaphases counted). This corresponds to 23% abnormal anaphases with

250 Kvp x-rays as determined from the dose-response curve and the RBE value at this point is 2+. Studies will be continued and cells will be irradiated at various points along the ascent to Bragg peak, at the Bragg peak, and beyond Bragg peak.

B. Effects of Dose-Rate on Biological Response of Mammalian Cells and Vascularized Tissues

Since previous studies have suggested a strongdose-rate dependence for high energy protons, we are undertaking dose-response and dose-rate effects of high energy protons on ascitic tumors grown as solid tumors in the hamster cheek pouch and on ascites cells themselves. In two runs to date, 120 tumors have been irradiated (dose rates 15,000 rads/min., 1500 rads/min. 150 rads/min. and doses of 500, 1000 and 2000 rads). The assay system is ideally suited to quantitative analysis since the cheek pouch can be everted for periodic measurements. It also allows us to fractionate the effect on different systems, i.e. irradiated cells in unirradiated pouch, unirradiated cells in irradiated pouch, and cells and pouch irradiated together. These findings will be correlated with histological data at the cell level (chromosome breakage) and histopathology of the whole cheek pouch. In addition, some pouches will be irradiated with protons and others with x-rays and microcinematographs will be made to ascertain effects on the microcirculation.

C. K. Levy
Boston University

PHYSICS

1. Dosimetry

The mapping of dose distributions resulting from proton beams of various configurations has been continued. Profiles for a 51 mm diameter beam and for a beam defined by a slit .75 mm wide have been measured at various depths in water. A computer program has been obtained from John Lyman at the Donner Laboratories and adapted to our use. This will allow the dose distributions measured for individual beams to be summed yielding the dose distribution in three dimensions for an arbitrary combination of beams.

A careful experiment has been carried out to measure the stopping power of skull bone. This parameter enters as a correction factor in controlling the depth of penetration of the proton beam into the skull. A report on the results is in preparation.

Further experimental checks have been made on the efficiency of the Faraday cup which is the absolute standard of all our dosimetry, and of the miniature silicon diodes which are being used more and more as dosimeter probes, especially for the smaller diameter beams.

A. M. Koehler
W. M. Preston

2. Collimation Apparatus

No major changes in collimation have been made. Redesign of the mounts for x-ray tube and headholder are underway.

G. Pollucci
A. M. Koehler

3. Radiation-sensitivity of various photographic films

A variety of photographic films which may be used in lunar orbiting reconnaissance satellites have been exposed to known fluxes of protons of energies from ~20Mev to 132 Mev. This project is being pursued by Dick Adams of NASA-Langley.

Quantitative Positron Scanning

The problem of determining quantitatively the distribution of a positron emitting isotope in tissue has been shown to be mathematically the same as the problem of determining the gamma-ray absorption coefficient of an inhomogeneous medium.[1] Because of this equivalence, and because the experimental techniques for the absorption problem are so simple, the absorption problem has been studied further.

The program written to obtain the first results[2] is being rewritten to improve its flexibility and accuracy, and to incorporate certain chi-squared tests of statistical significance.

It is important to know how counting statistics affect the results. Three sets of data have been obtained with statistical accuracies of 1%, 3.2% and 10%, and these are being used to study the effects of counting statistics.

A. M. Cormack
Tufts University

1 A. M. Cormack, Journal of Applied Physics, 34, 2722(1963).

2 A. M. Cormack, Journal of Applied Physics, in press.