PRNC 40 PUERTO RICO NUCLEAR CENTER RESONANCE IN RADIATION EFFECTS TECHNICAL REPORT NO. 2 [OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT NO. AT (40-1)-139 FOR U.S. ATOMIC ENERGY COMMISSION ---Page Break--- RESONANCE IN RADIATION EFFECTS Henry J. Conberg, Principal Investigator Robert A. Luse Florencio Vazquez Martínez Frank S. Koo Rosa J. Santiago de Morales Baltasar Cruz Vidal Technical Report #2 Work performed at Puerto Rico Nuclear Center under U.S. Atomic Energy Commission Contract AT(40-1)-1833, Project 14 May, 1966 ---Page Break--- n, um. Ww. vr. vn. vu. LIST OF FIGURES . LIST OF TABLES TABLE OF CONTENTS INTRODUCTION ... RESONANCE RADIATION EFFECTS IN CARBOXYPEPTIDASE A . PRODUCTION OF F-CENTERS IN ALKALI HALIDE CRYSTALS (NaCl, RBr).. 13 RESONANCE RADIATION EFFECTS IN PRODUCING CHROMOSOME ASERRATIONS (preliminary report by F. S. Koo) ... HIGH INTENSITY FIELD EMISSION X-RAY SOURCES ... INSTALLATION OF NEW FACILITIES ..... a) KeRay Spectrometer, GE Model XRD-6 ..... b) Low Temperature Irradiation Chamber ... c) Devices for F-Center Measurement . APPENDIX Abstract of paper presented at the Radiation Research Society Meeting, May, 1953... : Abstract of paper presented at Conference of Nuclear Spectroscopy and Solid State Physics, February, 1964 LITERATURE REFERENCES ---Page Break--- 1. REVISION ‘The aim of this project remains as originally stated: to answer the question "What are some of the unique effects of ionizing radiation on matter?” To this end, our research program has studied x in the 5-20 KeV energy range upon biological systems. This energy region is of considerable importance since it contains the K-absorption edges of the constituent atoms of most living systems. The biological system studied previously has been the enzyme catalase. Our previous Technical Report (1) dealt with the resonance radiation effects observed with this metalloenzyme and indicated the presence of an “action spectrum" in which the greatest biological effect (inactivation) was produced at or

near the K-absorption edge of the constituent iron. At that point of our research (approximately 12 months ago), it was felt that extra information regarding the resonance radiation effect could be obtained by study of carboxypeptidase A and its related esterases. These enzymes are unique in that the constituent metal can be removed by dialysis and then replaced by certain other metals. Accordingly, the original zinc-containing enzyme can be converted to esterases containing cobalt, nickel, tin, mercury, or molybdenum. A series of such enzymes, identical in composition except for the metal, are being irradiated at wavelengths including those corresponding to the various K-absorption edges. Observed resonance effects are tested for specific relationship to the metal and for their independence of the remainder of the molecule. For example, should the cobalt enzyme and the zinc enzyme be irradiated at energies corresponding to the K-edges of cobalt and of zinc, respectively, the resonance concept would predict that for equal doses there would be greater inactivation of the cobalt enzyme at energies corresponding to the K-edge of cobalt than at energies corresponding to the K-edge of zinc, and conversely for the zinc enzyme. Results obtained to date with zinc carboxypeptidase A indicate that inactivation is energy dependent. Further experiments are needed, however, to confirm the phenomenon; refinements in dosimetry and more intense sources are required to solve this problem. The biological studies of resonance effects have been extended into genetics by Dr. F. S. Koo. Chromosomes in onion root tissue were labeled with a nucleic acid analog, S-bromodeoxyuridine, and then irradiated at photon energies at or near the K-absorption edges of bromine. Preliminary results indicate that chromosome aberration in the cell nucleus occurs with highest frequency after irradiation at energies equal to or greater than the K-edge of bromine. It is hoped that these experiments will open new approaches for probing the nature of

radiation-induced mutations. Since only low-intensity monochromatic beams can be obtained from present equipment, much of our effort is directed at overcoming this limitation. Several steps have been taken: 1) A new x-ray spectrometer with higher current capacity has been installed, 2) fluorescent line emission produced by the original spectrometer has been utilized, and 3) new field emission type x-ray devices have been designed and are in construction. In addition, we plan to survey other biological systems which, because of their larger “amplification factors," may prove more suitable for the study of resonance effects. Since our project is concerned with clarifying the effects of radiation, from the initial physical event to a final change identifiable through biological or chemical means, it is necessary to understand clearly the nature of the primary event. For this, the relatively simpler inorganic crystal is an excellent subject for study. The literature on the subject reveals that the monochromatic x-ray technique has not previously been utilized. Only recently has similar work been begun in Dr. Swoluchowski's laboratory at Princeton University. For this reason, we feel that it would be reasonable to devote a modest fraction of our total effort to such studies. The bulk of this work is being done by a student conducting research towards his dissertation requirements at Harvard University. Other physical aspects of the project are 1) development of dosimetry techniques which will provide accurate measures of absorbed energy in the low-energy x-ray region and 2) completion of a vacuum spectrometer which will produce a high intensity of very low-energy x-rays (3-10 KeV) suitable for study of low atomic weight elements. The field mentioned earlier is for use with this spectrometer. At the present time, this project is fully operative in two laboratories, one in the Physics Department, University of Puerto Rico, Mayaguez, the other in the Bio-Medical Building, Puerto Rico Nuclear Center,

Rfo Piedras. The former is used primarily for physical studies, the latter entirely for biological research. Report submitted by: Dr. H.M.J. Gombers, Principal Investigator Dr. R.A. Lage Dr. F.B. Koo Dr. F. Vézquez Martine: M.B. Cruz Vidal Mrs. R.J. Santiago de Hora! ---Page Break--- 11, RESONANCE RADIATION EFFECTS IN CARBOXYPEPTIDASE A Carboxypeptidase A is a pancreatic exopeptidase having a molecular weight of 34,300 and 1 gram atom of zinc per mole of protein (i.e., 0.2% Zinc by weight) (2). This atom of zinc is an integral part of the enzymatic active site, as shown by the fact that its removal by dialysis results in a concomitant loss of enzymatic activity. This zinc atom is bound to a mercapto group; as the metal is removed, a reactive -SH group is disclosed (3). Carboxypeptidase A was obtained from the Worthington Biochemical Corp. as an aqueous suspension of material crystallized by the original method of Anson (4) and recrystallized by that of Putnam and Neurath (5). It was diluted to a concentration of approximately 9.3 mg/ml (1 x 10^-5) in 10% Lithium chloride solution and then immediately before use diluted 1:25 with 0.067M sodium phosphate buffer, pH 7.50. To avoid microbial action upon the enzyme protein during the long irradiation periods, the buffer was sterilized by "Millipore" filtration. The concentration of enzyme in the solutions for irradiation was approximately 4 x 10^-7 M, a concentration chosen as similar to that of catalysis used in previous studies by Banons (6) and by Luse (1). In this way, a comparison of irradiation effects can be made for these two enzymes at the same concentration (and also similar pH). Irradiation of carboxypeptidase A as a dilute solution was carried out in the cell shown in Figure 1. Sample compartments are of a width equal to that of the collimated x-ray beam; with appropriate solution depth, the whole beam is incident upon the sample solution. This cell, an improved version of the cell described in our previous report (1), has certain advantages.

features: ---Page Break--- All dimensions in mm. Figure |- Exploded view of irradiation sample holder (see description, next page) ---Page Break--- Description of Figure I A. Parts C and F are attached to A with five nylon screws; B. Cooling plate with water inlet and outlet (Ivette) Rear

window - 0.005" film (polyethylene, or other material) Sample holder with irradiation compartment and two control compartments (lucite, polyethylene, teflon, or other material) Front window ~ 0.002" film (polyethylene or other material) Front plate, with cut-outs to match sample compartments Cover for front plate, to provide insulating air pockets {A. Front of sample compartments (these prevent moisture condensation on chilled sample holder in path of x-ray beam) = 0.005" film (mylar, polyethylene, or other material) B, D, and F are sealed to plastic with halofluorocarbon grease Kel-? #90, (Minnesota Mining & Manufacturing Co.) ---Page Break--- 4) Increased chemical resistivity - Plate C, which forms the sides of the compartments may be of Ivette, polyethylene, teflon, etc. By selection of plastic films B and D, compartments could be all polyethylene or all teflon, should the solution to be irradiated require this. >) Variable solution thickness = By using different plates C of various thickness, solution thickness may be varied to match the absorption characteristics of the incident x-ray beam. ©) Versatility of cells ~ Plate C and its cover plate E may be removed from cooling plate A and replaced with other sample holders, as for any F108. After irradiation, solutions of carboxypeptidase A were assayed for remaining activity by the method of Neurath, Elkins, and Kaufman (7), in which the rate of hydrolysis of 0.025 M carbobenzoxyglycyl-L-phenylalanine was determined using the Moore and Stein colorimetric ninhydrin method (8). In this procedure, enzyme and substrate (Mann Research Laboratories material) were reacted at 37°C and pH 7.5; amounts of phenylalanine formed were determined after reaction times of 5, 10, and 15.

minutes. The mean reaction constant, k, was determined from the slope of lines obtained from plotting 100/(100 - percent hydrolysis) against the reaction time. The proteolytic constant, C, was calculated as (opt. of enzyme nitrogen per ml), assuming 18% N content for carboxypeptidase A. Remaining percent enzyme activity was estimated as (C x 100 of irradiated solution) / (average of intensity controls). The activity of such in situ controls (i.e., kept in the irradiation cell compartments): We found that slight amounts of ultraviolet light-absorbing material leached from mylar film during 20-50 hour contact with the 0.8N sulfuric acid solvent of the dosimeter solution. This causes erroneously high estimates of dose from absorbance measurements at 224 nm. ---Page Break--- compared to that of other portions of the non-irradiated enzyme solution stored in the refrigerator. The experimentally determined values of collimated x-ray beam intensity as measured by the ferrous dosimeter are presented in Table 1. Reproducibility is poor, probably due to long irradiation periods and small increases in optical absorbance over that of the control samples. It may be concluded that the beam intensity is approximately 2 x 10^11 photons/hr/ml of dosimeter in the 7.5 to 9.7 Kev region. This value is in general agreement with previous data obtained with the KRD-5 spectrometer at Mayaguez. The sharp decrease in intensity at 11.69 Kev is unexpected. Possible causes, such as related to the target position, or target damage, are being looked into. Since the spectrometer system is sharply collimated, small changes in the x-ray source position will have a marked effect on intensity at the test sample position. The x-irradiation of carboxypeptidase A with collimated beams has been carried out at three photon energies: 7.6 Kev, 8.68 Kev (the K-line of zinc), and 9.69 Kev (the K-absorption edge of zinc). Experimental results are as given in Table 2, where enzyme activities are expressed as the proteolytic constants C. Percent

of remaining enzyme activity is estimated from the ratio: (C of irradiated solution)/(C of in situ control). Percent of inactivation, of course, equals 100% less percent remaining activity. Such preliminary experimental data may be used as a 1 Koray integrity to a function of the applied current (mA), the applied voltage (as V), the atomic number of the target (Z), and general cube geometry. The XRD-6 spectrometer is capable of higher intensity because of its higher current ratings, but in the chromium target tube, a tungsten target is used instead of a tungsten target to avoid line emission near the zinc K-absorption edge. This difference in target material reduces

intensity by nearly one-third (Z0.5/2, * 26/74). ---Page Break--- Table 1. Measurement of Intensity of Monochromated X-Ray Beam, Using Ferrous Dosimeter Dosimetry techniques were as described in our previous Technical Report (1). The x-ray tube was operated at 18 kVp and 75 mA; its output was collimated with two Soller slits and LiF diffraction crystal. Concentration of ferric ion formed, C, = Increase in absorbance /1000E, moles/al where E has the value 4565 at 224 nm and 2196 at 304 nm, subject to temperature corrections, as given by Scharf and Lae (9). Intensity of absorbed radiation, as photons per hr per 1 ml of dosimeter concentration of ferric ion formed by 4.46 x 10^21 energy per photon, in KeV. We "target" Wave take Ferric ion intensity, µ per’ Tren. length A, over' cone. as photons crystal) photon period of A _ controls moles better KeV Hours a x10 yo 48.83" 7.47 27.0 228 0.037 oat 18 304 009 oa 0.9 47.83" 7.60 22.0 226 16 43 304 028 1s 3.5 iste goa 2s 26 046 1.0 2 304 025 Lt 2.6 4nst® 868 18.5 228 0320.70 Le 306 2016 63 La 37.17 9.69 20.0224 1844.0 9.2 30% 056 2.6 5.0 37.17" 9.692.028 926 5.4 306 2038 17 3.6 30.577 11.69 37.5 226 ors 0.61 0.62 308 OL 150 49 ---Page Break--- 10 basis of plots of inactivation vs. x-ray dose (“survival curves"). Estimates of absorbed dose are based on beam intensity data, for which the averaged value of 2 x 10^6.

Photons/hr/ml of solution was utilized. This best average fe value of intensity is subject to correction with further dosimetry. The survival curves, plotted in Figure 2, must be considered as of a preliminary nature, subject to future revision. ---Page Break--- yorqw0> yo 9 /torqw0s TAT skqddy soy z0uyy sakzue repso 28273 s29yn teTshtosphy oaesa9qne YO? - 7 Jo eBuex oq3 uy poansvon ‘(3x02 999) 4 tequeaeu0> uoya9¥er uve ox] PsuTEIO3ep 279M #3ULIEUCD SEOUL ay _ cena Yenbs 103 203839843303 992 Uy ‘BF 30 9 awwaev0> >4ahtoa2024 ‘oyaex ays seta OT eTeNbe eT UE aden jequaazed eFql ” 9s os oror 9s 068 escent 8 6 on en w sw 6 ave exfonstt ss > oy ve 7 ores e9/o/et ey us or ve 99 ues 29'8 wy e/efer zc ey ve sieve 6 96 09 tater eayerser saayaomey _£ayay328 “uros “p2ay Jo Yor3009 Jo. pfo038 50% nox ee (qea8ks9 ‘aus] Suyuyeme: xy wyauezeue) 2}34qos301g aw “1023009 swox0ud/a 451) 92" Kesey 30 Kayay22y “03 er WorarTpHU=x aFIMNOAyIOUOK YaFH ¥ esePTadedhcoqzeg Jo VORIPATIONU *Z STqEL | 8 ob 8 8 8 8 ee le lll lll ---Page Break--- 2 Figure 2-Course of Carboxypeptidase A Inactivation at Various Photon Energies 9.69 Kev 76 Kev & 8.68 Kev Percent Remaining Activity & ° 50, 100 Hours of Irradiation a oT a ° 1 2 Dose /mil. as photons x 10! (assuming = 2x10") ---Page Break--- B EI, PRODUCTION OF P-CENTERS IN ALKALI HALIDE CRYSTALS (NACL, KBE). 4) Introduction and Theoretical Background continues In the last few years, a considerable effort has been made to determine the nature of defects in ionic crystals and to reach an understanding of the mechanism by which such defects are introduced under the influence of ionizing radiation. The interest in this research stems from the fact that the typical alkali halide crystal is a remarkable medium for the study of radiation damage effects such as lattice imperfections. These crystals have a very simple structure and can easily be obtained in a high state of purity and perfection. A wealth of physical data can be obtained; among the feasible measurements are many related to the crystalline imperfections, the

existing dislocations, the density of electrons and holes and their mobility, the effects of impurity atoms, and the presence of vacant lattice sites. Our present research deals with the formation of color centers in alkali halides by x-rays in the energy region of the K absorption edges of the constituent atoms (cf. Table 3). X-ray irradiation effects have been obtained by other workers using the whole output spectrum of the x-ray tube; our study utilizes monochromatic x-ray beams. Experiments have centered upon the investigation of the P-center, which consists of trapped electrons in a halogen vacancy. Such P-centers are stable at temperatures (4K to 300K); at liquid helium temperature, they are associated with a neutral interstitial halogen atom (i-center). At such

low temperatures, other workers have found that the rate of formation of P-centers is a bulk property independent of tonte diffusion and of the presence of dislocations. The results at low temperature support Varley's mechanism for F-center formation (10) in which an initial multiple ionization of the halogen ion results in a positive halogen ion that is then ejected from its lattice position. An Auger process is likely to take part in the multiple ionization. For this reason, x-irradiation near the K, L, M, and N absorption edges of the halide is of special interest. Here, such questions arise as: 1) What is the x-ray photon energy dependence of the formation rate of F-centers, especially around the K, L, M... absorption edges? (This question is relevant because of the importance of the postulated Auger process.) 2) What is the time for recombination of electrons with the multiply ionized halogens? 3) What is the time required for the halogen to migrate a given distance? 4) What is the energy necessary to create one F-center? At room temperature, the

unsolved problems are even greater. As the temperature rises, thermal diffusion as well as the presence of lattice vacancies, impurities, and dislocations become increasingly important. Furthermore, the intensity of irradiation at room temperature has yet to explain some effect. All these effects tend to detract from the feasibility of various native processes. To obtain information on the density of F-centers, use the equation of Snakula (11): Ne = 1.29 × 10^7 × 1^2 Perr se ---Page Break--- Here is the number of F-centers per cm^3, E is the oscillator strength, n is the index of refraction, α is the optical absorption coefficient at the maximum of the F-band in cm^{-1}, and W is the width in electron volts of the band at half maximum. The number N is obtained from the relation for transmitted intensity at thickness d in terms of the incident intensity I■, viz, I = I■e^(-m), for light of a frequency equal to that of the maximum absorption. At a given temperature, neither E nor W changes as the density of color centers increases. Under these circumstances, N is proportional to an exponential function of irradiation time at room temperature. We have verified that N follows a curve of the general form shown in Fig. 3. b) Experimental Procedures Our samples consist of alkali halide crystals 1 to 2 cm thick and approximately 1.5 cm on each side. For work at room temperature, they are mounted on a holder designed to fit in the sample slide of our Beckman stable-beam DU spectrophotometer. The crystal is located at one of two openings in the holder. The other opening is left unoccupied so as to provide the air reference standard for the relative measurements of optical density. For low temperature work, we have designed a sample holder to be mounted in vacuum inside the cryostat. Irradiation of a KBr crystal has been carried out for 200 hours at 11.2 keV (selenium radiator) and for an additional 125 hours at 14.2 keV (strontium radiator), with the same intensity and at room temperature. For details of radiators and filters, see Tables 1 and 2.

5, Present intensity measurements have used the ferrous sulfate dosimeter as well as the proportional counter of the spectrometer (for measuring relative intensities and while monitoring intensities). In the ---Page Break--- Relative N, density of F-centers ” 1 Time of irradiation Figure 3- Typical density of F-centers against time of irradiation at room temperature. Typical values are t, 10 hours, ty 17 hours for a KBr crystal irradiated at 1.2 KeV, 4 x 10° photons emitted see ---Page Break--- Le 4, Characterization of Fluorescent Radiators for Alkali Halide crystal Irradiation Ky Energy (KeV) Chemical Form Physical form 2 element 35 Bromine 38 Strontium 39 Yttrium 40 Zirconium 27 Cobalt 2 Mt 34 Selenium 47 Silver 48 Cadmium 49 Indium 72 Hafnium 76 Osmium 78 Platinum 73 Gold 11.923 14.166 14.957 15.774 6.930 2477 a2 22.162 23.172 26.207 Lacey ENERGY (KeV) 7.098 8.910 9.4an om Ne or SF (03) ¥9(009)3 2 0 co Nt se as ca an Fine powder Metallic sheet cast Am AL planchee Metallic sheet Metallic sheet Metal, fine powder Metallic sheet eee ---Page Break--- Table 5. characteristics of X-ray Filters Used with Fluorescent radiators! Essay (EG) X-ray (Raw) mechanical PRT ayaa Radiator “Kc Kg, Filter used “Rabe form (np/eay 1 1

$$ Se UL22t 12.495 as 11.863 40303 29 Br 11.923 13.290 se 12.652 ge Pr Sr 16.166 15.836 ab 14.323 mbet 3 ¥ 14.957 16.736 sr 16.105 $0803). 29, ze 15.77% 17.666 se 16.106 " eee The appropriate filter for a given radiator was determined by using the emission line and absorption edge energies compiled by Fine and Hendes (12). The absorption edge of the filter must be between the Kα and Kβ lines of the radiator. The desired thickness of the filtering is given by means of the following equation: where Pr density of the filtering element (g/cm³) = thickness (cm) * absorption coefficient (cm²/g) ---Page Break--- In the future, our primary energy measurement standard will be a calorimeter obtained from the Michigan Memorial ~ Phoenix Project research group in Resonance in Radiation Effects. It consists of an evacuated steel

vessel with 4 mylar windows and beryllium window shielding totally absorbent (for the energies of interest) gold foil target. Two thermistors are attached to the gold foil target for measurement of the energy absorbed, and one has a thin heater for calibration. The calorimeter measures incident powers in the range from 4 to 30 milliwatts. The curve of density versus time of irradiation does not show significant variations at the two energies. Interpretation of our data, however, must take into consideration the variable penetrability (absorption coefficient) of radiation at different energies. Irradiation of KBr crystals under the same conditions (11.2 KeV, room temperature) showed that the change of slope occurred after 37 hours of irradiation at a beam intensity of 6 x 10^10 photons/en?/sec. We have concentrated attention on the slope and occurrence of the straight part of the density curve because the initial fast rise in photon concentration is dependent on the history of the crystal. At low temperatures, this initial rise does not appear. Likewise, the effects of impurities, lattice imperfections, and diffusion are minimal. The subsequent linear increase in the center density at room temperature arises solely from lattice displacement caused by the incident radiation. ---Page Break--- 1. RESONANCE RADIATION EFFECTS IN PRODUCING CHROMOSOME ABERRATIONS Preliminary Report by F. S. Koo Puerto Rico Nuclear Center, Mayaguez, Puerto Rico First results indicate that the x-radiation effect on a given biological system can be increased if the photons are delivered at the K edge absorption energy of a particular constituent atom of the system. Evidence supporting the hypothesis of radiation dependence was first obtained during studies of the enzyme catalase, which contains four atoms of iron in its porphyrin ring structure. Emons (6), P. Evoudekis (13), and Use (1) independently showed the enhanced inactivation of the metalloenzyme catalase by monochromatic x-rays at wavelengths (or photon energy) near the

Keabsorption edge of iron The choice of an enzyme that contains a heavy element has been the key factor in the tests of the resonance radiation effect hypothesis advanced by Gonberg. A critical test for the genetic system may be irradiation at resonant energies of DNA labeled with halogenated thymidine analogs. A first attempt has been made by investigating S-bromodeoxyuridine-labeled chromosomes in Allium cope root tips. Roots from germinated seeds were first treated with BUDR solution at a concentration of 15 µg/ml for 15 hours, and then irradiated with monochromatic x-rays for 1 hour. The dose intensity was approximately 5.9 x 10^10 photons per cm² per hour. X photon energies were applied to the whole series of samples, which were then left to recover for 24 hours before being fixed for cytological study. ---Page Break--- 2 In summarizing the data, which are now available at four photon energies, the chromosomal aberrations at metaphase in forms of chromatid and chromosome breakages, fragments, interchanges, etc., were scored and expressed as the number of chromosomal breakages per cell. The results are presented in Table 6 and Figure 4. ---Page Break--- Table 6. Chromosomal Breakages Produced by Monochromatic X-Rays at Various Photon Energy Levels in BUDR-Labeled Chromosomes of Allium cope Root Tip Cells Photon Energy No. of cells No. of

breaks No. of breaks applied studied observed per cell 25 85 7 08 13.2 92 45 16 3.43 50 36 29 3.7 now being studied 15.5 68 22 Although the data obtained at the present stage of research are rather limited, there are strong indications that the photon energies at or near the absorption edge of bromine cause a clear-cut increase in the production of breakages in BUDR-labeled chromosomes in Allium cope. Detailed reports on methods of material handling, chemical treatment, irradiation, cytological studies, etc., will be compiled and published upon completion of the whole study. ---Page Break--- No. of chromosomal breakages per cell Kabs edge Bromine 128 135

13s 155 Photon Energy (Kev) Figure 4. Resonance radiation effect of monochromatic x-rays on BUDR-labeled chromosomes in Allium cepa. ---Page Break--- 25 Y. HIGH INTENSITY FIELD EMISSION X-RAY SOURCES It appeared that one basic experimental difficulty which must still be faced in spite of the progress made in the course of the last year is the question of low beam intensities at the required energy. The following crude approximation may serve to illustrate the problem. We have found that the typical intensity output of a tungsten x-ray tube is of the order of 10^3 ev/en²-hour. For crystal structure research, this requires irradiation periods of about 50 hours to 160 hours during which the relative effect of intensity and the rate of color center formation is studied. Using a crystal monochromator, even at optimum geometrical alignment, the energy output after the second collimating solar slice is of the order of only 10^2 to 10^3 ev/en²-hour, i.e., four or five orders of magnitude less than for the tungsten tube. With the irradiator technique, on the other hand, output intensity is of the order of 10^7-10^8 ev/en²-hour, that is to say, one tenth to one hundredth of the total beam output. In view of the much higher intensities obtainable from irradiators, they have been used in the preliminary crystal studies. However, we still need the continuous adjustability of wavelength afforded by the crystal system. Presently available intensities result in long periods of irradiation which give rise to numerous experimental difficulties, with regard to both the alkali halide and the biological irradiations. A considerable improvement in experiment control and more reliable data could be obtained if the irradiation periods were shorter. These considerations have led to a study of x-ray sources other than the available commercial x-ray tubes in the hope of achieving a higher intensity output. The chief design of a high intensity x-ray source raises problems: ---Page Break--- 26 ion from the source 4) How to achieve

high electron exit: b) How to focus the emitted electron beam on the target; and c) How can the cathode and target design eliminate the high x-ray dispersion found in commercial tubes. The solution to the first problem lies in the principle of field emission. The use of a pointed cathode—entailing a very high field (intensity per unit area)—lowers the potential barrier to electron emission and produces electron current densities which are significantly higher than those obtained with hot filament cathodes. In field emission, the electrons leave the cold metal under the action of a strong electric field intensity. The emission mechanism, which could not be explained by classical physics, has been well described in terms of wave mechanics: free electrons tunnel through the metal surface potential barrier when the latter is decreased and thinned by the applied field. The general expression for field emission according to Sommerfeld and Bethe is given by J (electrons per unit area) = N(Z,E) D(F,B) a8 where N(Z,E) measures the relative number of electrons at a given absolute temperature T whose kinetic energy, based on the component of velocity normal to the surface, by Sommerfeld and Bethe, has the form similar to the strong electric field F, the potential barrier becomes thin and is reduced in height. Electrons impinging on this finite barrier from the inside of the metal have a certain probability of penetrating it and ---Page Break --- 2 appearing outside the metal. This probability is expressed by the transmission coefficient. Here ϕ is the electron work function in eV, E is the electric field in volts/em, E is in eV and f(y) is a

dimensionless elliptic function of the variable y = 3.79 x 10^-4 √(ϕ) introduced by Nordheim. C is the constant that has the form 47m kT/b^3, where m is the mass of the electron and h is Planck's constant. A plot of this formula (Figure 5) indicates the relationship between current density with respect to the applied field for a needle-shaped tungsten emitter at different

temperatures (16). Some significant properties of field emission cathodes are a) Current densities of up to 10^6 amp/cm², exceeding the temperature-limited currents of conventional pure tungsten cathodes by a factor of one million. b) No energy other than that stored in the field is required for emission. c) The emitted current density depends exponentially on the applied electric field and hence can be controlled. In view of these advantages, particularly the possibility of high current density and consequently, high x-ray intensity, we decided to investigate a field emission source for our x-ray spectrometer. At the present time, we are experimenting with commercial razor blades to be used as the cathodes. One of their sharp edges can create high field intensity by placing the anode close to the edge. The experimental x-ray tube and the field emission source now being tested are shown in Fig. 6. ---Page Break--- SQUARE CENTIMETER CURRENT DENSITY (AMPERES PER SQUARE CENTIMETER) APPLIED FIELD (VOLTS PER CENTIMETER) Fig. 8 From Sci Amer, an. 1968(06) 28 ---Page Break--- B3OYNOS NOISSIN3 G73l4 ONISN 38NL AVY-X WLINBWIY3dX3-9 914 4 anne Not aw enemy os 1991-49189 —O-- _ beam von Jo] Shy | whos L) ; | ---Page Break--- 3a The current measurements of the radius tip of commercial stainless steel razor blades indicate that radii of the order of 10^-5 cm are available. As the work function of iron is quite close to that of tungsten, razor blades should work well as field emitters. In fact, some work has already been done using iron needles instead of the more conventional materials. We will have to establish the influence of the other elements present in steel razor blades. In an x-ray tube using a field-emission electron source, the main causes of instability and short life are surface contamination and sputtering. The latter occurs when the cathode is bombarded by the residual gas ions in vacuums as good as 10^-20 am. Helium gas is one of the major problems. However, improving the vacuum and the clean-liness of the system may

result in very good stability. The field emission electron source further offers the possibility of an extended “area” source. The high gradient is established at the point - or edge - of a sharp source. A sharp source is about 1 x 10^-4 = 1 x 10^7.5 cm in diameter. Another sharp source 1 x 10^-1 cm from the first is, for field purposes, far away. Thus it should be possible to set up a group of points, or a parallel set of blades, to get the equivalent of an “area” source. It is true that initially, individual sharper points will emit first, possibly reducing the voltage at other points. In a short time, however, judicious “melting” can increase the tip radius and reduce the gradient to the point where other points begin to emit simultaneously. We plan to test this concept soon; our objective here is the creation of a simple, rugged high-output x-ray source. In our total program, though, we are not overlooking ---Page Break --- at the hot cathode sources. A conventional hot filament source is being made for the vacuum spectrometer now under construction in our shops and in which we plan to use the field emission source, if successful. Also, we have been following the development of the large filament-area, electron-focusing type sources as used by Dr. Burton Menke of Pomona College in California and Dr. William Gross of the Radiological Research Laboratory, Columbia University. ---Page Break --- 3 ‘VE. INSTALLATION OF NEW FACILITIES 1) KeyRay Spectrometer, GE Model 100-6 To expedite the biological phase of this program, an x-ray spectrometer was installed in the Rio Piedras Laboratory of PRNC for use by Dr. Luse. This spectrometer, model X8D-6 of the General Electric Co. (see Fig. 7), has higher current ratings than the spectrometer used in previous studies, so that a more intense beam of x-rays for a given target is available without sacrifice of beam purity. The x-ray tube, type EA-75, can be operated at up to

100 ma current in full wave rectified mode of operation, a value twice the rating of tube An our XRD-S.

spectrometer. ‘A high degree of beam resolution has been obtained in this new spectrometer by application of the system used in the original spectrometer, viz: a) collimation with a medium resolution soller slit of the beam emitted from the x-ray tube; b) diffraction from a crystal of the collimated beam; and c) further collimation with a high resolution soller slit of the diffracted beam before it impinges on the sample to be irradiated. With such a resolving system, 85% of the emergent beam energy is within ±30 eV of nominal energy, i.e., less than ±1% beam energy spread in the 5000-18000 eV range. For details of measurement, see Project Report 1 (1). The intensity distribution of the emergent x-ray beam produced by the XRD-6 equivalent has been measured; typical values are given in Table 7. >) Low-Temperature Irradiation Chamber The production by x-radiation of F-centers in alkali halide crystals can be measured quantitatively only at low temperature, where effects due to impurities, crystal dislocation, and thermal diffusion are absent. For this reason, a low temperature irradiation chamber was ---Page Break --- ---Page Break --- % Table 7, Intensity Profile of Collimated X-Ray Beam Photon intensity, Percent of Position highest value 1 - 2 33.8 3 4 96.6 3 88.0 6 92.7 7 93.0 8 95.1 : 10070 10 96.3 u 75.6 n 345 rf 26.3 1“ + 13 : The intensity of x-ray photons was measured by moving the SPC-6 detector in 0.86 um intervals horizontally across the face of the beam; a 0.1" (0.254 cm width) slit was used in front of the detector window. ‘The x-ray tube was operated at 18 KVP and 45 mA. Collimation is described for tests of energy resolution. The intensity profile was made with the Lip analyzer crystal set at 37.17° for selection of 9.69 KeV radiation. This is the energy of the K-absorption edge for zinc. Width of beam utilized in radiations is 0.92 mm, i.e., distance from position 2 through 13. The detector is a proportional type counter tube, xenon-filled, with a 0.010 in. beryllium window; its efficiency ta

80% for the 7.1 Kev energy photons here utilize: 2 for details, Fig. 11-6 and Fig. 11-5 of our previous report (1). ---Page Break--- constructed by modification of a two-liter experimental cryostat (manufactured by Superior Atr Products Corp.). In this cryostat (Fig. 8), the sample is held in contact with a metal rod which is in turn in contact with a reservoir containing liquid nitrogen or liquid helium. The metal rod can be encircled with a resistance heater coil, so that it is possible to vary the sample temperature from that of the reservoir liquid (4 K for helium, 90 K for nitrogen) to room temperature (300 K) or above. Such variation of temperature is essential in measurements of the temperature at which P-centers appear and of the range of their temperature stability. The crystal is irradiated through a port covered with a mylar film window; other of the four exit ports may be fitted with quartz windows for subsequent optical measurements. The sample chamber beneath the cold liquid reservoir is evacuated to about 10^-6 torr by a diffusion pump with a mechanical forepump (Vactronic Laboratory Equipment Co.). The same vacuum system provides heat insulation to the cold reservoir. Although this irradiation chamber is ideally used only for alkali halide crystals, it offers the potential for irradiation of enzyme films at low temperature in future research. ©) Devices for P-center Measurement Production of F-centers in alkali halide crystals via x-radiation as measured in the low temperature chamber described above. To accomplish this, the spectrophotometer (Beckman model DU) used for optical absorption determinations was modified by substituting for the usual 1.3 cm thick sample carrier compartment a compartment of sufficient size to accommodate the lower portion of the cryostat (Cf. Fig. 8). This light-tight compartment, 16 x 12 x 12 cm (L x W x H), is fitted between the monochromator housing and the phototube housing; within it the cryostat may be positioned so that the spectrophotometer light beam passes through two

port windows ---Page Break--- 38 and the sample crystal before reaching the photomultiplier. The spectrophotometer is equipped with a SERA attachment and a chart strip recorder which permit automatic plotting of transmitted energy as a function of wavelength. Present investigators have utilized potassium bromide crystals which have P-center absorption peaks at 6200 Å. For this wavelength, the mylar film windows covering the cryostat provide satisfactory light transmission, that is, the same port used for x-radiation may be used for optical measurements. However, for studies utilizing sodium chloride, with P-center peak at 2409 Å, optical measurements must be made through quartz windows which transmit in the ultraviolet region. Several devices have been designed to permit x-irradiation through mylar windows and optical measurements through quartz windows. The first of these is a mirror system by which the incident spectrophotometer beam enters a port other than the irradiation port, is bent to pass through the crystal, and then is bent again to emerge from the port opposite to its point of entry. A second device would permit turning the sample crystal 90° from the irradiation port so as to face the optical measurement port. The third device is designed to rotate an irradiation port which has two halves, one mylar, one quartz, from the mylar half (for x-irradiation) to the quartz half (for absorption measurements). A prototype of the first of the devices has been constructed and tested. The other devices may prove more simple and hence more reliable. ---Page Break--- ---Page Break--- 38 VIL. APPENDIX Resonance Radiation Effects of Low Energy Monochromatic X-Rays on the Metalloenzyme Catalase! Henry J. Gonberg and Robert A. Luse Puerto Rico Nuclear Center, Mayaguez, Puerto Rico To answer the question "what are the unique effects of ionizing radiation on matter?", our present research program has studied x-irradiation effects in the 5-20 KeV energy range upon biological systems. This energy region is of

considerable importance since it contains the K absorption edges of the constituent atoms of most living systems. X-radiation of these energies is produced from incident radiation of much higher energies (as cobalt-50 gamma, 250 keV x-rays) by degradation through Compton scatter. Biological systems chosen for study were those which are composed primarily of light elements with but traces of medium atomic weight elements. The metalloenzyme catalase has been studied extensively to date by three investigators. For this enzyme, which contains four atoms of iron in its porphyrin ring structure, the question was “Does radiation absorbed by the iron atom produce more damage (inactivation) per electron volt absorbed than radiation absorbed only by the light elements (carbon, hydrogen, oxygen, etc.) which make up the bulk of the catalase molecule?" Experiments were designed to show or disprove the presence of a true action spectrum of radiation in the kilovolt region. The presence of such a spectrum has been shown and indicates unique effects of such radiation, entirely divorced from the general “indirect” effects of radiation (which may be simulated chemically under conditions of far less energy absorption per molecule). (Work supported by U. S. Atomic Energy Commission.) Abstract of May, 1963. Presented at the Radiation Research Society Meeting, ---Page Break--- 39 F-center Production with Monochromatic X-Rays! F. Vazquez Matinee Puerto Rico Nuclear Center, Mayaguez, Puerto Rico Our present studies are concerned with the rate of F-center production as a function of the energy of incident x-ray photons. Sodium chloride crystals of 10 x 10 x .S mm were irradiated at room temperature. Fluorescent x-rays from three different targets were used to produce K-Lines at 7.477 eV (Kα), 11.221 eV (Se), and 23.172 eV (C4). A calorimeter was the primary standard to ensure line intensities. The x-ray tube was operated at 50 kV and various milliampere current settings to obtain the same beam intensity from all sources.

Saskula's formula is used to determine Pecenter concentration from data on optical absorption

obtained with a Beckman DU spectrophotometer. At low x-ray doses, the process of filling the normal vacancies of the crystal with electrons predominates, and no proportionality was observed between absorbance and energy. Irradiation times extended two days beyond the point where the region of linear relationship between F-F production and energy was reached. At higher doses, where the creation of new vacancies by the incident photon crisis occurs, the number of Pocenters produced is independent of the photon energy in the energy range under consideration. This supports Varley's mechanics (Phys. Chem. Solids, 23, 985 (1962)), explaining chlorine ion displacement by means of an Auger process of multiple ionization. Calculations confirm this hypothesis for x-rays well above the K-absorption edge of chlorine. Abstract of paper presented at the Conference of Nuclear Spectroscopy and Solid State Physics, February 1964. ---Page Break--- VII. LITERATURE REFERENCES 1. Gomberg, M. J., B. A. Luse, and P. Vézquen Martfner, “Resonance in Radiation Effects". Technical Report No. 1, PRNC Publication 12, 1953. 2. Vallee, B. L., J. A. Rupley, T. E. Coombs, and Y. Neurath, "The Role of Zinc in Carboxypeptidase”, J. Biol. Chem. 295, 64 (1950). 3. Vallee, B. L., T. S. Gooabs, and P. L. Hoch, "The Active site of Bovine Pancreatic Carboxypeptidase A”, J. Biol. Chem. 238, PC 4S (1950). 4. Anson, M. L., "Carboxypeptidase I. The Preparation of Crystalline Carboxypeptidase", J. Gen. Physiol. 20, 663 (1937). 5. Putnam, F. W. and N. Neurath, "Chemical and Enzymatic Properties of Crystalline Carboxypeptidase", J. Biol. Chem. 166, 603 (1966). 6. Emmons, A. H., "Resonance Radiation Effects of Low Energy Monochromatic X-Rays on Catalase", Doctoral Thesis, Univ. of Michigan, 1959. (Also Technical Report No. 2, Contract No. AT(II-1)-684.) 7. Neurath, H., E. Elkins, and S. Kaufman, "The Antipodal specificity and Inhibition of Crystalline Carboxypeptidase”, J.

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Chrono-tones of Allium cepa Root Tip Cells” 2B Intensity Profile of Collimated X-Ray Beam ™
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