RESONANCE IN RADIATION EFFECTS ‘Technical Report No.1 ---Page Break--- 11. CHARACTERIZATION OF A MONOCHROMATIC HIGH INTENSITY VARIABLE WAVELENGTH X-RAY SOURCE IN THE 5-20 KEV REGION Henry J. Gonberg, Principal Investigator Robert A. Luse Florencio Vézquez Martinez Progress Report #1 Work performed at Puerto Rico Nuclear Center Mayaguez, P.R., under U.S. Atomic Energy Commission Contract AT(40-1)-1833 (Project 14) January 1963 ---Page Break--- TABLE OF CONTENTS Preface Section I. Resonance Radiation Effects of Low Energy Monochromatic X-Rays on Catalase A. Summary 3 Introduction 3 Experimental Procedures 1. Development of Present Irradiation System 2. Development of Ferrous-Ferric Micro-dosimetry System 7 3. Assay for Catalase Activity 10 Experimental Results 1. Energy Output of X-irradiation System 17 2. Resonance Radiation Effects in Catalase 20 Conclusions and Discussion 23 1. Proposals for Future Research 25 G. ADDED 1. Notes on Catalase Assay Calculation 29 2. Survey of Radiation Levels About X-irradiation Equipment 30 3. Preliminary Experiments on the Culture of E. coli 31 4. Literature References Section II. Characterization of a Monochromatic High Intensity Variable Wavelength X-Ray Source in the 5-20 Kev Region A. Summary 3. Introduction C. General Properties of X-ray Production and Diffraction ---Page Break--- Intensity and Energy B. Characteristics of X-ray Tube. Analysis for Harmonies in X-ray Beam. A. Absorption Method 2. Double Diffraction 3. Application G. Orientation of X-ray Tube, Protractor, and Soller Slit for Optimum Intensity and Resolution 1. Tube Position 2. Small Protractor Posts: A List of Soller Slit H. Literature References List of Figures List of Tables ---Page Break--- PREFACE A. Reasonably definitive answers to the question; “What is the effect of ionizing radiation on catalase?" are now beginning to emerge. This new

approach involves study of effects produced by radiation from adjustable monochromatic sources. In general, x-radiation effects have been observed to vary slowly as a function of radiation energy. However, little work has been done in the region of x-ray energies below twenty kilovolts, a region of considerable importance since it contains the K-edge absorption energies of the constituents of most living systems. X-radiation of such energies is produced from incident radiation of higher energies (such as cobalt-60 gamma, 250 kW x-rays) by degradation through Compton scattering. It was felt important, therefore, to study x-radiation effects in the 5-20 kV energy range upon biological systems, which are comprised primarily of light elements with but traces of heavier atomic weight elements. As a first system for study, the enzyme catalase, containing four atoms of iron in its porphyrin ring structure, was chosen and the question asked: 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 damage in the kilovolt region. The presence of such a spectrum indicates unique effects of such radiation, entirely divorced from the general "indirect" effects of radiation (which may be simulated chemically). ---Page Break--- Work at the University under the direction of Dr. Henry Gosberg has been 200%: 18 problems for some. Two separate studies have been carried out by this group on the enzyme. One was done by Dr. Ardath H. Emons and another by Mr. Peter Pa Kevoudakis. Both demonstrate that 100 electron volts absorbed at photon energies just beyond the K absorption edge of iron produce a reaction with hydrogen peroxide; on the other hand, tests made by Dr. William Clendenning in the same group on the free radical yield in L-bromobutane, as examined by reaction with DPPH,

shoved a unique response as a function of energy. Likewise, studies made by Dr. Marvin Atkine on damage through radiation at the "L’ nége of certain mercury organo-metallic compounds yield negative results. Dr. Gouberg moved to Puerto Rico where a new group attacking the same

problem but with different equipment and under different environmental conditions was established. The Ann Arbor Group is continuing its study under the leadership of Dr. Hoyt Whipple. In Puerto Rico, Dr. Robert Luse has made a completely independent rerun of the cai fe experiment and has confirmed the existence of a uniquely high damage rate for photon energies in the vicinity of the K-absorption edge in catalase. In the course of this work, Dr. Vazquez Martinez, also of the staff, has developed extremely effective techniques for obtaining substantial yields of sonochromatic x-rays for irradiation purposes from Andaré General Electric Co. XRD-type spectrographs. ---Page Break--- An incidental, but significant problem has been solved or is being worked on in the course of this research. These include a new calorimeter for low energy x-rays, being developed in Ann Arbor, and high sensitivity chemical dosimetry, being developed in Puerto Rico. Work is now in progress on the x-ray action spectrum of carboxypeptidase (a zinc metalloenzyme) and on E. coli. ---Page Break--- RESONANCE RADIATION SPECTRUM OF LOW ENERGY MONOCHROMATIC X-RAYS ON CATALASE Robert A. Luse A summary of experiments using monochromatic x-radiation in the energy range 6.4 - 8.3 Kev have shown increased inactivation of the metalloenzyme catalase at or near the K-absorption edge of iron (7.11 Kev). This is taken to confirm the resonance radiation hypothesis of Gosberg and previous experimental work of Emons and Paraskevoudakis. Radiation intensities of 2 x 10^14 photons per hour have been measured in the sample holder with Fricke ferrous ammonium sulfate dosimeter. A more sensitive method for detection of the ferric ion product has been developed, using the ferric-hexacyanate.

complex. INTRODUCTION Previous work by Emmons (3) and by Paraskevoudakis (8) has indicated that there is an enhanced inactivation of the macromolecule enzyme catalase by monochromatic x-rays at wavelengths near the K absorption of Argon. Indeed, a plot of enzyme inactivation as a function of the x-radiation wavelength (or photon energy) follows closely the normalized sass absorption spectrum for iron (see 2438. I-L and 1-2). Since no such resonance radiation effects have been reported by other workers, it was desired to confirm the work of Emmons and of Paraskevoudakis using other equipment and personnel. The present report is concerned with such confirmatory experiences. ---Page Break--- EXPERIMENTAL PROCEDURES 1. Development of present irradiation system. To obtain x-radiation of precise energy, the irradiation system described in this report by Vézquez Martínez was utilized. Where a portion of the x-ray energy produced in the x-ray tube was selected by collimation and crystal diffraction, so that a beam of monochromatic x-rays having photon energies within the 4-50 KeV range and with a high purity of energy (+ 50 eV) was available. The characterization of this radiation defined the requirements for the holder in which the enzyme solutions were placed, viz: irradiation chamber dimensions 9 - wide, and 4 cm deep; vertical solution height not to exceed 6 cm. Irradiation of solutions of catalase was carried out in the sample holder sketched in Fig. 1-3. This holder was constructed of measurement of the beam area and position was done by placing a 4 x 4 cm sheet of X-ray film (in light-proof envelope) directly in front of the sample holder. After a short irradiation period, the position of the film relative to the holder was marked by piercing the envelope, film, and holder with a sharp scribe. After photographic development of the film, it was replaced on the holder, and the holder adjusted to coincide with the darkened area of the film. Later, very accurate characterization of the distribution of energy across

the X-ray beam was done by Vasquez (15). During the course of early work, three other ‘staple holders’ having the characteristics tabulated below were utilized. Experience gained in their use allowed the design of the present holder.

Construction Capacity Solution thickness

A round polyethylene bottle top 1.5
a block of lucite 10
16
A block with small components ("Michigan cell") 0.2
Cells A and B had Mylar windows, Cell C was covered with a scotch tape until it was found that contact with such of catalase solution

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Percent of Catalase Activity Lost
Activity Lost Percent of Catalase Note: (24)
0/9
Catalase Solution Lots of Acts X-ray Energy. Data of Eamons, Ref. 1,2, absorption curve given by the broken curve.

Activity as a Function of Normalized iron-base
Fe
Pig. 1-2. Refer to caption above. Data of Paraskevoudaki. For Fig. 1-2, 1.2 x 10^19 x-rays/ea?, was 9.5 x 10^10 x-rays/ead-ne. For Fig. 1, values ranged from 1.5 x 10^10 to x-rays/ea at 10 keV. For Fig. 1-L, each sample absorbed a dose of 1.8 x 10^12. The incident dose rate TL, for Fig. 12 was from 3 keV to 7.0 x 10H

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Fig. 13, Distance between chamber and sample compartments = 2.5 Sketch of sample holder

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Polystyrene plastic (polystyrene) with 4.001 inch thick Du Pont Mylar polyester film reaches 29 ice face; Loose catalase is relatively transparent to low-energy x-rays and is non-reactive chemically with the biological sample. Compartment thickness was determined by balancing the factors of solution absorption and sample size: a sample thickness was measured to absorb approximately 95% of incident 7 keV photons, yet this thickness would permit only a 100 µl sample, too small for accurate manipulation and analysis. A depth of 4 cm permitted a 220 µl. and conditions of complete absorption were avoided by mechanical stirring with a glass rod inserted into the solution and rotated at 88 rpm.

To minimize

Evaporation and inactivation of the catalase solution, samples were covered and maintained at 5°C by passing water from a ‘constant temperature refrigerated bath through the holder block. 2. Development of ferrous-ferric micro-dosimetry system. The prime requirement specified for the dosimeter system was the ability of direct substitution for the sample, so that values of radiation intensity measured with the dosimeter correspond directly with those absorbed by the biological sample. Other considerations were simplicity of use and reliability in the low dose ranges involved in this work. The Fricke ferrous-ferric dosimeter is the most commonly used and best characterized “secondary standard” available. This dosimeter relies on the oxidation by ionizing radiation of ferrous ion to ferric ion, and determination of the concentration of ferric ion formed by comparison

with primary standards such as colloidal solutions. Summary of that (7) ---Page Break--- The light absorption is 50% at (ref. see: sd Alten, 12). Application of this dosimeter is primarily via methods for ferric ion analysis. Recent work by Scharf and Lee (11) has shown that a more sensitive assay for ferric ion measurement is the absorbance at 226 nm; here the molar absorptivity of ferric ion is 4565 L·mol■¹·cm■¹ compared to the value 2196 at 204 nm wavelength. That the absorption spectrum for the ferric ammonium sulfate solution used in this work coincides with that of the ferric sulfate solution used by Scharf and Lee was confirmed by laboratory measurements. A considerably more sensitive assay of ferric ion concentration is measurement of the absorbance of the red-orange ferric-thiocyanate complex; the molar absorptivity of this complex is 10,000 - 14,000 L·mol■¹·cm■¹ at 480 nm (see Fig. 1-4). Pribicevic, Gal, and Draganic (10) have characterized this complex formation and proposed the use of a dosimetry system in the 300 - 1000 x6 dose range. Not all their results could be confirmed, so that further characterization and modification of the system were undertaken.

Differences were found in the location of the absorption maximum (480 nm, not 470 nm) and in the potassium thiocyanate concentration yielding greatest complex-absorbance (2.08, not 0.658). The changes were incorporated into the procedure developed. In addition, to avoid dilution of the irradiated solution, optical measurements were taken with the Lambda DU spectrophotometer in cuvettes of 3 cm x 10 cm x 25 cm chamber dimensions; as little as 0.22 mL of solution may be assayed in such cuvettes. The term molar absorptivity (ε) is equivalent to the older extinction coefficient and molar absorption coefficient, following the preferred usage of the editors of A. ---Page Break --- De 480 nm (when thiocyanate) Absorbance ! A= 224 nm X= 304 nm 2 + © @ 0 Concentration of ferric ion at (moles/L) x 37> Fig. 1-4 Comparison of sensitivity of various methods for determining ferric ion in solution: ---Page Break --- The procedure for assay of various concentrations was as follows: To the four aliquots were added portions (approximately 0.23 mL in volume) of 5 x 10^3 mM ferrous ammonium sulfate in 0.8 M sulfuric acid, treated as: a) not irradiated, stored in refrigerator, b) irradiated for the specified time at 5°C, with mechanical stirring, c) and d) controls 1 and 2, kept in colder compartment B and C during irradiation. Measurements of absorbance at 224 and 306 nm were made using the stored control blank. One of the controls was then set aside, and 220 µL of the ferrous solution containing ferric ammonium sulfate to the extent of 1 x 10^-3 M ferric ion was placed in the cuvette. Fifty mg portions of potassium thiocyanate were then added to the cell and dissolved by shaking. Absorbance at 480 nm was measured after 10 min. The value of the molar absorptivity was calculated from the standard ferric solution and used to estimate the ferric ion concentration in the irradiated and control samples. 3. Assay for catalase activity. Catalase concentrations were determined using essentially the standard assay developed by Beers and Sizer (2), in which the disappearance of

hydrogen peroxide was followed spectrophotometrically at 212 nm. Catalase activity was expressed in units of enzyme (v) per minute. These authors used 240 nm wavelength for measurement to show that any wavelength in the 200 - 300 nm region is appropriate. The 212 nm wavelength was used for two reasons: to repeat the work of Eanons and Paraskevoudakis, and to utilize the larger (clearly five times) molar absorptivity of hydrogen peroxide at the lower wavelength. ---Page Break--- The amount of protein, where one unit will catalyze the decomposition of 2 moles of hydrogen peroxide per minute under specified conditions. Assay reagents were as follows: Enzyme solution - An approximately 4.5 mg % solution of catalase (Worthington Biochemical Co, lyophilized material, lot no. CTL 8535) was prepared in 0.067 M potassium phosphate buffer, pH 6.80 (made from 0.067 M solutions of KH2PO4 and Na2HPO4). Such solutions were stored at 5°C in red glass volumetric flasks. Calculation of enzyme concentration by

spectrophotometric measurement was done using the molar absorptivity value 340 nm and 405 nm for horse liver catalase. Substrate solution - A 0.03% (8.8 x 10^-4) solution of hydrogen peroxide was prepared from Fisher reagent grade 30% material by 1:1000 dilution with 0.067 M phosphate buffer, pH 6.80. This solution was prepared fresh daily before use, as dilute peroxide solutions are not stable at room temperature. Water used for enzyme and substrate solutions was distilled in glass from previously demineralized water. Buffers and water were stored in polyethylene bottles to minimize trace metal contamination. Such terminology follows recommendations made by the Commission for Enzymes of the International Union of Biochemistry (see Thompson, 13). Such a calculation indicates that a 4.80 mg. % solution, nominally 2.1 x 10^-7 M (molecular weight of 225,000) was actually 1.0 x 10^-7 M. This means that the lyophilized material has to approximately half its total weight due to moisture. ---Page Break--- The as

ny procedure was as follows: The sample of cathecol solution (normally 0.1 - 0.2 ml in volume) was placed in a specialized cuvette (1.0 cm path length, 4 ml capacity, fused silica for ultraviolet transmission). At zero reaction time, 2 ml of substrate was rapidly pipetted into the cuvette, and the change in percent transmittance at 212 nm of the solution measured over the first 90 sec. of reaction time. This change in transmittance was measured using a Servograph strip chart recorder attached to the Beckman DU spectrophotometer with a Beckman energy recording attachment (ERA). The limits of pen travel on the chart were defined prior to this measurement by adjusting the pen to 0% transmittance with the spectrophotometer dark current adjusted with no light striking the photocell, and then to 100% transmittance with the ERA "100% adjust knob" with light passing through a solution of buffer only. No attempt was made to add the hydrogen peroxide to the enzyme and to start the recorder simultaneously; the substrate was added at zero time (as judged from a sweep second hand timer), the spectrophotometer shutter was opened, and the recorder was started, in a sequence requiring about seven seconds. The ninety-second interval was accurately measured, at which point the shutter was closed causing a rapid deflection of the chart pen to 2410. Points on the chart corresponding to 60 sec. were determined by carefully measuring back from the deflection point with a ruler. Since chart speed was 18.3 cm min^-1, the 60-90 sec interval was equivalent to 9.15 cm. In some measurements, increased sensitivity in the chart recording was achieved by setting the 100% T margin with a solution of uric acid approximately 0.9. By this method, absorbances in the 0.9 - 2 region were more accurately measured, and the slope of the curve ET vs. time increased by a factor of approximately eight. The values of 12 cm from the cacodetsip second were converted to absorbance values by the relationship absorbance = log (100/percent transmittance).

oan. and have absorbance values used in the equation fenzyne unite per mg. = A per nature x 1000 Aes eirbat pee at. Of reaction mixture (2) where 146 is the molar absorptivity of hydrogen peroxide at 212 nm (measured in this Laboratory). At the same time that the molar absorptivity at 240 nm was measured and found to agree with published values ---Page Break--- The intensity of x radiation in the 6.5 - 7.5 keV photon energy used by the ferrous dosimeter, is shown in Fig. 1-5, based on the following equations were in calculating this data: Amount of ferric ion (QEDIBes sample selected for { }- blank at) of measurement. in sample, as moles/L, x 10^3 is the absorptivity of ferric ion at the measurement or, c = A/1000ε (eq. 3) Molar absorptivity of ferric ion at 224 and 306 nm (2196 and 4965 L·mol■[1]·cm■[1] respectively at 25°C). The molar absorptivity of the thiocyanate complex at 480 nm was estimated from ferric standards, described previously. 6.02 x 10^{23} can be used by the sample, in erg “! the cone , Cc rate of radiation absorption where © = concentration of ferric ion, moles/L. £ = factor for conversion of electron volts to erg, 1.6 x 10■[1]■ erg/eV. The ferrous ion yield (G value), equal to 0.135 moles oxidized per electron volt, in the 8 - 10

keV range (Cottin and La Porte, 4) the period of irradiation, in hours of, 1 (as ergs at the level S + 7.16 x 10■³ (eq. 42) 1, furthermore, as photons al") are c 4 © Wper pions, L TEx Coste, 503 ---Page Break--- absorbed in sample heal x 10" mean intensity ‘ae (photons - 7 a Photon energy, as keV Fig. T-5. Monochromatic x-ray beam intensity ‘as a function of photon energy ---Page Break--- “CUD 201 paw sagas 50 v2¥p Boyen sanz¥reder 303 pey221209 9100 sonyeA AIFATIALONGE WTO vw WSLBATSOG SORTA ONTSN “ALISKRINE HVE AVH-X OTLLYMOWHOONOA 40 INEKEANSVEK °T aTEVE ---Page Break--- (eq. 535 1, as photons 2 sosgie = = (1, as photons. at nel, x (sample volume in al.) (eqtn. 6) Total dose delivered to sample arose (eq. 7) The most important features of these data are a) that the

beam Ancensity is approximately 2x 10^11 photons per hour in the 6.4 - 7.2 KeV photon energy region, b) that the intensity is increasing with increasing photon energy (as found by Paraskevoudakis), and c) that there are no resonance radiation effects in the ferrous dosimeter at the absorption edge of iron. This finding is as expected, since the radiation effect measured for an oxidation, the value for which is energy independent over at least one hundred orders of magnitude. 2. Resonance radiation effects in catalase. The extent of catalase inactivation by monochromatic x-radiation is shown graphically in Fig. 1-6. This curve is based only on data from experiments in which inactivation of control catalase solutions by scatter radiation was minimized by additional shielding (Table 2). That there is enhancement of catalase inactivation by radiation of energies at or near the K and K-adsorption edges of iron is obvious and confirms the findings of Emons and Paraskevoulakis. In general, the shape of the curve approximates that of the iron-mass absorption curve. Vertical arms from these points indicate the extent of difference in duplicate assays on irradiated samples. Horizontal areas indicate the variation in photon energies within the x-ray beams (+ 50 electron volts), ---Page Break--- Percent catalase inactivation 65 70 75 80 as Photon energy, KeV Fig. 1-6. Resonance radiation effect of monochromatic arrays on catalase. Total dose absorbed by sample = 1.4 x 10^29 photons (except for starred point, where dose = 0.2 x 10^-10) ---Page Break--- 06 36'e8 course | see by 99 co" 96 ras ore o'sy sa 698 68 uw | see vos | zx/over 09'r9 98 s6'06 wu] we eis | esverer as'e9 1988 18°96 ui | cs sons | esvover! 06 96 6 a | ove | asces | zosertt WY PRAT TOY TH IIOD TAY THING | TATE UY [AAT | THIET |G posed | yng | art] fesey sua o NOLLVEGYE-X DLLYMOWHOONOR AB NOLIVALIOWNI 2S¥I¥LND. “2 318¥E ---Page Break--- -20- CONCLUSIONS AND DISCUSSION The interesting comparison of present dose rates and control doses with those of Emons and Paraskevoudakis, A

Summary of these data is given in Table 3. Emons reports dose rates which are 0.1 to 0.025 those found in his work, and total doses which are 0.02-0.03.

TABLE 3. SUMMARY OF DOSE RATES AND TOTAL DOSES ASSORTED IN CATALASE SAMPLES

Dose rate	Total dose	Sample volume	Investigator	Activity	Se'photone
Emons - first order	4.5 x 10^7 to 5 x 10^7	0.18	1.957 Pehrnteneet	2.2 cat	
Luge - first order	2 x 10^11 to 1 x 10^12	+23	Vazquez - first order	1.7 x 10^9	-	-

Comparison of the dose rate values measured by Luge with the Fricke similar electronic efficiency is approximately one percent, that is, the counter registers one out of every 100 incident counts. A possible reason for the ten-fold difference is that the surface area of Emons' dosimeter was not matched to the x-ray beam area, so that the apparent energy flux per cm² was in error. For example, it seems that Emons used a sample holder in his dosimetry studies having a surface area

equal to 2.26 cm², while the beam area may have been only about 0.26 cm² (roughly defined by the collimating slits used, approximately 0.6 x 0.3 cm). In the present work, this discrepancy is eliminated since the sample surface exposed to the x-ray beam is exactly the same as the beam area in simplified form. The scheme below illustrates this situation:

Source emits 100 photons; SPC-1 detector registers 1 photon in beam area of 1 cm²; dosimeter absorbs 100 hv/A = 100/A; counter efficiency = 1/100.
In case 2, dosimeter absorbs 100 hv/A' = 10 hv/A; apparent counter efficiency = 1/10.

An apparent quantum yield for catalase inactivation at the K edge absorption edge of iron may be calculated from present.

experimental details, vie: Quantum yield = molecules inactivated / photons absorbed, where molecules of catalase inactivated = (x 10^77 aolee/1) x (0.25/1000 liters) x (1/3 inactivation) x (6.02 x 10^23) photons of 7.11 keV energy absorbed = (x 10^n e/ar) x 72 bee. Hence the parent quantum yield (x 10^2) = (1.4 x 10^19), or 0.36 molecules/photon. The quantum yield calculated is 200-fold less than the 70 molecules inactivated per photon reported by Emons using dry catalase. Quantum yields considerably greater than unity have not been reported for enzymes in the many studies of non-resonance radiation effects. A part of this discrepancy may be due to error in estimation of doses absorbed in the sample.

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It must be realized that this apparent quantum yield is not the true quantum yield, since the above is not corrected for any absorption by the solvent. An estimate of the relative absorption of 7 keV photons by solvent and solute in a 1 x 10^-3 M catalase solution may be made as follows (cf. data of Banon Total Absorption, Material Coefficient, nt/s. concentration, g/l. catalase 0.022 0.26 0.087 + 13.60 13.7 1000 13,700 8902) absorbs 1/57,000 the energy absorbed by the solution. An exact measure of the quantum yield can be obtained only from experiments using dry enzyme preparations. However, rough estimates may possibly be made by extrapolation from a series of enzyme concentrations. Calculation also may be made of the G value for catalase based on the results, since 0.36 molecules are inactivated per photon of 7100 eV energy absorbed by the solution the G value equals 0.005 molecules/100 eV. This is approximately half the value of 0.009 previously reported for x-radiation of much higher energies where the resonant effect is not present.

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PROPOSALS FOR FUTURE RESEARCH

As present results confirm the presence of resonant effect in the x-ray inactivation of catalase, it is proposed to enter the second phase of the research--examination of second metalloenzyme, carboxy peptidase A.

has been chosen for study for several reasons, viz: 1) The radiosensitivity should allow much shorter irradiation periods so that use of the present low-intensity x-ray source can be facilitated; 2) The physical chemical characterization has been well developed by Vallee and coworkers (3, 14), such that it provides a rather well-defined enzyme for study; 3) It is presently unique in that the single zinc atom present per molecule, which is necessary for peptidase and esterase activity, may

be replaced with mercury, cadmium, lead, cobalt, nickel, or manganese to give a series of new metallocarboxypeptidases displaying specific activities toward a certain set of new substrate characteristics of the particular metal which is incorporated. The ability of substitution of the metal atom associated with the active site of carboxypeptidase permits the following experiment: Irradiation with monochromatic x-rays of carboxypeptidases containing different metals, e.g., zinc and nickel, at the K-absorption edges of the respective metals. Enhanced inactivation of the zinc enzyme would be expected at 9.66 KeV (zinc Kα), inactivation of the irradiation of catalase at higher doses would be awaited until a more intense x-ray source is available. ---Page Break--- The nickel enzyme at 8.33 KeV (nickel Kα); no inactivation should be produced in the zinc enzyme by photons of 23 KeV, nor in the nickel enzyme by photons of 9.66 KeV energy. Phase three of the experimental program will be a study of living systems where the resonance effect may be observed. Where the single-celled bacterium Escherichia coli provides a well-characterized test organism. In addition, it contains the zinc metalloenzyme alkaline phosphatase, which is obtainable in pure form (9). It is proposed to compare the resonance radiation effect in this enzyme (inactivation vs. photon energy) with the resonance radiation effect in the bacterium (percent survival vs. photon energy). Should the enzyme be vital for bacterial reproduction, the two effects should be static. A whole new range

of experiments can be initiated when vacuum x-ray equipment becomes available. With photons of 2.1 and 2.5 keV energies, the resonance radiation effects may be studied in sulfur and phosphorus ions respectively. At this point, the importance of disulfide bonds to three-dimensional enzyme structure and the role of inorganic phosphate ions in the active site of certain enzymes (alkaline phosphatase) may be studied. It is hoped that such resonance radiation studies will introduce a new dimension of specificity in the field of radiobiology. Low-energy monochromatic x-radiation offers a tool as “clean” as monochromatic ultraviolet light, but in an energy range one to three thousand-fold that of UV. With such a tool, a host of new radiobiological studies in the important field of enzyme structure and function becomes possible. It is interesting to speculate on enzyme-nucleic acid interrelations at this point. ---Page Break--- -s- 6. approx 1. Notes on catalase activity calculation The method of calculating catalase activity given in this report differs from that used by Emmons (and presumably, Paraskevoudakis), who both use the following relationships to (90-2 2 cn sda Gee Ge. 9 and fy = 1.2 x 10^2 (concentration: 8 even. 2) Ky was determined in each case by plotting the percent transmittance values measured by the recording system as log (100 - T) and determining the value of this line over the first 60 sec. of enzyme reaction, - (any value at a point on the) = 13g ee (eqn. 3) The values so calculated are negative hence unrecognized by Emmons. An empirical linear relationship was found between log x and log enzyme concentration in the 0.3 - 2 x 10■ M range. The equation best fitting this line (eqn. 2) was subsequently used to estimate enzyme concentration in control and irradiated samples. This method was rejected because it is not an accurate expression of the fundamental kinetics of the enzyme reaction. The reaction of catalase with hydrogen peroxide is described by first-order kinetics during the initial part.

of the reaction (before product concentration reaches @ point where the enzyme is poisoned). The equations describing such first-order reactions are Ky = L loge (S■/S■) where S■ and S■ = substrate concentrations at time 0 and time 1, respectively. Keq = 4) ---Page Break--- = and Ky = k (enzyme concentration) Keq. 3) Beers and Sizer (2) have shown that 0.01 = 0.02 hydro peroxide substrate follows Beer's Law for wavelengths of 210 - 300 nm, i.e. A = 4a proportional to $, hence Ky = loge (o/A). (data. 6) Substitution of percent transmittance for absorbance results in an equation of the form = loge (10/100) . loge (100) = Log (a) 4° S08 Glog Cyto "eM Gsptoo eS (data. 7) (the term (Log 100 - log T) is not equal to log (100-1). By, if T= 22%, log 100 - log T= 0.688; Log

(100 - 1) = 0.892). Comparison of the shapes of the curves obtained by plotting equations I and 9 against enzyme concentration may be made using simplified data. See Table 4 and Page 1-7 and 1. That means obtained a straight line relation between log Ky and log & (8 fortuitous and some indication of the insensitivity of a log function of T. Such insensitivity to the nature of enzyme concentration may be responsible for the wide variations in enzyme inactivation reported, ---Page Break--- oar TABLE 4. ILLUSTRATION OF DIFFERENT METHODS OF HANDLING KINETIC DATA FROM CATALASE REACTION Let h = 20000, A = 0.3, 0.6, 0 by 0.2 for various concentrations of com a. ktaeteceey ies) Let a = tot ggg ca6% | toa eee tn = 1827 333 ao | as coef as - | ---Page Break--- loge (A■/A■) of Toke 28 Rowe (ho/A■) log, (200-71) * (oo-t) 2 + 6 ® Enzyme concentration (arbitrary units) Figs 1-7. Comparison of absorbance and percent transmittance plots vs. enzyme concentration ---Page Break--- Apparent rate constant, K■ (negative values are 10) 08 ° 10 log, of enzyme concentration (arbitrary units) Fig. 1-8. Apparent velocity constant (simplified data) 20 generation ---Page Break--- = 30+ 2, Survey of radiation levels about x-radiation equipment A survey of the radiation levels existing about the

x-ray 476c prior to its routine use for irradiations and laces after additional shielding was found necessary in the vicinity of the sample holder. A Nuclear-Chicago model 2612 survey meter equipped with a thin-window probe for beta-counting was used, and results found in Table 5, related ‘TABLE 5. RADIATION LEVELS ABOUT X-IRRADIATION EQUIPMENT monitored counts min^-1 Geiger counter table, right end, 0-24" above table 100-1500. Geiger counter table, left end, 0-24" above table 100-150 0.30.5 Geiger counter table, front, 0-24" above table 100-150, Geiger counter table, in line with the collimator slit, 0-26" above table ‘5000-20000 ‘At sample holder, with single shield (3/16" Lead sheet) at diffraction crystal 60,000 20 At sample holder, with additional shield at crystal and over second collimator, horizontal 350 La Ditto, vertical measurement . 1500 5 Ditto, with additional shielding over crystal and first collimator 1200 ‘To minimize the effects of scatter radiation striking the controls and irradiated sample from above, lead shields 1/16" thick were placed atop the compartment in the sample holder: ---Page Break--- 3. Preliminary experiments on the culture of E. coli! It is desired in the future to test the effect of radioactive radiation in in vivo systems. In preparation for this work, the conditions for culture of the unicellular bacterium Escherichia coli were A culture of E. coli obtained from the U.S. Biology Department was inoculated in a sterile medium containing 1000 ml. water, 7.0 g. K_2HPO_4, 3.0 g. Na_3PO_4, 0.5 g. sodium citrate .2120, 0.1 g. $MgSO_4.7H_2O$, 1.0 g. $(NH_4)_2SO_4$, and 2.0 g. glucose. After 15 days growth at 35°C the culture was stored at 5°C to inhibit further growth. For purposes of counting the cells/ml of this suspension, a 0.1 ml. aliquot was diluted 1:100, 1:1000, 1:10,000, 1:100,000 and 1:10,000,000. A 0.1 ml. aliquot of each of these dilutions was inoculated with the tip of a pipet on peptone-beef extract agar (Baltimore Bacteriological Laboratories nutrient no. 01-125) in Petri dishes. Samples of

0.1 ml of sterilized distilled water were used as control. Counts of visually observable colonies after 2-day incubation at 35°C indicated 1:100,000 dilution would give the most accurate plate counts within the standard region of measurement (30-300 colonies per plate). Repetition of this initial experiment using the pour plate modification yielded the data in Table 6; the precision between replicate plates is high, so that this technique should permit a rather sensitive measure of resonant energy effects carried out by Luis X. Vargas of U.P.R, Chemistry Department. ---Page Break--- =: | TABLE 6. PLATE COUNT REPLICATION IN E. COLI CULTURING \ Sample Dilution Colonies/plate 1:105 110 1:106 168 1:107 120 1:108 control 1 ---Page Break--- » 2» » » » » 8%) » =a l. LITERATURE REFERENCES Anon. Spectrophotometry Nomenclature, Anal. Chemistry 34, 1852 (1961). W. Sizer, "A Spectrophotometric Method for Measuring the Breakdown of Hydrogen

Peroxide by Catalase", J. Biol. Chem. 195, 133 (1952). Coombs, T. Le, J. P. Felder, and B. L. Vallee, "Metallocarboxypeptidases; Mechanism of Inhibition by Chelating Agents, Mercaptans, Metal Ions", Biochemistry 1, 699 (1962). Cotton, M. and M. Lefort, "Lonnage Absolu Du Dosimètre Au Sulfate Ferreux, Rayons X mous de 10 à 8 Kev", 267 (1956). Bomons, A. H., "Resonance Radiation Effects of Low Energy Monochromatic X-rays on Catalase", Doctoral Thesis, University of Michigan 1959 (also Technical Report #2, contract No. AT (11-1)-684). Engstrom, L. "Further Studies on the Incorporation of Inorganic Phosphate into Calf-intestinal Alkaline Phosphatase", Biochim. Biophys. Acta, 32, 179-185 (1961). "Measurement Systems for High-Level Dosimetry", Proc. 2nd U.N. Intl. Conf. on Peaceful Uses of Atomic Energy, Geneva, 1958, Vol. 21, 188-93. Revoudakie, J. "Resonance in Radiation Effects", Technical Report #5, Contract No. AT (11+1)-686, November 1960. Plocke, D. J., C. Levinthal, and B. L. Vallee, "Alkaline Phosphatase of...

Zacherichta cold: A Zine Necaltooazyse", 3iocheats © 1, 373 (4982), ---Page Break--- 1) ny ry aw 1%) 1) 16) ou Pribicevic, $.M., 0. S. Gal, and I. G. Draganic, “Use of the yellow 1060 + Report NR-O01-0022-1961 Inst. of Nuclear Sciences "yorte xidrich". Scharf, Ke, and RM. Ls “Investigation of the Spectrophotometric Method of Measuring the Ferric Ion Yield in the Ferrous Sulfate Dosimeter", Radiation Research 16, 115, (1962). Schuler, R. H., and A. 0, ALM + Method of the Ferrous Sulfate Dosimeter", J. Chem. Phys. 24, 56-9 (1956), ‘Thompson, R. Hs S., “Classification and Nomenclature of Enzymes", Science 137, 405-8 (1962) Vallee, B. L., “The "Active catalytic Site, an Approach Through Metalloenzymes", Ion Proceedings 20, 71-80 (1961). Vizques-Martines, F., Progress Report No. 1, December, 1962. Westheimer, F. H., in The Enzymes 1, 295 (1939), edited by P. D. Boyer, H. Lardy, and K. Myrback, Academic New York. ---Page Break--- LL el =a. CHARACTERIZATION OF A MONOCHROMATIC HIGH INTENSITY VARIABLE WAVELENGTH X-RAY SOURCE IN THE 5-20 GV GION Plorencio Vizquez AL summary The x-ray radiation system utilized for the present resonance radiation studies has been characterized quantitatively as to intensity and photon energy distribution, and second harmonic contamination. The monochromatic x-ray beam resulting from crystal diffraction and collimation was analyzed horizontally across its front for a) intensity distribution, utilizing 4 special moving slit devices and b) photon energy distribution, using double diffraction by a second analyzer crystal. Deduction of the extent of second harmonic energies was made from a) absorption measurements relying on the different mass absorption coefficients at the first and second harmonic wavelengths and b) double diffraction measurements in which photons with second harmonic energies were analyzed separately. Correction for percentage of reflection by second harmonic energies also was determined by the double diffractor method. Contamination by higher harmonics was shown to be

considerable at higher operating voltages; monochromatic beams can be obtained only by proper selection of tube potential. The effects on the beam of positioning the various components of the X-ray system (tube, diffraction crystal, two Soller slits) were determined and we selected a system which provides high uniformity of photon energy distribution. As a result, a diffraction system was developed which permits irradiation with photons of uniform energy distribution (+ 50 eV, in 6000 - 9000). Such selectivity of photon energies is not possible with fluorescent excitation systems. ---Page Break--- = 36 - 8. INTRODUCTION ' To study the effect of X-ray irradiation on matter, it is important to study the effects of the X-ray energy, the total dose transformations involved and, on that basis, a clear definition of the mechanism for the production of change by irradiation. In particular, we have concentrated on the study of effects produced as a function of X-ray energy. Resonant effects may occur, and it is necessary to utilize an X-ray source in which photon energy can be changed slowly and by appropriate steps. We must also be concerned with the setting of

the irradiating beam at the proper energy and with obtaining the maximum possible intensity. Once these prerequisites are present, we should be able to deliver the required dose in a reasonable time. Two other important conditions must be met here: (1) Setting the dose as wide as possible in order to avoid the problems arising from very small irradiated samples, and (2) obtaining a beam of uniform energy and intensity so as to deliver to all particles in the sample, no matter their position, a homogeneous external radiation field. It is difficult to meet all the above requirements simultaneously because some of them are contradictory. For example, the higher the energy resolution of the beam, the lower the intensity. Also, the continuous range of energies is only possible with lower intensities than those available when discrete excitation X-ray energies are utilized.

‘This problem has given rise to two different monochromatic irradiation approaches: ---Page Break--- 0. 2) The use of high intensities at discrete x-ray points. ‘This method was used by M.C. Atking and ¥. R. Clandiaing.. 9) The use of lower intensities, but with almost continuous x-ray adjustment. This is the technique studied in the present report. ALL the work reported was done with a General Electric X-ray diffraction unit XR0-5 shown in Fig. 1-1 located in the Laboratories of the Department of Physics of the University of Puerto Rico at Mayaguez. Nevertheless, our results may be applied to any other similar machine, ---Page Break--- S-OK 2700 vosa9NAT FFG Ans-K opsv09Tm YesAVAD Jo géesBorONE “T-IT “ATA ---Page Break--- eu“ el =». C. GENERAL PROPERTIES OF X-RAY PRODUCTION AND DIFFRACTION Each factor entering into the technique of radiation, and the relative importance of each factor must be known. Brief descriptions of the most interesting problems involved in X-ray production and diffraction are included here, 1. X-ray production occurs in an X-ray tube, a voltage Vo is applied between the target and the cathode, all electrons coming from the cathode impinge on the target in such a way that photons are produced, with a distribution of intensities and energies. If we plot the intensities against the energies as shown in Fig. 11-2, we get, in general, a continuous distribution of intensity starting at point "A" which peaks at definite energies appearing afterward, intensity 606. to take — wavelength at the enemy ev, Fig. 1-2. X-ray em ---Page Break--- there are two reasons for these two superimposed distributions. When an electron with energy "E" impinges on the target, it may lose some energy by emitting photons with energies according to the equation or) This is the so-called Bremsstrahlung effect. Since the maximum energy of the electron is (V.e), the maximum possible energy of photons is given by: E = Vo and is shown in point A, defined by the applied voltage Vo. ‘The

Distribution extends from cha Liatt hv = hv co hv =o and 8 independent of the target material. There is also the possibility that the impinging electron may eject another electron from any orbit of the target atoms. As soon as the vacancy is produced, an exterior orbital electron falls into it and a photon is produced with an energy given by the difference of the energies of the two corresponding orbits. This last effect is known as the fluorescence effect and is the reason for the high-intensity peaks shown in Fig. 11-2. For this case, we will get a particular peak if the impinging electron has enough energy to eject the electron of a definite orbit. The peak distribution depends on the electron orbits of the target. Being interested in radiations with a continuous change of energies around a definite value and with constant intensity, we use radiation from the continuous part of the X-ray spectra. Let us summarize some properties of this part of the spectra: 1. The intensity in a narrow band of energy d(3¥) is proportional to the tube power (V.4.) ---Page Break--- 2. The total intensity and the intensity in a narrow band are proportional to Z (atomic number of the target). 3. The limit wavelength of the spectra at point a (Fig. 11-2) has to be given in Angstrom units when expressed in volts. For a particular energy (h¥), according to the voltage applied, the tube can, at

harmonics of the first energy, give h¥, 3h¥, and so on. 4. As the maximum voltage applied to the tube is increased, the spectra shift to the left as can be seen in Fig. 11-2. The peaks in this figure do not correspond to any specific atom but are representative. It can be seen that the useful zones of irradiation are those labelled x, y, and z. For these, the intensity distributions are not uniform, with maximum unevenness near the spectral limit, as should be the case. 5. X-ray diffraction: An X-ray beam incident on a crystal is diffracted according to Bragg's Law: $n\lambda = 2d \sin \theta$, where n is an integer, λ is the wavelength of the diffracted X-ray, and d is the interplanar spacing.

Berveen successive atomic planes in the crystal is the angle between the atomic plane and both the incident and the reflected beam. The beam obtained after diffraction is monochromatic with the following limitations: ---Page Break--- -a- The incident angle is defined by the slice with a defined width; the diffracted beam also has a band of photon energies tv + Q according to the incident angles. The crystal produces dispersion which also causes energy diffraction. If there are photons in the incident beam with energies ZV, hv = abv, they are also diffracted. So the intensity of the incident beam is strongly reduced. The percentage diffracted is quite dependent on the type of crystal. Also, depending on the crystal, the percentage diffracted is quite different for fundamental, second, and third harmonics, in such a way that some crystals strongly reduce diffracted harmonics. The Bragg equation limits the maxima wavelength that can be measured with a given crystal since sin θ cannot exceed unity. A relation between the width of the beam and the length of the crystal is easily observed. As seen in Fig. 11, the analyzer crystal, by its atomic structure, determines the efficiency of the crystal; it is not very high, as it can vary substantially from one crystal type to another. Selection of the crystal is therefore an important decision. Two theories have been developed to explain analyzer crystal reflecting power. The first, developed by Darwin and quoted in Janes, assumes that the crystal consists of perfect parallel planes of atoms with equal spacing between planes. The reflecting power of the "perfect" crystal is given by the equation where R is the reflecting power of the perfect crystal, λ is the wavelength diffracted, N is the number of atoms per unit volume, and F is the atomic scattering factor for the diffracted wave direction. The maximum value of F is for angle $2\theta = 0$, and decreases as 2θ increases, related to the classical “electron radius.” ---Page Break--- Comparison. Let’s consider the worm.

11-3, Crystal Length ---Page Break--- structure in the crystal; the crystal consists of small "crystallites," each slightly skewed from the average direction for the group as a whole: 2242 Se" et Ty! Where 4 is the reflecting power of the "mosaic" crystal 4 is the linear absorption coefficient of the crystal for x-ray energy, ---Page Break--- =e Ns the number of unit structural groups (cxy ae 90 vate votuse Ysa the scattering factor for the structural group and may be written Tate oo M, ace (4a the scattering factor of the "(" atom, and approaches 2 for small angles provided A is much shorter than the absorption wavelengths of the atom. The term eM corrects for the temperature of the crystal. For our work, this factor may be neglected. Note that Rp contains the number of atoms (or electrons) per unit volume and the scattering factor £, which is also proportional to 2, doveve fm contains both factors to the 2nd power. Thus, Re will always be much larger than Rp. In nature, most crystals are much closer to "mosaic" in structure. Adopting 2, calculations were made for crystals of Lithium Fluoride, Aluminum, Sodium Chloride, and Quartz. The corresponding values of Ra LiF (200) = 1543 x 10^-6 AL QM = 670 x 10^78 Nac (200) - 360 x 10^6 Quartz (1011) 490 x 10^6 (experimental) These indicate that, for a wavelength of 1.739 Å (K absorption edge of 1100), Lithium Fluoride crystal is superior. In this case, p, the linear absorption coefficient is small, and the number of atoms per unit volume is big. This is sufficient to compensate for the smaller number of electrons per atom. However, the diffracted energy is still only a small part of the incident energy. ---Page Break--- |. INTENSITY AND ENERGY DISTRIBUTION Most of the studies in this section

were made with two special devices built in our lab. The first provides for detailed measurement of intensity distribution across the diffracted x-ray beam. The second device is used to analyze the photon energy content at all points across the beam. Both instruments are described.

below. 1, Intensity distribution [A photograph of the device used is shown in Fig. 11-4 with the corresponding sketch in Fig. 11-5. The device replaces the normal support of the X-ray detector Geiger counter with a moving section mechanism. The lower section is fixed to the goniometer protractor and the upper section carries the Geiger counter in such a way as to provide a sliding motion normal to the X-ray beam. A lead screw moves the counter to the desired position. The window of the counter is covered with a shield containing a well-defined slit. Photons from the beam enter the counter only through this slit. By sliding the window slit across the beam and counting at different points, the intensity distribution can be established. If the width of the window slit is much less than the width of the individual medium resolution (MR) collimator slits, we can also get the intensity distribution across the individual collimator slits, as shown in Fig. 11-6. Here, each axis corresponds to the center of a collimator slit. If the width of the window slit is similar to that of the MR collimator slits, we obtain a similar intensity distribution across the beam like that drawn in Fig. 11-6. Figs 11H + Ch, show, at the bottom, a cross-section of the collimator slit cavity and, at the top, a file negative taken at the ---Page Break--- ---Page Break--- <4 woeye soysep FurprTs “SIT “Bra wonzas w-¥ Wana wee) 3708 ROULMTOSA HOM {cours ronnie wose] EBSA aioe toon Ee ---Page Break--- distribution with {2° aur : } ' ae oetue | 2 lone ' i 8 ! ‘ 2 1 t é ' t : ' { 2 ' ' 2 ! 1 distribution with j2" sur 82 {We Stam DISTRIBUTION with [2° suit 4 We84 mm. Bs = i ; gn q a ‘ q ~ ae ‘ i 1 SreTmBUTION TAKEN WITH Ata! ano2 kes a at) ope redadade Fig. 11-6, Intensity distribution ---Page Break--- <4 sample position, 1€ can be seen how the shadows in the film correspond with the MR collimator. Fig. + Ags By and Cp, show similar diagrams for the case of the high resolution collimator slit system. Here, 19 maxima occur, corresponding to 19 collimator slits. The MR collimator contains only 9.

corresponding elite, ‘the intensity of the whole beam can be obtained by integrating under the curves. If the slit has a width w, the distance between two consecutive counting points is b, and the measured intensities are given by S1y, S2y --> Say, then the total intensity is: S = E S1 + E S2. It should be noted that we obtained the same results, within ± 0.3% accuracy, with the narrower slit with the wider slits, when corrections for the dead time of the counter were made. Using reading periods of 10 seconds duration, the intensity of the whole beam was obtained with good statistical accuracy. A similar procedure was used in comparing intensities for the acetone cases. 2. Double diffraction - Photon energy analysis. In order to determine the energy distribution of the beam, a special crystal analyzer device was used. It is shown in Fig. 11-7 by a photograph, and in Fig. 11-8 by a sketch. The device consists of a wedge that can move in direction 2 (horizontal) across the beam. A rotating crystal holder, which is an accessory commonly used in spectrometer techniques, is mounted on top of the plate. After fixing the beam, the crystal is moved into position. A beam is directed by means of a lead screw, providing exact axial positioning motion in direction “a-a". Precise setting of the corresponding angle desired is obtained from the rotary table of the crystal holder. A support bracket holds a shield and slit in such a way that photons which arrive at the second crystal have come from the first crystal without intervening scatter. A counter is mounted on the plate, in such a way as to permit rotation around the crystal axis. It is turned by means of a circular steel strip and a screw, as shown in Fig. 11-8. Another slit and shield are attached to the counter to block scattered radiation from the first crystal. MEDIUM RESOLUTION SOLLER SLIT (between Mares) HIGH RESOLUTION SOLLER SLIT (between waves) SLIT ATTACHED TO SHIELD MOUNT ATTACHED TO PLATE ‘A’ Double

diffraction device ---Page Break--- SAE 4. CHARACTERISTICS OF X-RAY 232 Much of the research in this study has been done by irradiating at energies close to the K adsorption edge of a particular atomic element. Once the X-ray energy around which we are to irradiate is decided, the next problem is selection of the appropriate X-ray tube anode. Some of the criteria for the selection are commented on briefly below. 1. Selection of anode material The higher the atomic number of the anode, the higher the radiation intensity, at any fixed values of anode voltage and current. However, since we want to adjust the energy of the radiation searching the material under study, the output should change slowly as a function of energy. The anode material should be free of strong sharp emission lines in the energy region under study. (See Fig. 11-2) 2. Operating voltage A precise determination of the anode voltage to be used can be made only after the permissible second harmonic contamination has been established. As a starting point, we can apply a voltage giving twice the minima photon energy desired, since up to this value, no second harmonic is generated for the energies under study. This is discussed in detail in a later section. 3. Operating current Once the voltage and the anode material have been selected, the maximum operating current is determined on the basis of either the maximum allowable anode-power dissipation or the maximum filament current permitted. These vary with the tube type. Our present equipment allows 40 to 50 mA anode current. We plan to install new 100 mA equipment soon, ---Page Break--- -3- 4. Output pattern and collimation The next step is the measurement of the output pattern, which in turn determines the arrangement of collimators in the diffraction unit. In the usual diffraction study, small areas and narrow slit openings are used to obtain high spatial resolution. In emission studies, narrow slit systems are used to resolve emission lines. However, our objective is maximum flux commensurate with

good energy ce resolution. We have used sodium resolution softer slits before the crystal and a high resolution slit after the crystal. The slits are few in the vertical position. This arrangement yields maximum useful output. The monochromaticity of the output was checked using double diffraction as described above. 5. Anode shape and orientation The importance of the atomic number of the anode material was mentioned earlier. We will now consider the form of the anode surface. The General Electric Ch-7 tube has a target which is 0.8 x 15 cm, and windows which are disposed as shown in Fig. 11-9. The surface of the target is perpendicular to the longitudinal axis of the tube. Fig. 11-9 also shows the emission pattern; taken with two files at one position of the first goller slit. We found that with window 3 the dollar was not well filled, but with windows A and C, the results were much improved. The General Electric AEC 50-T tube has a target which is 5x5 cm in projection and forms an angle of 70 with the tube axis. This angle is important from the viewpoint of getting higher intensities, a question which will be considered in the next section. The effect of a large projected area across the width of the cooler slit is to promote various scenarios ---Page Break--- as section ey el bi l CA-7 TUBE oh (2, TUNE WINDOWS! dy ca me _- |. - sea SECTION tesJ “souLen) Tanger AEG-SOT TUBE Cr 1 VU ———— Std ion tsactEat —taRser MW PaazECTION 2), Target emission patterns ---Page Break--- eo | ANALYSIS FOR HARMONICS IN X-RAY 3zAH, As was stated earlier, the higher the voltage applied to the tube, the higher the intensity obtained. However, there exists a potential Vg above which the beam contains higher harmonics, which are not wanted. Thus, therefore, determine the maximum voltage that can be applied, taking into account the percentage of second harmonic which can be tolerated. Jas were used to find the percentage of second harmonic present. The first one is based on the different mass absorption coefficients of an absorber for

two 4 ffeccue waveleagehs. The second ie based on double if fraction. Le Absorpeion method Let

us suppose that the diffraction unit ta arranged for irradi~ ation as shows a Fig, 11-10. ‘the probles 4s co find at a point A, where the sample is to be placed, the percentage of the second harmonic as 4 function of volcag Sioce che position of che counter 18 close co potne A, the tntansicy of the seus Ls che sane as EAC received by the counter. hence 1 T, is the intensity a# measured by the covacer, thea @ Gry + ws where of and @ are the efficienet of the counter for energies ¥ and 2 oocaknes fom we put sa piace "B", an absorber with a Large ¢ifference in the absozption coefficient for the energies ¥ and 2¥, and in which energy ¥ 16 pace strongly absorbed, then the intensity ax given by tha = EDM cay ED ay ---Page Break--- = 56 te vateh: . and (8 D Dy ed 8), ‘energies v and 2¥ sre the mase absorption coeéfictente for >) p i4 absorber density © 4 4s sbeorber entekaces rom equations (1) and (2) we get &, s apy Aaa tyes bo. F . ’ a - BM Thy With d fixed, values of che factors A and B may be calcula: 1d of tables of monochromatic absorption coefficients or by ueasure- seent, using the X-ray unit, If divece measurements a: ‘made for a given frequency, ¥ , the X-ray unit mst be operated initially at a voltage lover than Vo, at vhich the second haronie will appear, The intensities T and To, with and without the absorber od to cheat, T/ig-3 Pe Pe Zo obtata the second t4ctor, the apectromtter angle 18 set for frequency 2 and a voltage above Vp but Less than 2p t4 uses Once ve have the evo £4ctore seeded and the ot, A efficiencies of fhe counter, determinations are made of 12/11 at ditterent veltages oo 4s to obtain the percentay of second harmonic present, ---Page Break--- ore counen race of snares sane oem {i Mero oENE Oi Pig. 11-10, Component orientation for second harnonie studies (abeorption method) Ss cbectate Te PROTORS SEN EER + Dnetm ee wm” ae x New poomon ron Fp ane Pig. Lieli, Componant orientation for second

aarmonic scudi (double diffraction setso3) ---Page Break--- = 2, Double diffraction For this method, the proper arrangement of the main diffraction axis and the added double diffraction unit are shown in Fig. II-L1. Let us suppose Line A-A to correspond to the front surface of the sample, and let us select with a slit in point 4 a fraction of the total beam which we are to analyze. Also, let us suppose the mixed beam to be composed of intensities I1 and I2. Placing a second crystal with appropriate angle θp(v) to diffract energy Y, and measuring I1(v) intensity at the counter, we have I(v) = Ne(θ) (Y) where N(θ) is the crystal percentage of reflection and A(θ) is the counter efficiency. In the same way, with the crystal in position G2 (24) we get I2 = N2(θ) I1 + B. Hence the percentage of the second harmonic present is given by I2 = HAMA A(θ) I1. For precision measurements, it is necessary to take into account the different absorption by air of photons with energies Y and 2Y between Point A and the counter. However, by computation the above was found to be negligible due to the short distance between the crystal and the counter. Another important correction must be made for the second harmonic photons measured in I1(Y). The correct equation should be TDD = I1 + T1A' I2 MA' (20) ---Page Break--- PR type na experiment Mem at any feature YM in energy is not accounted for in the tenability. It is important to make corrections for the different absorption of photons in the method. It is necessary to consider the stability of the measurement. The curves were taken with a starting point for second harmonic production at 14.22 kV.

the crystal set co diffract 7.111 Kev energy protons. Both curves change slope at about 21 KVP and 28.5 KVP, which indicates the co of the third and fourth harmonics at these operating voltages. Both curves are similar at the lower voltages, hence both methods cross-check each other. For the higher voltages it is necessary to use Fig. 4 instead of Fig. 1. In the present problem where we were interested in avoiding all second harmonics, the voltage 14 KVP was chosen for all irradiations. ---Page Break--- -a- G. ORIENTATION OF X-RAY TUBE, PROTRACTOR, AND SOLLER SLITS FOR OPTIMAL INTENSITY AND RESOLUTION In the following sections the relative positions of the spectrometer elements are studied with the general objective of getting the

highest possible uniform distribution of intensity. Particular attention is given to obtaining maximum energy resolution. Analysis of the general procedure is made by following the steps used in the particular case of irradiating at the K absorption of iron. 1. Tube position The X-ray tube must be positioned correctly to obtain a symmetrical intensity distribution. To obtain symmetrical distribution along the height of the soller slit, it is necessary to place the tube with the target surface parallel to the rotation axis of the crystal. The centering of the soller must be at the same level as the center of the target. This adjustment was made with screw A, My P, @ and with the leveling screw AL By 0, as shown in Fig. 11-13. The cube position may be changed, in addition, by rotating a few degrees around an axis perpendicular to the plane of the figure. This last possibility is limited by the tube holder walls and by the insulation of the high tension cable. The advantage gained in putting the cube in such a skewed position is a slightly larger angle between the target surface and beam center line, thus increasing the flux into the soller collimator. When the target plane of the cube is perpendicular to the tube axis, this device may be useful. There are other techniques

16 adjustments for this type of tube, described in later sections, which also may be used to generate useful output. ---Page Break--- Fixed To THE TABLE Nook Fixed AT TO* FROM TUBE AXIS DIRECTION UT ROTATING AROUND © ease pare Fines To The TABLE fp \ cae cece sexes ig. 11-13, Tube and diffraction wall with small protractor ---Page Break--- In the case of a cube with the target plane at an angle of soous =<, the additional angle obtained in the stew position is insignificant. The C. £ Aeeay cube CA-7 has a flat anode. This tube is ordinarily used for crystal structure diffraction studies. The tube with the anode at 70° is the Machlett AEG 50-T, which is ordinarily used to excite characteristic radiation in samples to be analyzed. Usually, this cube is placed so that the radiation passes downward into a box containing the specimen to be analyzed. For our work, the tube is rotated 90° along the cube axis to obtain side discharge, as shown in Fig. 11-13. Another adjustment available in tube position is placement along the cube axis. The tube is moved forward in small steps until the output pattern reaching the crystal position is symmetrical around the crystal axis. This determination may be made by surveying with the Geiger counter, covered by a mask with a thin slit, small protractor position. When using a CA-7 tube in the ARD-5 diffraction setup, it is possible to change the angle between the surface of the target and the direction of the beam by rotating the base plate around point A, as shown in Fig. 1-14. The omission intensity pattern of the CA-7 cube is similar to that shown in Fig. 11-14. If we need a beam angle of "" degrees, to fill one width of the Soller slit, it is better to obtain the beam at the higher angle so that higher intensity 12, 1'2, and better distances are obtained. For our problem, the higher angle of the small protractor was always used. ---Page Break--- as the use of a Soller slit. The 100-5 is equipped with Soller slit collimators to which the plates are stacked one above the other, with each

plate parallel to the horizontal plane. As supplied by the manufacturer, the soller slits are designed to minimize vertical dispersion of the beam. Resolution is determined by separate vertical slit systems, which allow for beams with angular spread of 3°, 1%, 0.4%, etc. The small angle slit system transmits such a smaller number of photons, but spatial resolution is high. This system is used with characteristic emission radiation from a tube to study, for example, powder diffraction or crystal lattice spacing. For our problem, the need is to direct a relatively parallel beam of white radiation at an analyzer crystal, and then to accept a relatively monochromatic beam from that crystal to be directed against a target. This latter beam may be fairly large, about 0.5 cm square, but it should be as monochromatic as possible, commensurate with intensity. It was decided to try soller slit systems to provide the initial parallel beam and also to collimate the monochromatic beam. The soller slit system provides in effect several parallel narrow slits. Medium and high

resolution (WR and HR) soller slits were available with dimensions as shown in Fig. TT-15. As indicated previously, provision is made by the manufacturer only for their use with plates horizontal. In our work, they were used with the plates vertical. For test purposes they were fixed to the support, which has positive alignment grooves, with masking tape wrapped around the soller slit cage and the support. The positions occupied by the soller slits and the arrangement used for measuring the energy ---Page Break --- compared as 9tt0s “t-te sta nouns ¥-¥ (osu Mm sim3e 188 ---Page Break --- are resolution as shown in Fig. 11-15, the arrangement tested, the output from each individual slit of the soller slit system in the number 2 position was measured, obtaining intensity as a function of photot results for combination of the High Resolution slit system in position 2B, near the target, and the Medium Resolution slit system in position 1A, near the cube.

window, are shown in Fig. 11-16. Five different soller slit system arrangements were tested in this way, with the results shown in Figs. 11-17 and 11-18. The horizontal straight lines on the energy scale indicate the spread between all the intensity points. In these studies, it was apparent that for the different combinations, the total transmitted intensity varied to some extent about 2 percent. Moreover, there were noticeable differences in the photon energy distribution within the beam, with the combination used in that case producing the smallest energy spread. Referring to Fig. 11-15, we find that for the HR soller slit system in the 2B position, the dispersion angle is: $\theta = 8.3$ rad = 0.0566 rad. If we assume that the system is adjusted to accept 7.11 KeV photons for the excess (gesting) position, the anticipated wavelength (and average) spectrum can be estimated. $\lambda = 24 \cos(\theta) + 28 \cos(51.2°) = 1.763$ m or 7.111 keV, $\lambda = 24 \cos(\theta + A) = 2d \cos(51.20° + 0.3) = 1.743$ x 262251. If $\theta + A = 3$, then it equals 1.7463 m or 21.752 at 0 or 7.157 KeV. ---Page Break--- The anticipated spread is thus about ±46 eV. The experimental values in Fig. 11-16 show no more than 90 eV spread to attain maximum for any one slit. Finally, for the case in Fig. 11-16, the energy distribution for the whole beam was obtained by graphical integration. The beam intensity as a function of photon energy is represented in Fig. 11-19a. This is the result for one slit. In Fig. 11-19b, the beam intensity as a function of its position in the beam cross-section is shown. Combining the information in these curves for each measurement position in the beam cross-section, we obtain Fig. 11-20, the energy distribution of the photons in the whole beam. The energy resolution is quite good. The spread to the half-intensity point is only ±35 eV within ±50 eV, 81.6 percent of all the energy is found. Thus, meaningful radiation at intervals of 100 eV can be carried out. The interval between the $K\alpha$ emission lines for elements around iron is about 500 eV. This

18 important in comparing the use of the “sonochromatic" radiation available from crystal diffraction experimentation. The closest unit with energy available by use of fluorescence emission Line to the K absorption edge of iron at 7.111 Kev is the cobalt K Line at 6.930 Kev. If the effect being sought has a strong dependence on energy, and is associated with the absorption edge, it could easily be missed if only fluorescence radiation were used. ---Page Break--- a e x PE WR IM POSITION 20 Ain im Position 1A pens 3 3 fu * Geoae/eawen aNeaas / umes ‘AaiSndans A AeeNaa MT Pig. 11-16. energy distribution in different points across the beam ---Page Break--- Tora eon? pr waiw ronten et wot mot suct suotegrene MR IN position 28 TOTAL 7310” gage. mio anio ania? RR IN POSITION Te 1d Puoton MR IN POSITION 24 SECOND rom. trad ror, MA GW POSITION 24 Association distribution intensity distribution fig. ion and intensity distribution across the beam ---Page Break--- one Tea IN Position 28 wot aio snot snot prong MAIN POSITION 1A RESOLUTION DISTRIBUTION INTENSITY bls TALBUTION PG. 11nd. results and intensity distribution across the beam ---Page Break--- got t0n00 eoitoore + 0020 BRODIE SINTOS ‘GROSIET SINCE ---Page Break--- -n- A. LITERATURE REFERENCES Clencinning, W. a and Atkins, M. C. Methods of Yonohronacie Ireadiations and Absolute Dosimetry with soft + Resonance

in radiation effects Report No. 3, The University of Michigan, Ann Arbor, (1960). Compton, A. H. and Allison, S. X. X-rays in Theory and Experiment. New York: Van Nostrand Company, Inc., (1935). Emons, A. H. Resonance Radiation Effects of Low Energy X-Rays on Ph.D. Thesis, The University of Michigan, Ann Arbor, (1939). Clark, G. L. Applied X-Rays. New York; McGraw-Hill Book Co., (1955). Meyer, Charles F. The Diffraction of Light, X-rays and Neutral Particles, J. W. Edwards, Ann Arbor, Michigan, (1963). Advances in Spectroscopy. Edited by Interscience Publishers Inc. W. Thompson, vol. I. Rooksby, H. Py Wilson, A. J. C., “X-Ray Diffraction in Materials,” Chapman and Hall.

Lisited, Reinbold Publishing Corporation, New York, (1960). Janes, R. W. The Crystalline State. Vol. 12, G. Bell and Sons Ltd. (1955). Klug, Harold P., Alexander, Leroy E. X-Ray Diffraction Procedures John Wiley and Sons, Inc. New York, Chapman and Hall, Ltd., London, 1958). ---Page Break--- 13. 1. 15. 16. ve. 12, 1-4, ito, mi. -m List of Protones Resonance radiation effects on catalase - data of zamons see > Resonance radiation effects on catalase - data of Paraskevoudakis, Seessserese 5 Sketch of sample holder .... 6 Comparison of sensitivity of various methods for determining ferric ion in dosimeter.... +e 9 Monochromatic x-ray beam intensity as function of photon energy => Sarees 1s Resonance radiation effect of monochromatic x-rays wees ws Comparison of absorbance and percent transmission plots ve. eneyee concentration vesecesseee 23 Apparent velocity constant va. enzyme concentration (Simplified data)ecceceeeseres 2 Photograph of General Electric X-ray diffraction unit MADSS tee 3 X-ray attenuation distribution ..... 8 Crystal Length seseeeeseeeene 62 Photograph of the diffraction unit with counter on sliding device 46 Sliding device sketch a Intensity distribution « . “8 device Photograph of diffraction unit with double diffraction device.... 30 Double diffraction device... 3 Target excitation pattern . 3A Component orientation for second harmonic studies (absorption method) vase onenen 37 Component orientation for second harmonic studies (coupled diffraction method) 3 ---Page Break--- Target angular intensity distribution ..... Soller site positions «2... Znecky Distribution in different points across the beam Resolution and intensity distribution across the beam. Resolution : Yield, Total intensity distribution and energy distribution at point A= 2 11-20, energy Distribution of photons in the whole Beam 2 n ---Page Break---