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PUERTO RICO NUCLEAR CENTER

RESONANCE IN RADIATION EFFECTS

TECHNICAL REPORT NO. 2

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RESONANCE IN RADIATION EFFECTS

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Technical Report #2

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TABLE OF CONTENTS

VII. APPENDIX

I. INTRODUCTION

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-radiation 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.

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 this point of our research (approximately 12 months ago), it was felt that maximum 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

K_{abs} of cobalt than at energies corresponding to K_{abs} 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, 5-bromodexyuridine, 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 K_{abs} of bromine. It is hoped that these experiments will open new approaches for probing the nature of radiation-induced mutations.

Since only low-intensity monochromated 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. Smoluchowski'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 emission source 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, Río Piedras. The former is used primarily for the physical studies, the latter entirely for the biological research.

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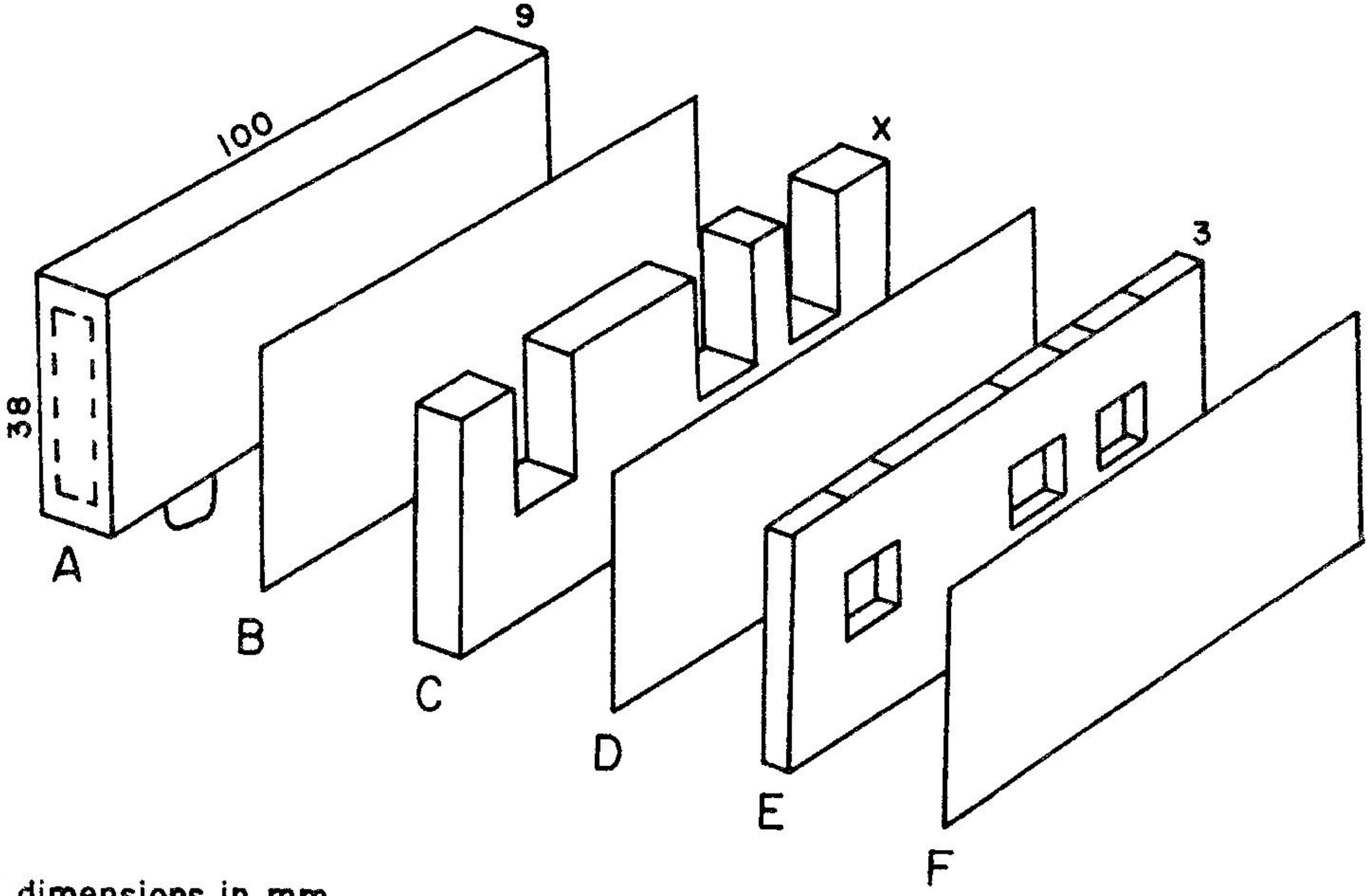
II. RESONANCE RADIATION EFFECTS IN CARBOXYPEPTIDASE A

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 0.3 mg/ml (1×10^{-5}M) 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 4×10^{-7}M, a concentration chosen as similar to that of catalase used in previous studies by Emmons (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 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 advantageous features:



All dimensions in mm.

Figure 1 - Exploded view of irradiation sample holder (see description, next page)

Description of Figure 1

A - Cooling plate with water inlet and outlet (lucite)

B - Rear window - 0.005" film (polyethylene, or other material)

C - Sample holder with irradiation compartment and two control compartments (lucite, polyethylene, teflon, or other material)

D - Front window - 0.002" film (polyethylene or other material)

E - Front plate, with cut-outs to match sample compartments

F - Cover for front plate, to provide insulating air-pockets in 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)

Parts C and F are attached to A with five nylon screws; Films B, D, and F are sealed to plastic with halofluorocarbon grease Kel-F #90 (Minnesota Mining & Manufacturing Co.)

a) Increased chemical resistivity - Plate C which forms the sides of the compartments may be of lucite, 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.[1]

b) 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.

c) 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 dry films.

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 carbobenzoxyglicyl-L-phenylalanine is 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 when $\log_{10}$ of 100/(100 - percent hydrolysis) was plotted vs. the reaction time. The proteolytic constant, C, was calculated as k/ (mg. 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 C of <u>in situ</u> controls). The activity of such <u>in situ</u> controls (i. e., kept in the irradiation cell compartments)

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[1] We found that slight amounts of ultraviolet light-absorbing material are leached from mylar films 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 mμ.

was 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 XRD-5 spectrometer at Mayaguez.[1] The sharp decrease in intensity at 11.69 Kev is unexpected. Possible causes, such as changes within the x-ray tube associated with 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 <u>in situ</u> control). Percent of inactivation, of course, equals 100% less percent remaining activity. Such preliminary experimental data may be used as a

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[1] X-ray intensity is a function of the applied current (ma), the applied voltage (as V^2), the atomic number of the target (Z), and general tube geometry. The XRD-6 spectrometer is capable of higher intensity because of its higher current ratings, but in the present system a chromium target tube is used instead of a tungsten target tube in order to avoid line emission near the zinc K-absorption edge. This difference in target material reduces intensity by nearly one-third ($Z_{Cr}/Z_W = 24/74$).

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 Kup and 75 ma; its output was collimated with two soller slits and LiF diffraction crystal.

Concentration of ferric ion formed, C, as moles/ml
= increase in absorbance /1000E,

where E has the value 4565 at 224 mμ and 2196 at 304 mμ, subject to temperature corrections, as given by Scharf and Lee (9).

Intensity of absorbed radiation, as photons per hr per 1 ml of dosimeter

$$= \frac{\text{concentration of ferric ion formed}}{\text{energy per photon, in Kev}} \times 4.46 \times 10^{21}$$

2θ (LiF crystal)	Energy per photon	Irrn. period	Wave length of A	Inc. in A, over controls	Ferric ion conc., as moles/ml.	Intensity, as photons $hr^{-1}ml^{-1}$
	Kev	Hours	mμ		x 10^8	x 10^{-11}
48.83°	7.47	27.0	224	0.037	0.81	1.8
			304	.009	.41	0.9
47.83°	7.60	22.0	224	.073	1.6	4.3
			304	.028	1.3	3.5
41.51°	8.68	21.5	224	.046	1.0	2.4
			304	.025	1.1	2.6
41.51°	8.68	18.5	224	.032	0.70	1.9
			304	.014	.63	1.7
37.17°	9.69	20.0	224	.184	4.0	9.2
			304	.056	2.6	6.0
37.17°	9.69	22.0	224	.119	2.6	5.4
			304	.038	1.7	3.6
30.57°	11.69	37.5	224	.028	0.61	0.62
			304	.011	.50	.49

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 2×10^{11} photons/hr/ml of solution was utilized. This best average 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.

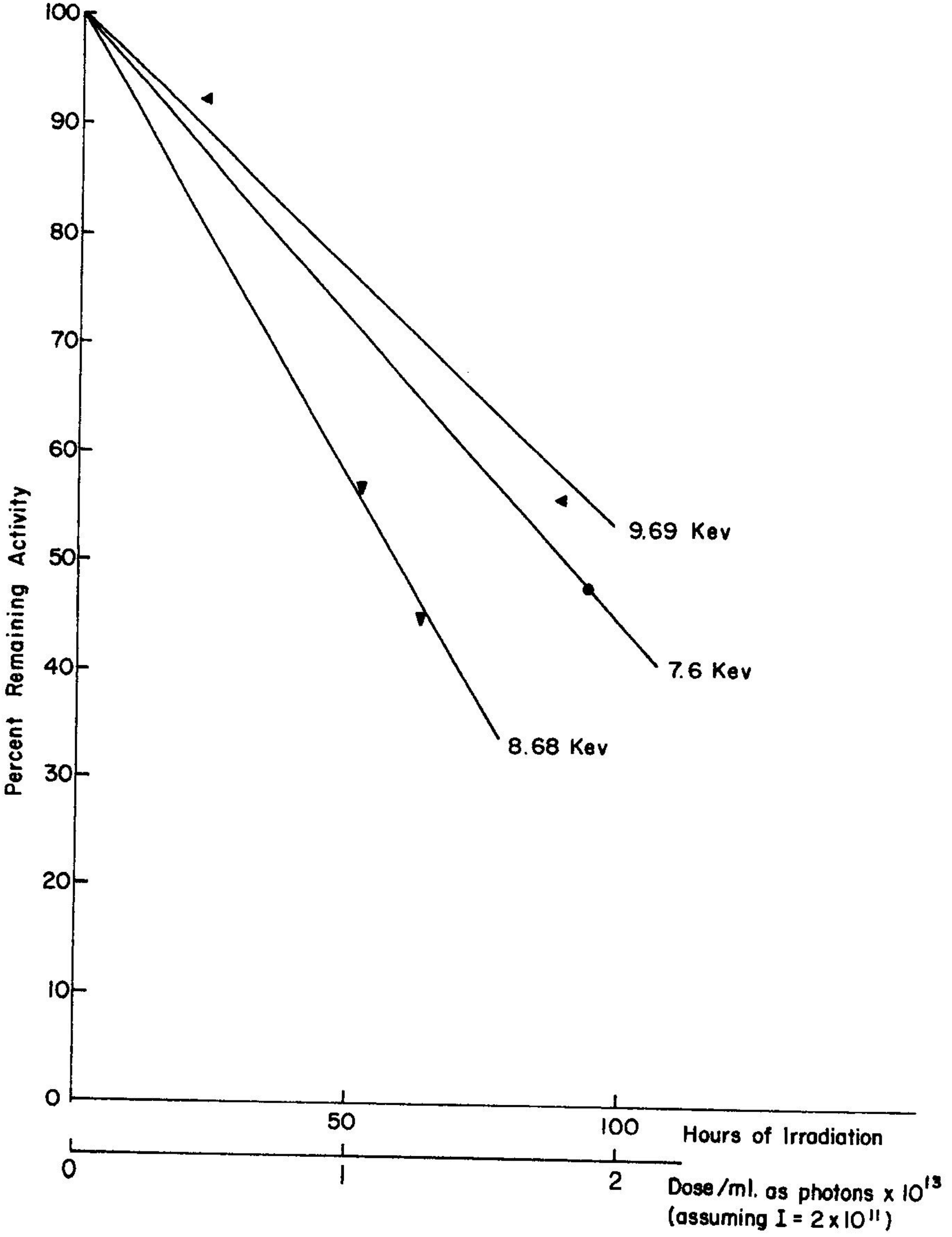
Table 2. Inactivation of Carboxypeptidase A with Monochromatic X-Radiation

Assay Date	2 θ (LiF crystal)	E/photon, as Kev	Irrn. period, in hrs.	Activity of control, as % of stock*	Proteolytic of control	Constant** of irrd. soln.	% remaining activity	% enz. inactivn.
12/19/63	47.83°	7.60	94.4	69	ca. 8.5	4.1	ca. 48	ca. 52
12/2/63	41.51	8.68	52.2	66	8.4	4.8	57	43
12/6/63	"	"	63.0	76	8.7	4.0	45	55
11/20/63	37.17	9.69	22.5	81	12.7	11.7	92	8
11/23/63	"	"	89.0	56	10.0	5.6	56	44

* This percentage equals 100 times the ratio: proteolytic constant C of *in situ* control/ C of control kept in flask in the refrigerator for equal time.

** These constants were determined from mean reaction constants, k (see text), measured in the range of 2 - 20% substrate hydrolysis, where first order enzyme kinetics apply.

Figure 2 - Course of Carboxypeptidase A Inactivation at Various Photon Energies.



III. PRODUCTION OF F-CENTERS IN ALKALI HALIDE CRYSTALS (NaCl, KBr).

a) Introduction and Theoretical Background

In the last few years 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-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 investigation of the F-center, which consists of an electron trapped in a halogen ion vacancy. Such F-centers are temperature stable (4°K to 300°K); at liquid helium temperature, they are associated with a neutral interstitial halogen atom (H-center). At such low temperatures, other workers have found that the rate of formation of F-centers is a bulk property independent of ionic 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

Table 3. K-Edges of Elements in Alkali Halides

Element	Z	K_{abs}	K_{α_1}	K_{β_1}
		Kev	Kev	Kev
Sodium	11	1.08	1.04	1.07
Chlorine	17	2.83	2.62	2.82
Potassium	19	3.61	3.31	3.59
Bromine	35	13.48	11.92	13.29
Iodine	53	33.16	28.61	32.29

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, and M-absorption edges of the halide is of special interest. Here, such questions arise as:

a) 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.)

b) What is the time for recombination of electrons and the multiply ionized halogens?

c) What is the time required for the halogen to migrate a given distance?

d) What is the energy necessary to create one F-center?

If we consider the same general problem of F-center formation 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 some as yet unexplained effect. All these effects tend to detract from the feasibility of Varley's mechanism and lead to a consideration of alternative processes.

To obtain information on the density of F-centers, use is made of the equation of Smakula (11):

$$N = 1.29 \times 10^{17} \ \frac{1}{f} \ \frac{n}{(n^2 + 2)^2} \alpha_m W$$

Here N is the number of F-centers per cm^3, f is the oscillator strength, n the index of refraction, α_m the optical absorption coefficient at the maximum of the F-band in cm^{-1}, and W the width in electron volts of the F-band at half maximum. The number α_m is obtained from the relation for transmitted intensity at a thickness d in terms of the incident intensity I_0, viz., $I = I_0 e^{-\alpha_m d}$, for light of a frequency equal to that of the maximum absorption. At a given temperature neither f nor W changes as the density of color centers increases. Under these circumstances N is proportional to α_m. As a 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 mm 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 single-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 4 and 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

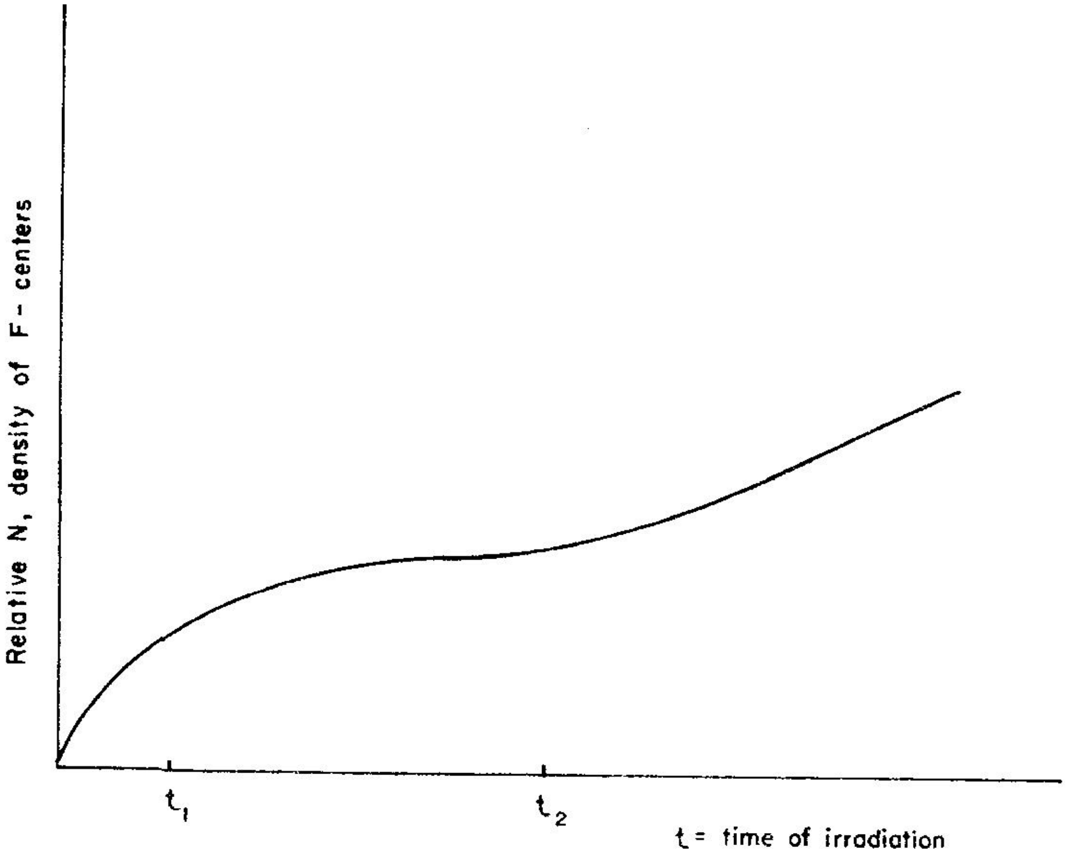


Figure 3- Typical density of F-centers against time of irradiation at room temperature.

Typical values are $t_1 = 10$ hours, $t_2 = 37$ hours for a K-Br crystal irradiated at 11.2 Kev, 4×10^9 photons / cm^2/ sec.

Table 4. Characteristics of Fluorescent Radiators for Alkali Halide Crystal Irradiation

Z	Element	K_{α}, Energy (Kev)	Chemical Form	Physical Form
35	Bromine	11.923	Na Br	Fine powder
38	Strontium	14.164	$Sr(NO_3)_2$	" "
39	Yttrium	14.957	$Y_2(CO_3)_3$	" "
40	Zirconium	15.774	ZrO_2	" "
27	Cobalt	6.930	Co	Metallic sheet
28	Ni	7.477	Ni	" "
34	Selenium	11.221	Se	Cast in Al planchet
47	Silver	22.162	Ag	Metallic sheet
48	Cadmium	23.172	Cd	" "
49	Indium	24.207	In	" "
		L_{α_1}, Energy (Kev)		
72	Hafnium	7.898	Hf	Metallic sheet
76	Osmium	8.910	Os	Metal, fine powder
78	Platinum	9.441	Pt	Metallic sheet
79	Gold	9.711	Au	" "

Table 5. Characteristics of X-Ray Filters Used with Fluorescent Radiators[1]

Radiator	Energy (Kev) K_{α_1}	Energy (Kev) K_{β_1}	Filter used	Energy (Kev) K abs	Chemical form	Filter density (mg/cm^2)
Se	11.221	12.495	As	11.863	As_2O_3	29
Br	11.923	13.290	Se	12.652	Se	29
Sr	14.164	15.834	Rb	14.323	RbCl	33
Y	14.957	16.736	Sr	16.106	$Sr(NO_3)_2$	29
Zr	15.774	17.666	Sr	16.106	"	"

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[1] The appropriate filter for a given radiator was determined by using the emission line and absorption edge energies compiled by Fine and Hendee (12). The absorption edge of the filter must be between the K_α and K_β lines of the radiator. The desired thickness of the filtering element has been calculated by means of the following equation:

$$\rho d = \frac{\ln 20}{\frac{\mu}{\rho}_\beta - \frac{\mu}{\rho}_\alpha}$$

where:

ρ = density of the filtering element (g/cm^3)
d = thickness (cm)
$\frac{\mu}{\rho}$ = mass absorption coefficient (cm^2/g)

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 a mylar window and a beryllium window shielding a 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 it has a thin heater coil for calibration. The calorimeter measures incident powers in the range from 4 to 30 micro-watts.

The curve of density against 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 4×10^9 photons/cm^2/sec. We have concentrated attention on the slope and occurrence of the straight part of the density curve because the initial fast rise in F-center 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 F-center density at room temperature arises solely from lattice displacement caused by the incident radiation.

IV. RESONANCE RADIATION EFFECTS IN PRODUCING CHROMOSOME ABERRATIONS

Preliminary Report

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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. Emmons (6), Paraskevoudakis (13), and Luse (1) independently showed the enhanced inactivation of the metalloenzyme catalase by monochromatic x-rays at wavelengths (or photon energy) near the K-absorption 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 Gomberg. 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 5-bromodeoxyuridine-labeled chromosomes in *Allium cepa* root tips. Roots from germinated seeds were first treated with BUDR solution at a concentration of 15 ug/ml. for 15 hours, and then irradiated with monochromatic x-rays for 3 hours. The beam intensity was approximately 5.9 x 10^{10} photons per cm^2 per hour. Six 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.

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.

Table 6. Chromosomal Breakages Produced by Monochromatic X-Rays at Various Photon Energy Levels in BUDR-Labeled Chromosomes of *Allium cepa* Root Tip Cells

Photon en. applied	No. of cells studied	No. of breaks observed	No. of breaks per cell
12.5	85	7	.08
13.2	92	15	.16
13.48	122	36	.29
13.7	----	---	--- now being
14.1	---	---	--- studied
15.5	68	15	.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 K-absorption edge of bromine cause a clear-cut increase in the production of breakages in BUDR-labeled chromosomes in *Allium cepa*.

Detailed reports on methods of material handling, chemical treatment, irradiation, cytological studies, etc., will be compiled and published upon completion of the whole study.

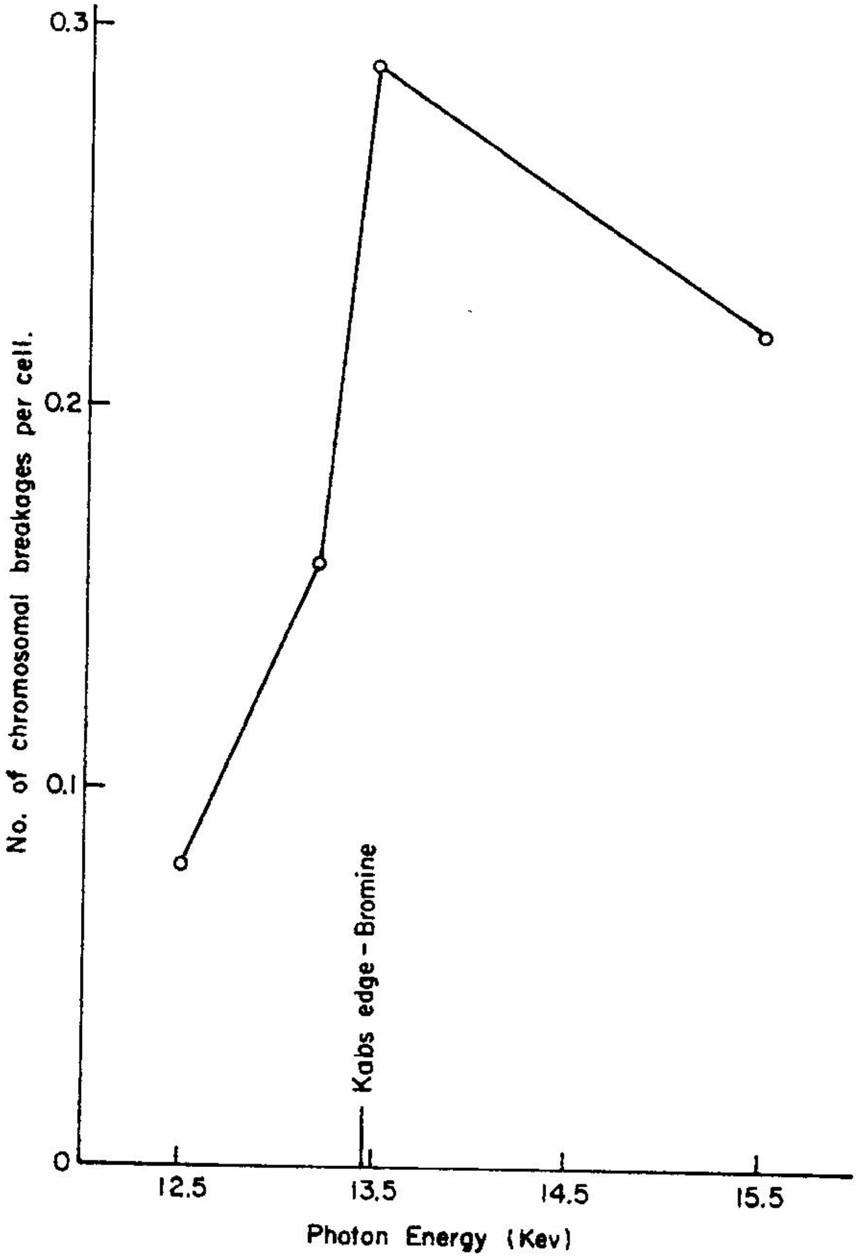

Figure 4 - Resonance radiation effect of monochromatic x-rays on BUDR-labeled chromosomes in *Allium cepa.*

V. 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^{19} ev/cm^2-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 soller slit is of the order of only 10^{14} to 10^{15} ev/cm^2-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^{17}-10^{18} ev/cm^2-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 wave length 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 design of a high intensity x-ray source raises three basic problems:

a) How to achieve high electron emission from the source;

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 of 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 (14) is

$$J\ (\text{electrons/cm}^2\text{sec}) = \int_{-\infty}^{+\infty} c\ N(T,E)\ D\ (F,\ E,\ \phi)\ dE$$

where N(T,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, has the value E. This function, as used by Sommerfeld and Bethe, has the form

$$N\ (T,\ E) = \ln \left[1 + \exp(-E/kT) \right]$$

where k is the Boltzmann constant. In the presence of a sufficiently 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 appearing outside the metal. This probability is expressed by the transmission coefficient

$$D\ (F,\ E,\ \phi) = \exp\left[-\ 6.83 \times 10^{7} \quad (\phi - E)^{3/2}\ f(y)/F\right]$$

Here ϕ is the electron work function in ev, F is the electric field in volt/cm, E is in ev and f(y) is a dimensionless elliptic function of the variable $y = 3.79 \times 10^{-4}\ F^{1/2}/(\phi - E)$ introduced by Nordheim (15). C is a constant that has the form $4\pi\ m\ kT/h^3$, where m is the mass of the electron and h is Planck's constant.

A plot of this formula (Figure 5) indicates the drastic increase in 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 10^8 amp/cm^2, 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. Because of their sharp edges, it is relatively easy to 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.

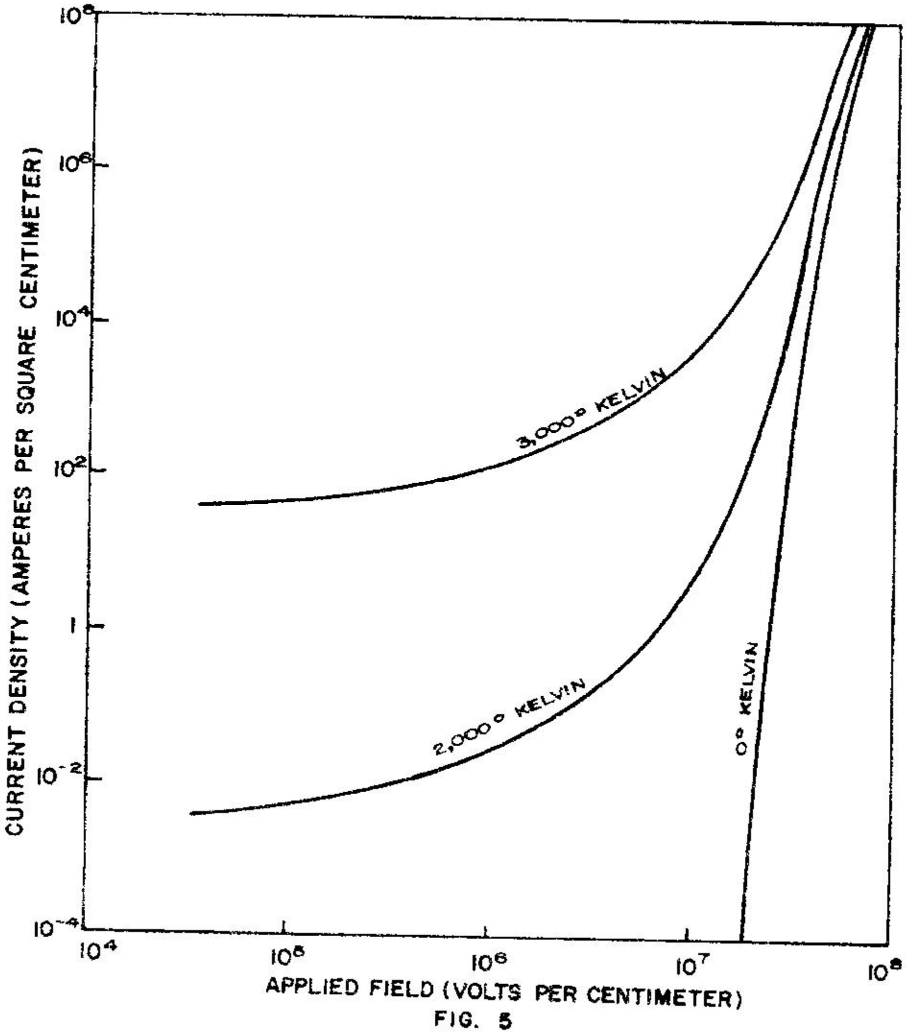

FIG. 5

From Scientific American, Jan. 1964 (16)

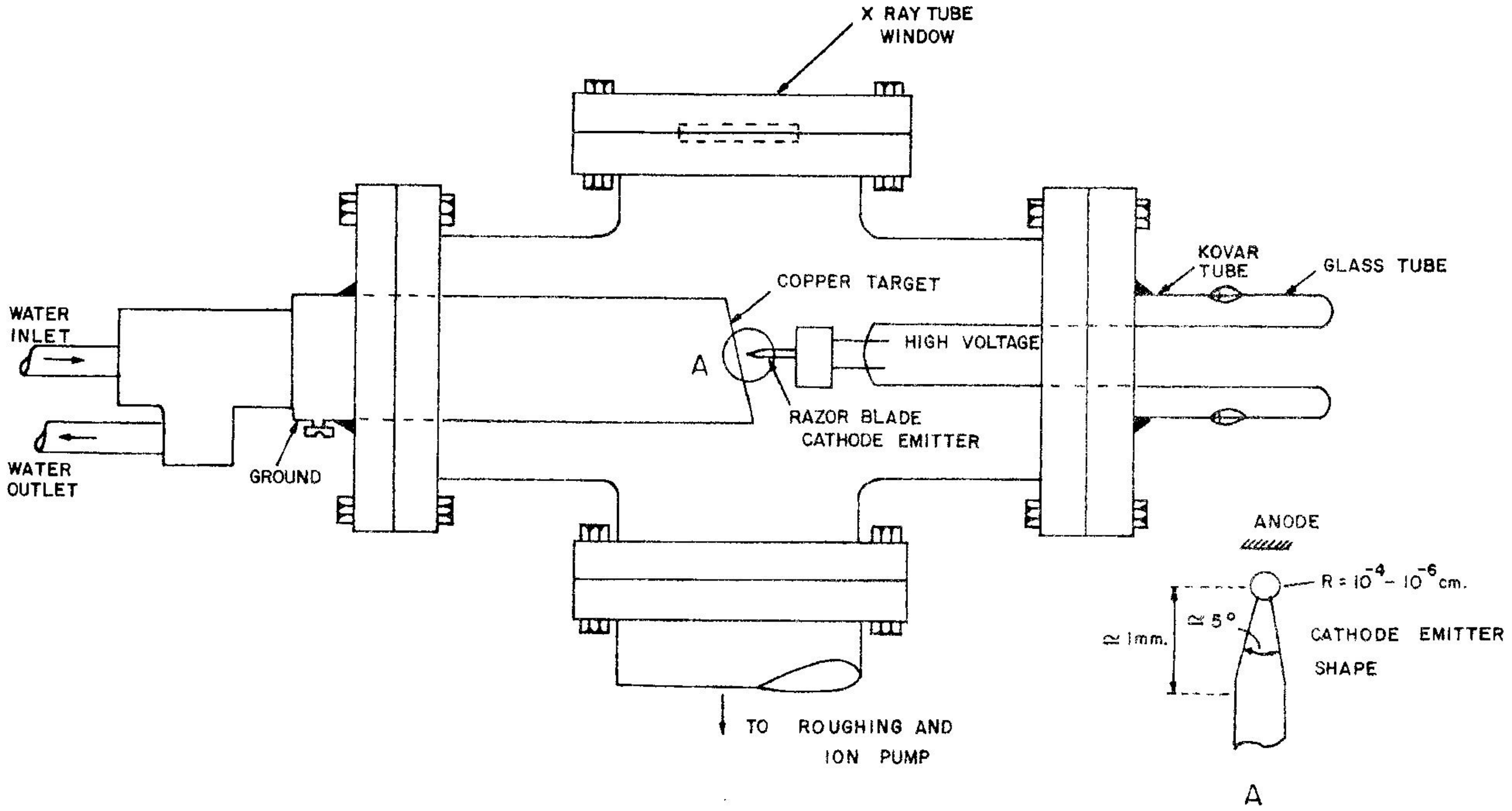

FIG. 6 - EXPERIMENTAL X-RAY TUBE USING FIELD EMISSION SOURCE

The first 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 tungsten. In our case, 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^{-10} mm. of Hg. Helium gas is one of the major problems. However, improving the vacuum and the cleanliness 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 1×10^{-4} - 1×10^{-5} cm in diameter. Another sharp source 1×10^{-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 blade edges, 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 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-focussing type sources as used by Dr. Burton Henke of Pamona College in California and Dr. William Gross of the Radiological Research Laboratory, Columbia University.

VI. INSTALLATION OF NEW FACILITIES

a) X-Ray Spectrometer, GE Model XRD-6

To expedite the biological phase of this program, an x-ray spectrometer was installed in the Río Piedras laboratory of PRNC for use by Dr. Luse. This spectrometer, model XRD-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 tubes in our XRD-5 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 ± 50 e.v. of nominal energy, i.e., less than ± 1% beam energy spread in the 5000-18,000 e.v. range. For details of measurement, see Project Report I (1). The intensity distribution of the emergent x-ray beam produced by the XRD-6 equipment has been measured; typical values are given in Table 7.

b) Low-Temperature Irradiation Chamber

The production by x-radiation of F-centers in alkali halide crystals can be assessed 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

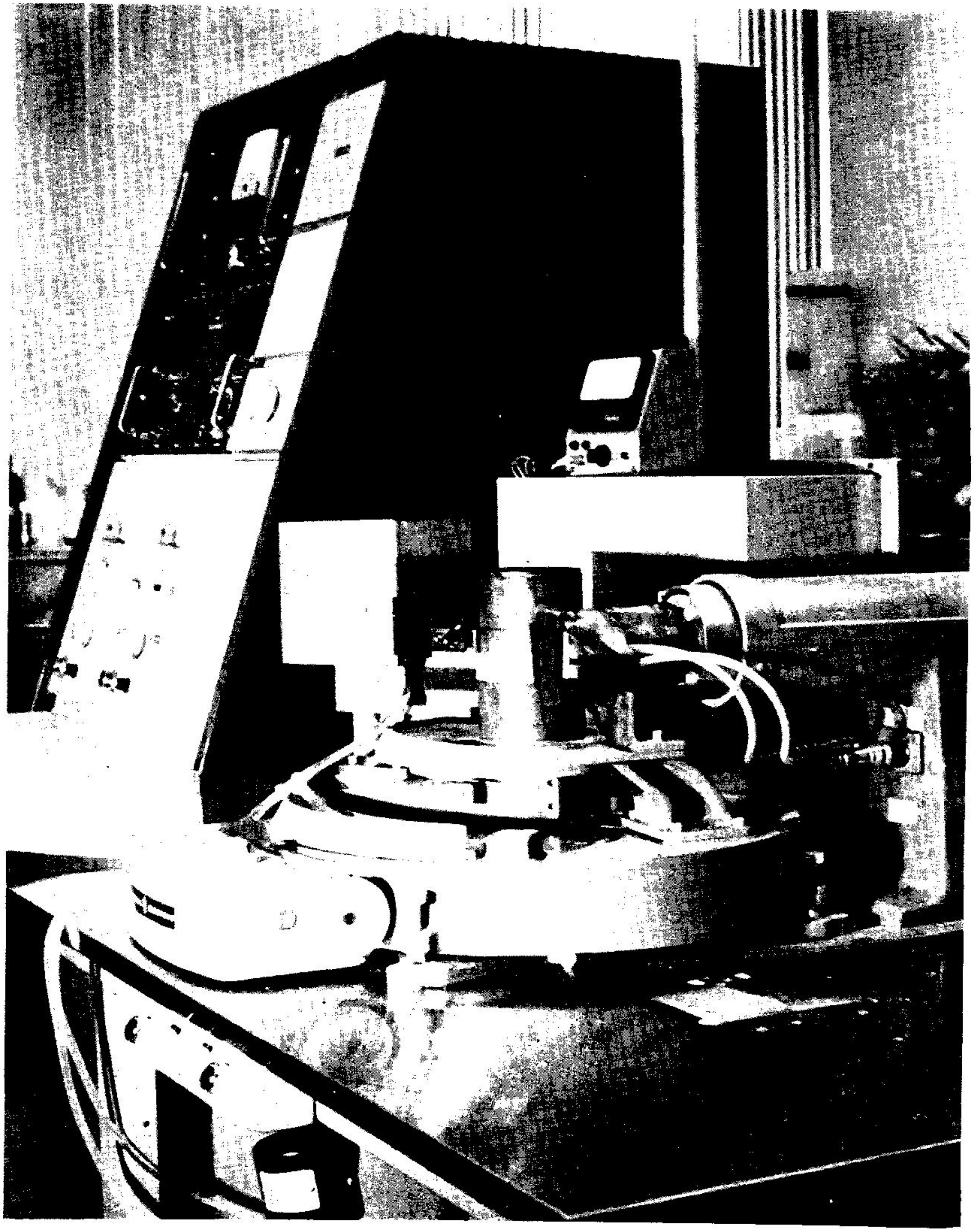

FIGURE 7. View of XRD-6 x-ray spectrometer showing x-ray tube, beam collimators, and shielded crystal diffraction unit atop the goniometer. Sample cell is attached to detector housing. The power supply unit, which also houses the detector scaler-timer and related power supply, is shown in the background.

Table 7. Intensity Profile of Collimated X-Ray Beam

Position	Photon intensity, as averaged cps	Percent of highest value
1	2	-
2	6,352	33.8
3	7,816	41.6
4	17,782	94.6
5	16,532	88.0
6	17,430	92.7
7	17,480	93.0
8	17,884	95.1
9	18,811	100.0
10	17,733	94.3
11	14,229	75.6
12	6,487	34.5
13	4,951	26.3
14	960	-
15	26	-

The intensity of x-ray photons was measured by moving the SPG-6 detector[1] in 0.86 mm intervals horizontally across the face of the beam[2]. A 0.1° (0.254 mm width) slit was used in front of the detector window.

The x-ray tube was operated at 18 KVP, and 45 ma. Collimation was as described for tests of energy resolution. The intensity profile was made with the LiF 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 irradiations is 0.92 mm, i.e., distance from position 2 through 13.

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[1] The detector is a proportional type counter tube, xenon-filled, with a 0.010 in. beryllium window; its efficiency is 80% for the 7.1 Kev energy photons here utilized.

[2] For details, see Fig. II-4 and Fig. II-5 of our previous report (1).

constructed by modification of a two-liter experimental cryostat (manufactured by Superior Air 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 resistance heater coils, 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 F-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 cryostat ports may be fitted with quartz windows for subsequent optical measurements. The sample chamber beneath the cold liquid reservoir is evacuated to about 10^{-4} torr by a diffusion pump with mechanical forepump (Vactronic Laboratory Equipment Co.). The same vacuum system provides heat insulation to the cold reservoir.

Although this irradiation chamber is presently in use only for alkali halide crystals, it offers the potential for irradiation of dry enzyme films at low temperature in future research.

c) Devices for F-Center Measurement

Production of F-centers in alkali halide crystals via x-radiation is 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 accomodate 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 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 F-center absorption peaks at 6200 A. 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 F-center peak at 2400 A, optical measurements must be made through quartz windows which transmit in the ultraviolet region. Several devices have been designed so as 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 these devices has been constructed and tested. The other devices may prove more simple and hence more reliable.

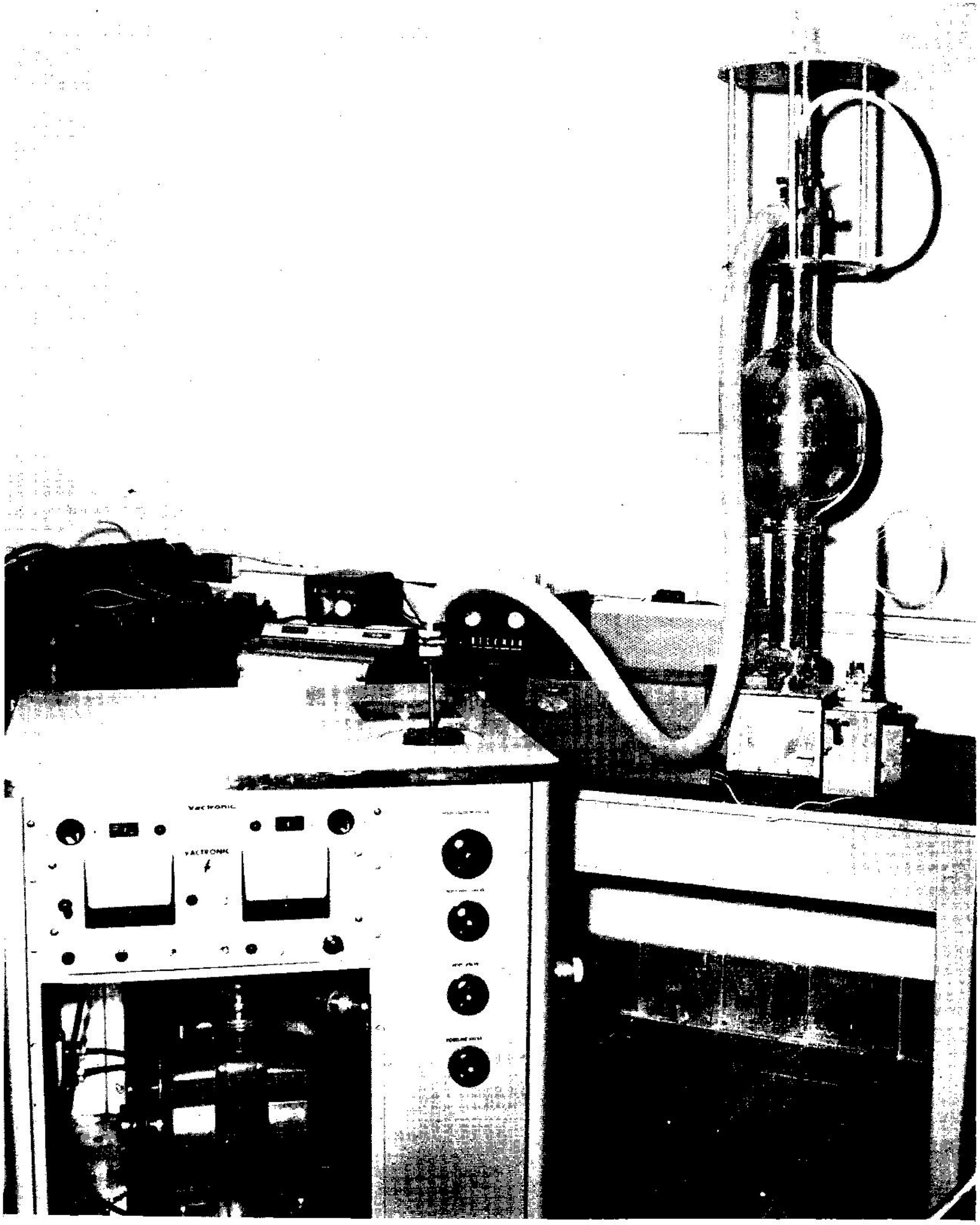

FIGURE 8. System for low temperature crystal x-irradiation and F-center measurement. The cryostat here is suspended from a counterbalanced support, which permits moving it from the x-radiation system to the optical measurement system shown in the background. The vacuum system is in the foreground on the left.

VII. APPENDIX

Resonance Radiation Effects of Low Energy Monochromatic X-Rays on the Metalloenzyme Catalase[1]

Henry J. Gomberg and Robert A. Luse

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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-60 gammas, 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 atoms 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.)

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[1] Abstract of paper presented at the Radiation Research Society Meeting, May, 1963.

F-Center Production with Monochromatic X-Rays[1]

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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 .5 mm. were irradiated at room temperature. Fluorescent x-rays from three different targets were used to produce K-lines at 7.477 ev (Ni), 11.221 ev (Se), and 23.172 ev (Cd). A microcalorimeter was used as primary standard to measure 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. Smakula's formula was used to determine F-center 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-center production and energy was reached. At higher doses, where the creation of new vacancies by the incident photons arises, the number of F-centers produced is independent of the photon energy in the energy range under consideration. This supports Varley's mechanism (Phys. Chem. Solids, <u>23</u>, 985 (1962)), explaining chlorine ion displacement by means of an Auger process of multiple ionization. Calculations confirm this hypothesis for x-ray energies well above the K-absorption edge of chlorine.

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[1] Abstract of paper presented at Conference of Nuclear Spectroscopy and Solid State Physics, February, 1964.

VIII. LITERATURE REFERENCES

1. Gomberg, H. J., R. A. Luse, and F. Vázquez Martínez, "Resonance in Radiation Effects". Technical Report No. 1, PRNC Publication 12, 1963.

2. Vallee, B. L., J. A. Rupley, T. L. Coombs, and H. Neurath, "The Role of Zinc in Carboxypeptidase", J. Biol. Chem. <u>235</u>, 64 (1960).

3. Vallee, B. L., T. L. Coombs, and F. L. Hoch, "The Active Site of Bovine Pancreatic Carboxypeptidase A", J. Biol. Chem. <u>235</u>, PC 45 (1960).

4. Anson, M. L., "Carboxypeptidase I. The Preparation of Crystalline Carboxypeptidase", J. Gen. Physiol. <u>20</u>, 663 (1937).

5. Putnam, F. W., and H. Neurath, "Chemical and Enzymatic Properties of Crystalline Carboxypeptidase", J. Biol. Chem. <u>166</u>, 603 (1946).

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(11-1)-684.)

7. Neurath, H., E. Elkins, and S. Kaufman, "The Antipodal Specificity and Inhibition of Crystalline Carboxypeptidase", J. Biol. Chem. <u>170</u>, 221 (1947).

8. Moore, S., and W. H. Stein, "Photometric Ninhydrin Method for Use in the Chromatography of Amino Acids", J. Biol. Chem. <u>176</u>, 367 (1948).

9. Scharf, K., and R. M. Lee, "Investigation of the Spectrophotometric Method of Measuring the Ferric Ion Yield in the Ferrous Sulfate Dosimeter", Radiation Research <u>16</u>, 115 (1962).

10. Varley, J. H. O., "Discussion of Some Mechanisms of F-Centre Formation in Alkali Halides", J. Phys. Chem. Solids <u>23</u>, 985 (1962).

11. Dexter, D. L., Phys. Rev. <u>101</u>, 48 (1956).

12. Fine, F., and C. F. Hendee, "X-Ray Critical-Absorption and the Emission Energies in Kev", Nucleonics, <u>13</u>, 36 (1955).

13. Paraskevoudakis, P., "Progress Reports", The University of Michigan, School of Public Health, 1962-1963.

14. Sommerfeld, A., and H. Bethe, Handbuch der Physik, <u>24</u>, Part 2, 441, Springer, Berlin (1933).

15. Nordheim, L. W., Proc. Roy. Soc. <u>A 121</u>, 626 (1928).

16. Dyke, W. P., "Advances in Field Emission", Scientific American, <u>210</u>, No. 1, 108, (1964). Also, Dyke, W. P., and W. W. Dolan, "Field Emission", Advances in Electronics and Electron Physics, <u>8</u>, 89-185, ed. by L. Marton, Academic Press (1956).

LIST OF FIGURES

42

LIST OF TABLES