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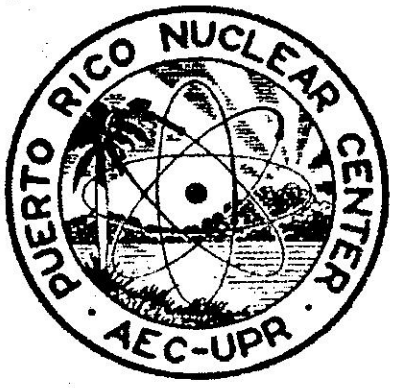
PUERTO RICO NUCLEAR CENTER

RESONANCE IN RADIATION EFFECTS

TECHNICAL REPORT NO. 3



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RESONANCE IN RADIATION EFFECTS

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I. INTRODUCTION

The Resonance in Radiation project has been devoted to evaluating the hypothesis that radiation damage in a molecule can be a function of the site at which the photon is initially absorbed. The hypothesis is based on the observation by Snell and Pleasanton (1) that xenon is left in a highly ionized state after internal conversion. From this observation it is postulated that 1) absorption of an X-ray photon in the K shell of an atom will produce a highly ionized atom and 2) the high state of ionization will lead to major disruption of the molecule at the site of photon absorption.

Using monochromatic X rays, molecules were irradiated at energies above and below the K absorption edge of selected target atoms. Damage was evaluated on the basis of effect observed per unit energy absorbed, or per photon absorbed, in the molecular system. We have shown that in enzymes and in labelled chromosomes, the efficiency of damage production is a sensitive function of the energy. These results are reported in Sections II and IV of this report. The limited data now available appears to confirm the original hypothesis.

In addition to our own results, substantial encouragement has been derived from the work of Carlson and White at ORNL (2) in which they demonstrated the complete disruption of the molecule CHI_3 by removal of inner shell electrons, particularly from the iodine atom.

Problems of technique in measuring the incident radiation and in producing high intensities of monochromatic radiation have also been worked on. Absolute measuremente of photon fluxes in the energy range we commonly use (5-15 kev) is difficult with available equipment. However, progress has been made on a total absorption calorimeter which will make absolute measurements possible. This is discussed in Section V.

To help meet the problem of low radiation intensity as discussed in Section II, higher intensity sources are being developed using field emission techniques. The results to date are good and are reported in Section VI.

In the search for appropriate systems to test the resonance hypothesis, two characteristics are desirable: 1) sensitivity to radiation so as to keep the required dose within the range of our equipment and 2) unambiguous evidence of radiation damage. The single celled bacterium Escherichia coli is being studied as a test system for evaluating the energy dependence of radiation damage. Preliminary studies on growth techniques and on radiation effects using cobalt 60 are reported in Section III. Monochromatic radiation studies are now beginning.

While the work being carried on under this program is devoted to effects on biological systems and to supporting techniques, the resonance effect should be observable also in physical systems. The radiation equipment we use in Mayaguez is the property of the Physics Department of the University of Puerto Rico. Research on effects due to monochromatic radiation in alkali halide crystals being carried on in this laboratory is reported briefly in an appendix. The research was carried out as part of a doctoral thesis by an ORINS Fellow, and, while not part of the Resonance in Radiation project, it is included here for information.

II. RESONANCE RADIATION EFFECTS IN CARBOXYPEPTIDASE A

R. A. Luse

In the previous Technical Report of this project (3), it was pointed out that the metalloenzyme carboxypeptidase A is an especially attractive metal-bearing biological system for test of the resonance in radiation phenomenon. This is because the single atom of zinc present in the native enzyme may be replaced by other metals, such as cobalt, nickel, mercury, tin, or molybdenum, to result in related enzymes showing similar (though in some cases different) enzymatic activities. Irradiation of carboxypeptidases containing different metals with low energy monochromatic X rays should answer the basic questions: 1) Is there a resonance in radiation effect in this biological system? and 2) If so, is it specifically related to the metal and independent of the remainder of the molecule? According to the resonance concept, irradiation of a zinc carboxypeptidase and of a cobalt carboxypeptidase with equal doses of X-radiation at wavelengths corresponding to the K-absorption edges of zinc and of cobalt, respectively, would produce greater inactivation of the zinc enzyme at the zinc K-edge than at the cobalt K-edge, and conversely for the cobalt enzyme. It has been difficult to accrue quantitative experimental data. For this reason several experimental approaches have been studied. This report contains a discussion of the irradiation of the zinc enzyme as dilute solution and as dry film. Preliminary spectra of electron spin resonance obtained during irradiation of dry enzyme with high energy X rays are reported.

A. Irradiation of carboxypeptidase A as solution

Within this report period, irradiation of the enzyme in solution was carried out to investigate two factors:

1) enzyme concentration and 2) zinc ion concentration in the medium.

Basic to the consideration of the first factor was the observation that the inactivation per unit absorbed dose, i.e., the yield, for some enzymes rises with increasing enzyme concentration in solution (4). In such cases plots may be drawn of the reciprocal of total yield versus reciprocal of solute concentration, to result in straight lines. This indicates that either inactivation is produced predominantly by one radical species, or else the probabilities for the inactivating radicals are all the same (5). Investigation of this factor should demonstrate the importance of radical effects, if any, in the inactivation of carboxypeptidase with low energy X-radiation.

The second factor, that of zinc ion concentration in the medium during irradiation of the zinc enzyme, was studied to determine whether or not the presence of additional target zinc atoms might enhance carboxypeptidase inactivation by X rays of wavelength corresponding to the K-edge of zinc. Such an experiment is designed to explore for our system the effects of the series of events which follow the absorption by a zinc atom of such an X-ray photon. These are known to include ejection of a K-shell electron, orbital electron rearrangement ("cascade"), and the production of numerous singly or multiply charged species (1). Indeed, experiments done by Carlson and White (2) on methyl iodide at low pressure have indicated that coulombic repulsion of the charged species produced by such electron vacancy cascades results in an "explosion" of this simple molecule into H^+, C^{n+} (primarily, n = 2), and I^{n+} (most frequently, n = 5). The average recoil energies involved (10 to 40 ev) are more than enough to break chemical bonds. Essentially, the aim of increasing the concentration of zinc ions in the vicinity of the enzyme and of its active site was to see if some estimate could be

obtained of distances through which such repulsion forces act.

The experimental methods of irradiation were as described previously (3). The XRD-6 spectrometer was operated at 78 ma current, 19 kvp, using a chromium target X-ray tube, lithium fluoride diffraction crystal, and a single HR soller slit placed between the crystal and the sample. Enzyme solutions were placed in the variable thickness irradiation cell also previously described, using polyethylene windows. A solution thickness of 6 mm was used, so that X-ray absorption of 8-10 kev photons was nearly total. Solutions were maintained in tightly covered compartments at 3-5°C to minimize activity losses and evaporation. After irradiation, the residual enzyme activity of the irradiated solution was assayed and compared with that of the in situ controls (replicate aliquots of solution kept in identical compartments in the irradiation cell but not irradiated) and with that of a control kept in the refrigerator. In early work reported here, the assay was that reported previously, i. e., measuring the rate of hydrolysis of carbobenzoxy-glycyl-L-phenylalanine by ninhydrin analysis of the phenylalanine produced (6). Difficulties with substrate solubility in this assay led to the adoption of another, much simpler assay, namely, measurement of the rate of hydrolysis of hippuryl-L-phenylalanine by increase in absorbency at 254 mu, the point of peak absorption of the hippuric acid formed stoichiometrically (7). Results of both types of assay are comparable, although the numerical expression of the carboxypeptidase activity is different.

Experimental results are given in Table 1. Briefly summarized, enzyme concentration varied over 30-fold, from 3.5×10^{-7}M to 1.2×10^{-5}M. (experiments a, b, c, and d). Unfortunately, it is not possible to draw significant conclusions as to relative rates of inactivation, since little

Table 1. Inactivation of Carboxypeptidase A in Solution by Monochromatic X-radiation

Irrad. date	Energy/hr, as kev	Irrad. period in hrs	Enzyme conc. as mg/ml	Irrad. medium	Enzyme activity, as proteolytic Coefficient[1] refrigerator control	In situ control 1	In situ control 2	Irradiated sample	Percent remaining activity
a. Apr. 15,'64	9.69	91.0	0.0120	buffer	10.0 (100%)	7.8 (78%)	8.4 (84%)	8.0 (80%)	ca. 100
b. Feb. 23,'65	9.69	66.5	0.0871	buffer	7.2 (100%)	5.8 (80%)	6.7 (93%)	5.0 (70%)	ca. 82
					Specific enzyme activity, as units/mg[2]				
c. Apr. 8,'65	9.69	85.0	0.402	buffer	47 (100%)	10 (21%)	14 (30%)	12 (26%)	ca. 100
d. Apr. 30,'65	9.69	69.0	0.396	buffer	41, 46[3] (100%)	32, 36 (78%)	36, 37 (84%)	32, 39 (82%)	100
e. May 17,'65	8.69	91.0	0.358	buffer	39, 41 (100%)	31, 32 (79%)	29, 33 (78%)	25, 25 (63%)	80
f. May 29,'65	9.69	69.0	0.401	buffer + M/100 $ZnCl_2$	40, 40 (100%)[4]	34, 39 (91%)	33, 36 (86%)	34, 38 (90%)	100

[1] Coefficient = $\frac{\text{mean reaction constant k}}{\text{mg of enzyme nitrogen per ml}}$

[2] Unit/mg = 1 micromole of substrate hydrolyzed per minute at 25°C and pH 7.5, per mg. enzyme per ml reaction mixture.

[3] 17% loss of initial activity during 69 hr period

[4] 2% loss of initial activity during 69 hr period

if any inactivation occurred in these experiments. (The basic problem, that of low beam intensity, is discussed below.) Likewise, in experiment f, where 0.01 M zinc ion was present in the solution of carboxypeptidase during irradiation, no inactivation over controls was observed, so results cannot be compared with those of experiment d, where zinc-free buffer was used.

The failure in these experiments to obtain significant enzyme inactivation, even over 90 hour irradiation periods, is due to the very low intensity of the monochromatic X-ray beam. Measurements made using the SPG-4 scintillation detector indicated that the emergent beam intensity was only approximately 7×10^9 photons per hour in the above experiments. This incident intensity corresponds to one roentgen per hour, clearly inadequate for present experiments. Meaningful dosimetry by the Fricke ferrous sulfate solution proved impossible as production of ferric ions by air oxidation is far greater than production of ferric ions by the ionizing radiation. Dosimetry was done by using the lithium fluoride thermoluminescent powders described by Cameron et al. (8). Here the irradiation compartment was filled with the LiF powder so as to match the dimensions of the incident beam (6 mm x 9 mm). After irradiation with 9.69 kev X rays for 19 hr, the powder was mixed and subdivided into 58 mg portions (standard capsule contents). The light emitted by each portion during heating was measured using the Con-Rad equipment. Rough corrections for effects in the 10 kev region were made by extrapolating curves for higher energy X and gamma-radiation. While subject to various refinements, the results,3.5 r/hr, or 2×10^{10} photons per hour, are in rather good agreement with actual photon count.

As the low intensity has severely limited the results of these experiments, current efforts are directed to increasing beam intensity.

Various techniques are available: replacement of the chromium target X-ray tube with a tungsten-target tube should increase intensity by three-fold, and use of a bent diffraction crystal should raise this by a similar factor.

B. Preparation of dry carboxypeptidase films

Inactivation of carboxypeptidase A by low-energy X rays should be enhanced by irradiation of the enzyme in dry form, since in such a case absorption of incident X radiation by the solvent water is eliminated and more photon energy is absorbed by the enzyme itself. This may overcome the difficulty of achieving significant inactivation of the enzyme with low intensity monochromatic X-ray beams.

The purpose of the present experiments was to find those conditions under which the enzyme could be dried to a very low moisture content, maintained dry for a period of hours, and then redissolved--all with minimal loss of its initial activity. The general experimental procedure was to apply a 100 microliter portion of carboxypeptidase A solution (ca. 10^{-6}M enzyme in 0.067 M sodium phosphate buffer, pH 7.5) onto a solid support. Such supports were 6 mm by 9 mm in size, to match the dimensions of the monochromatic X-ray beam emitted by the X-ray spectrometer. The solvent was then removed under low vacuum (about 0.3 mm of mercury) over calcium chloride dessicant. The resulting dry enzyme preparations were stored in vacuo in the cold for varying periods of time. Activity which remained after such drying and storage was assayed after dissolving the enzyme in a few milliliters of 10% lithium chloride solution and then diluting with the phosphate buffer. Assay was by measurement of the hydrolysis rate of substrate carbobenzoxy-glycyl-L-phenylalanine (6). Controls were 100 microliter portions of the same enzyme solution from which portions were taken for drying, stored as solution at 5°C and assayed at the same time as the redissolved dried sample.

The types of supports and the drying conditions tested are summarized in Table 2. The term "percent remaining activity" is determined by comparison of the activity displayed by the dried enzyme with that of the non-dried controls. The data indicate that a variety of plastic materials may be used. Coating plastics such as Mylar with hydrophobic films seem to decrease the loss of enzyme activity during drying, presumably by insuring desorption of the enzyme at the re-solution step. For this reason, Teflon chips were adopted as the most suitable non-wetted supports. Other factors which minimized loss of enzyme activity are a) maintenance of 5-8°C temperature and b) use of a relatively low vacuum. Freezing of solution and high vacuum (at least initially) are to be avoided, as mechanical loss of light dry material results.

Based on these experiments, a procedure has been found for producing dry enzyme films with minimal loss of initial activity. Such films may be irradiated with monochromatic X rays at a series of wavelengths to bracket the K-absorption edge of the zinc present in carboxypeptidase A. In this way it will be possible to prove or disprove the resonance effect for this enzyme. Similar techniques are applicable to the other enzymes under consideration in this research. A more general radiobiological aim may be served by comparing the radiation doses required for equal enzyme inactivation in solutions and in dry systems, to show the relative importance of free radicals produced by such low energy X-radiation in water to the inactivation of the enzyme.

C. Preparation of Cobalt-Carboxypeptidase

During this report period, a small quantity of pro-carboxypeptidase A was prepared from beef pancreas by the methods of Neurath and co-workers (9,10). This material has been converted to enzymatically active delta-carboxypeptidase, the only enzymatic species in which the constituent zinc atom may be replaced

Table 2. Preparation of dry carboxypeptidase A films

Support	Drying conditions	Percent remaining activity*
Mylar film	8°C, 14 hrs	28
Polyethylene film	8°C, 20 hrs	25
Mylar film	8°C, 30 hrs	ca. 20
Mylar film	8°C, 63 hrs	24
Mylar film	25°C, 25 hrs	11
Mylar film	-5°C, 4 hrs (high vacuum)	ca. 5
Mylar film	2°C, 2 hrs, then 8°C for 42 hrs	40
Mylar film with Desicoat	2°C, 2 hrs, then 8°C for 42 hrs	55
Mylar film with silicone grease	2°C, 2 hrs, then 8°C for 42 hrs	50
Teflon surface	2°C, 2 hrs, then 8°C for 42 hrs	65
Teflon chip	8°C, 66 hrs	85

*Equal to $\frac{\text{Activity of 100 µl solution dried t hrs, redissolved and assayed}}{\text{Activity of 100 µl solution stored t hrs, and then assayed}}$

by other metals. Conversion of this zinc enzyme to the cobalt-enzyme by simple dialysis procedures (11) awaits the availability of more intense monochromatic X-ray beams.

III. IRRADIATION OF E. COLI WITH MONOCHROMATIC X RAYS

R. A. Luse

A biological system undergoing rapid cell division should prove to have higher radiosensitivity, since the molecular replication associated with doubling the number of cells results in biological amplification of radiation damage. Setlow and Pollard (12) have emphasized this point, pointing out that damage to a few hundred nucleic acid molecules results in loss of thousands of such molecules after a few cell divisions. Similarly, protein molecules may be inactivated by radiation, so that interrelated enzymatic reactions may fail, frequently with increasing effect. Such amplification of small radiation effects over several cell divisions may be an important adjunct for our present low-intensity monochromatic X-ray beams. The biological system chosen for these studies was the single celled bacterium Escherichia coli, as this organism is simple to maintain under our experimental conditions. Irradiation was done at 9.69 kev, since this wavelength, the K-absorption edge of zinc, was considered to have maximum effect on zinc-containing enzymes, including alkaline phosphatase. Related studies are planned with this enzyme, which is isolated from E. coli and available in pure form from commercial sources. The biological character measured to assess radiation effect was the ability of the cell to undergo sustained cell division, i.e., to form visible colonies on nutrient medium.

In this experiment, a culture of E. coli was grown for 16 hr at 24°C in nutrient broth. An inoculating loopful of this culture was then transferred to 50 ml of minimal nutrient solution (inorganic salts plus glucose), where growth was taken to early log phase by a 7 hr incubation period at 24°C, with shaking. Careful adherence to this routine produced E. coli cell suspension having reproducible concentrations of cells undergoing rapid division. Portions of the stock suspension were designated as two

in situ controls. They were placed in the irradiation chamber compartments along with the irradiated sample. The compartments had been sterilized prior to this step and were covered during the irradiation period; no contamination of the cultures was noted after 22-41 hr in these compartments. Controls were the same cell culture incubated in a flask at the same temperature for the same period, and assayed at the same time as the irradiated sample and the in situ controls. Measurement of cell population at the end of the irradiation period was done by serial dilution of 0.10 ml portions with sterile saline and pour plating with nutrient agar. Visible colonies were counted after plate incubation at 37°C for two to three days.

Results of the experiments carried out to date are summarized in Table 3. In all cases, growth of in situ controls was equal to the controls grown in flasks. Unfortunately, because of the very low radiation doses (20-40 roentgens) no significant radiation effect on cell growth was noted. However, it is important to note that the system has proven trouble-free and reproducible. By use of low temperatures (as 12°C) division time may be lengthened, so that long periods of irradiation are feasible. It is likely that with more intense X-ray beams, the radiation damage caused during the production of 20 to 70 millions of cells will become evident.

It may be of some value to postulate the minimum dose of monochromatic X rays required to produce significant effects in E. coli. Lohmann et al. (13) have reported that the D_{37} for catalase in dilute solution is 5000 r of cobalt-60 gamma radiation. Under very similar conditions of irradiation (same enzyme concentration, pH, buffer, aeration), the D_{37} with 7.1 kev X rays (iron K-edge energy) is approximately 500 r, i. e., ten-fold more effective for inactivation of this enzyme. If a similar efficiency holds

Table 3. Multiplication of E. coli cells during irradiation period.

Trial:	A	B	C
Irradiation period, hrs	22	23	41
Culture temperature, °C	24	24	12.5
Cell number initially, per 0.10 ml	9×10^3	1×10^4	1×10^4
Cell number terminally, per 0.10 ml	$2\text{-}3 \times 10^7$	7×10^7	$6\text{-}7 \times 10^7$
Multiplication factor	3×10^3	7×10^3	7×10^3
Number of cell divisions	12	13	13
Time of cell division, min.	110	100	190

for E. coli, approximately 100 r of monochromatic X radiation will produce roughly the same effect as 1000 r of gamma radiation-namely, 20% cell death.

IV. RESONANCE RADIATION EFFECTS IN CHROMOSOMES INCORPORATED WITH 5-BROMODEOXYURIDINE

F. K. S. Koo

The effect of electromagnetic radiation on matter is often a function of the energy (or wavelength) of the incident radiation. One of the best known examples is the induction of mutations and chromosome aberrations by ultraviolet light. Light of about 2600 A wavelength is most effective in producing genetic changes since this wavelength corresponds to peak light absorption for nucleic acid (14). However, the relative efficiency of the UV quantum at 2600 A in producing genetic changes, based on mutations or chromosome aberrations produced per unit energy absorbed, does not appear to change with wavelength in the 2000-3000 A region. The change in effectiveness would thus appear to be due to the increased absorption of the 2600 A photons rather than the greater efficiency of the absorbed photon or energy.

Higher energy radiation such as X rays also may be characteristically absorbed as determined by the constituent atoms of the system being irradiated. The interaction between photons and electrons by the photoelectric process is strongest with the most strongly bound electrons, i.e., the K-shell electrons. Furthermore, for a given shell the interaction reaches the peak at the photon energies just above the ionization potential for the shell, and it falls off rapidly with increasing energies. We are particularly interested in the dependence of observable radiation effects in genetic systems on the energy of the incident radiation. Any change with energy may be due either to change in absorption, such as occurs with UV light, or to a change in efficiency (effect per unit energy absorbed) as a function of energy.

The chemical composition of DNA is known but that of nucleoprotein aggregates and chromosomes is not completely understood. Nevertheless the chromosomes are believed to contain elements with low atomic numbers. To facilitate this investigation, heavier elements such as bromine and iodine in form of halogenated thymidine analogues may be introduced into DNA and chromosomes. These heavier target atoms can be conveniently irradiated with commercially available equipment at the K-absorption edge energies of these elements.

The target atom chosen for this study is bromine, which can be incorporated into chromosomes through the use of 5-bromodeoxyuridine (5-bromouracil deoxyriboside or BUDR). The chromosomes thus treated contain 5-bromouracil which replaces the base thymine in the DNA. The 5-bromouracil differs from thymine by having bromine in the place of the methyl group. Although the actual incorporation study was not performed in our experiment, it is believed there was BUDR-incorporation in the onion root chromosomes in view of the success of other incorporation experiments. It has been shown that the halogenated deoxyuridines including BUDR can be incorporated into DNA during replication in microorganisms, human cells, and plant species (15). The evidence of BUDR-incorporation into the DNA of Allium cepa root cells has also been reported recently by Fučik and Kára (16).

This report summarizes the results on the resonant actions of X rays in the energy region of 12.5 - 15.5 kev on chromosomes which have been treated with 5-bromodeoxyuridine. For a more extensive report, see (17).

Materials and Method - Onion seeds were germinated on wet filter paper in petri dishes at 25°C. Roots 6-8 mm long were treated with BUDR solution at a concentration of 15 μg/ml for 15 hours. For irradiation the BUDR-treated roots and the control were washed and arranged in an

exposure area of 6 x 9 mm at the center of a Plexiglass sample holder. The exposure area and the surroundings were first padded with wet filter papers and then the treated and control roots were arranged in two separate rows (upper and lower) with tips opposite to each other. The roots were covered with wet filter paper. To prevent drying, the holder and roots were wrapped in Saran Wrap. The exposure area was aligned with the beam delivered from a General Electric X-ray Diffraction Unit XRD-5 operated at 25 kv and 25 ma. The irradiation system employed a combination of collimators and a LiF diffraction crystal to produce a beam of monochromatic X rays with a high purity of energy ($\pm$ 50 ev) (18). The samples were irradiated for three hours at a beam intensity of approximately 5.9×10^{10} photons per cm^2 per hr. Six photon energies, 12.5, 13.2, 13.48, 13.7, 14.1 and 15.5 kev., respectively, were utilized. After irradiation, roots were returned to petri dishes for recovery for 21 hours at 25°C, followed with 0.2% colchicine solution treatment for 3 hours before being fixed in Carnoy's solution. For cytological examination, the material was treated with 4% pectinase for 2 hours and the root tips, approximately 1.5 mm long, were squashed in a combination of aceto-orcein and aceto-carmine staining. Roots treated with BUDR but not irradiated were also prepared as controls of the effect of BUDR alone.

Experimental results and discussion - Chromosome aberrations observed at metaphase in the root tip cells of Allium cepa in both treated and control series include chromatid and chromosome breaks, free acentric fragments, interchanges, and others. In summarizing the data, all aberrations involving breakages are grouped together as chromosome breakages and are presented in Table 4. The occurrence of interchanges was extremely infrequent. Only

Energy per photon (kev)	2θ (LiF crystal)	BUDR-treated roots				Control roots			
		No. cells examined	Total no. breakages	No. breakages per 100 cells		No. cells examined	Total no. breakages	No. breakages per 100 cells	
				Actual	Adjusted+			Actual	Adjusted+
12.5	28.42°	135	12	8.9	8.9	64	6	9.4	9.4
13.2	26.90°	96	11	11.5	12.9	121	13	10.7	12.2
13.48	26.33°	183	51	27.9	30.9	149	15	10.1	12.2
13.7	25.88°	209	46	22.0	25.1	124	14	11.3	14.1
14.1	25.16°	191	35	18.3	22.5	174	16	9.2	12.5
15.5	22.86°	88	17	19.3	30.1	76	6	7.9	13.8

TABLE 4 - CHROMOSOME BREAKAGES PRODUCED BY MONOCHROMATIC X RAYS AT VARIOUS PHOTON ENERGIES IN 5-BROMODEOXYURIDINE-TREATED AND CONTROL ROOT TIP CELLS OF ALLIUM CEPA.

For roots treated with BUDR, but not irradiated, 5 breaks were found in 218 cells examined.

+ The number of breakages is adjusted by assuming that all the treatments absorbed the same amount of X radiation.

two were observed in some 240 aberrations scored. In four out of twelve series, the number of cells examined was less than 100. For comparison, aberrations in all series are expressed in terms of the number of breakages per 100 cells. In the BUDR-treated material (see Table 4, column 5), the number of breakages at the photon energy 12.5 kev was 8.9 per 100 cells but it increased with increasing photon energies. The amount of breakage rose sharply to a maximum of 27.9 breaks per 100 cells, showing a 3-fold increase at the K-absorption edge energy of bromine (13.48 kev), and then decreased slowly as the photon energies were raised still higher, with the exception that the aberration yield increased again at 15.5 kev. In contrast, there was no evidence of resonance radiation effect in the control material (without BUDR-treatment) which was irradiated at the same time with the same series of photon energies as the BUDR-treated material. The variation in aberration yield from treatment to treatment was relatively small (see Table 4, column 9).

In this experiment, variation of photon energy changes the spatial distribution of the energy absorbed by the chromosomes. When the impinging photon energy is lower than that of the K-absorption edge of the target atom, the atom is relatively 'transparent', and the energy is fairly well distributed over the molecule. When the impinging photon energy is at or above that of the target bromine atom, its absorption coefficient increases by a factor of about 7.5. However, the coefficients for the other atoms are essentially unchanged. Thus, there is a significant change in the spatial energy absorption pattern over chromosomes. When the target atom is present at low concentration, its change in absorption coefficient will have no significant change in the very large fraction of the energy absorbed by the rest of the chromosome. The effects due to energy absorbed by the

non-target atoms remain essentially unchanged. However, the large relative increase in the energy absorbed in the target atom will show whether or not the energy absorbed on that site is of unusual significance or efficiency.

To determine if there is a discrete photon energy of X rays capable of producing genetic damage in excess of that produced by photons with energies slightly higher and slightly lower, the mass absorption coefficient of the gross target material must be computed. For the calculation the chromosome as an entity instead of the whole cell was chosen because it was believed that chromosomes were the target of the direct action of the X rays on one hand and the effects assayed were chromosomal on the other hand. As a prerequisite, the knowledge of the organization of the chromosomes in general and their chemical composition in particular should be at hand. Unfortunately, information of this nature is far from complete and often uncertain, so the calculation at best is only a rough approximation. In the present study it was assumed that the Allium cepa chromosomes contain approximately 44% DNA and 46% histone, 8.5% residual protein and 1.5% RNA.[1] Other components such as Ca and Mg in an unknown trace quantity were not considered in the calculation. It was also assumed that the chromosomes contain water in the same amount by weight as all the macromolecules combined.[2]

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[1]Since the information on chemical composition of plant chromosomes is not available, we have used the results on calf chromosomes reported by several investigators (cf. Swanson's Cytology and Cytogenetics) as reference for our calculation. The percentages for the constituent macromolecules in the onion chromosomes have been arbitrarily set by considering these findings.

[2]No information is available on the water content of the chromosomes. Here we have considered water as the integrated part of the chromosomes. The estimate that in a chromosome water weighs the same as all macromolecules combined may be conservative.

The AT content in the DNA of Allium cepa is 64%.[3] The replacement of thymine by bromouracil during one replication of DNA in the BUDR-treated chromosomes was assumed to be about 20%. Based on the above-mentioned information, the chromosomes were estimated to contain approximately 0.18% of bromine. The mass absorption coefficients were calculated in terms of cm^2/gram which, while not representing the actual situation of the exposure area and the bulk mass of the root tips exposed, are relative and valid for the corrections made.

The mass absorption coefficients for bromine, and for the control chromosomes and those treated with BUDR are plotted as a function of photon energy in Fig. (1). For bromine the coefficient curve showed a sharp discontinuity at 13.48 kev, corresponding to the bromine K-absorption edge (see A). However, the coefficient curve for the chromosomes containing approximately 0.18% bromine (C) exhibited only a small fluctuation at the bromine K-absorption edge; less than 10% increase in photon absorption. As expected, the curve for the control chromosomes showed no fluctuation (see B). From these curves it is noted that the absorption coefficients at energy levels other than the K-absorption edge also differ considerably. Therefore, in comparing the efficiencies of different photon energies it is necessary to correct the differences in the amount of photons absorbed by the chromosomes at all energy levels. In Table 4 columns 6 and 10 are listed for the BUDR-treated and control roots, respectively. The number of breakages per 100 cells is adjusted by assuming that all the chromosomes at all energy levels absorbed the same amount of X radiation. These adjusted numbers are plotted as a function of photon energy in Fig. (2).

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[3] N. Sueoka reported GC content in the DNA of Allium cepa is 36% (J. Mol. Biol., 3, 31; 1961).

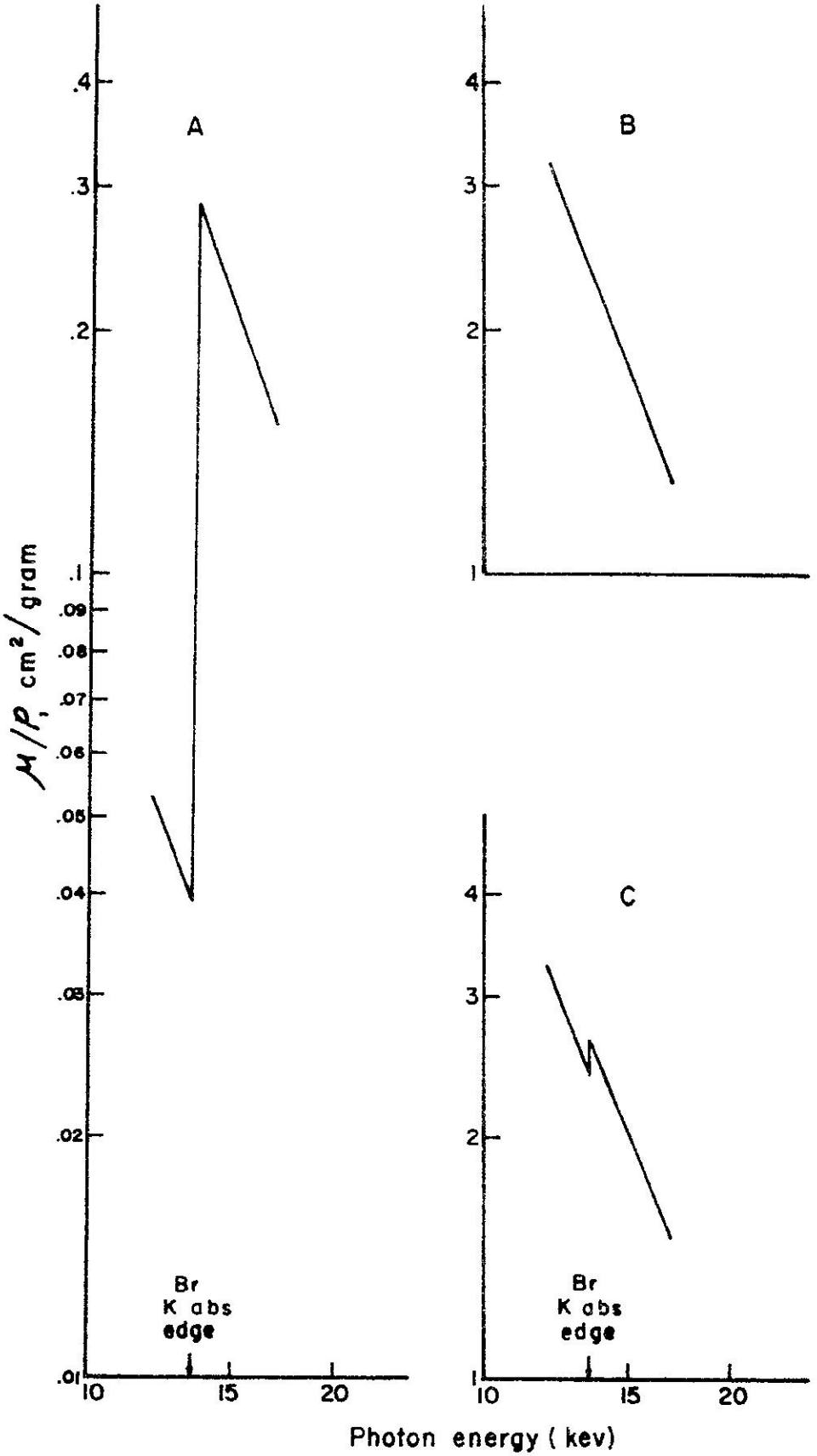

FIG. 1- MASS ABSORPTION COEFFICIENTS FOR BROMINE

A IN CHROMOSOMES

B IN CHROMOSOMES NOT TREATED WITH 5-BROMODEOXYURIDINE

C IN CHROMOSOMES TREATED WITH 5-BROMODEOXYURIDINE

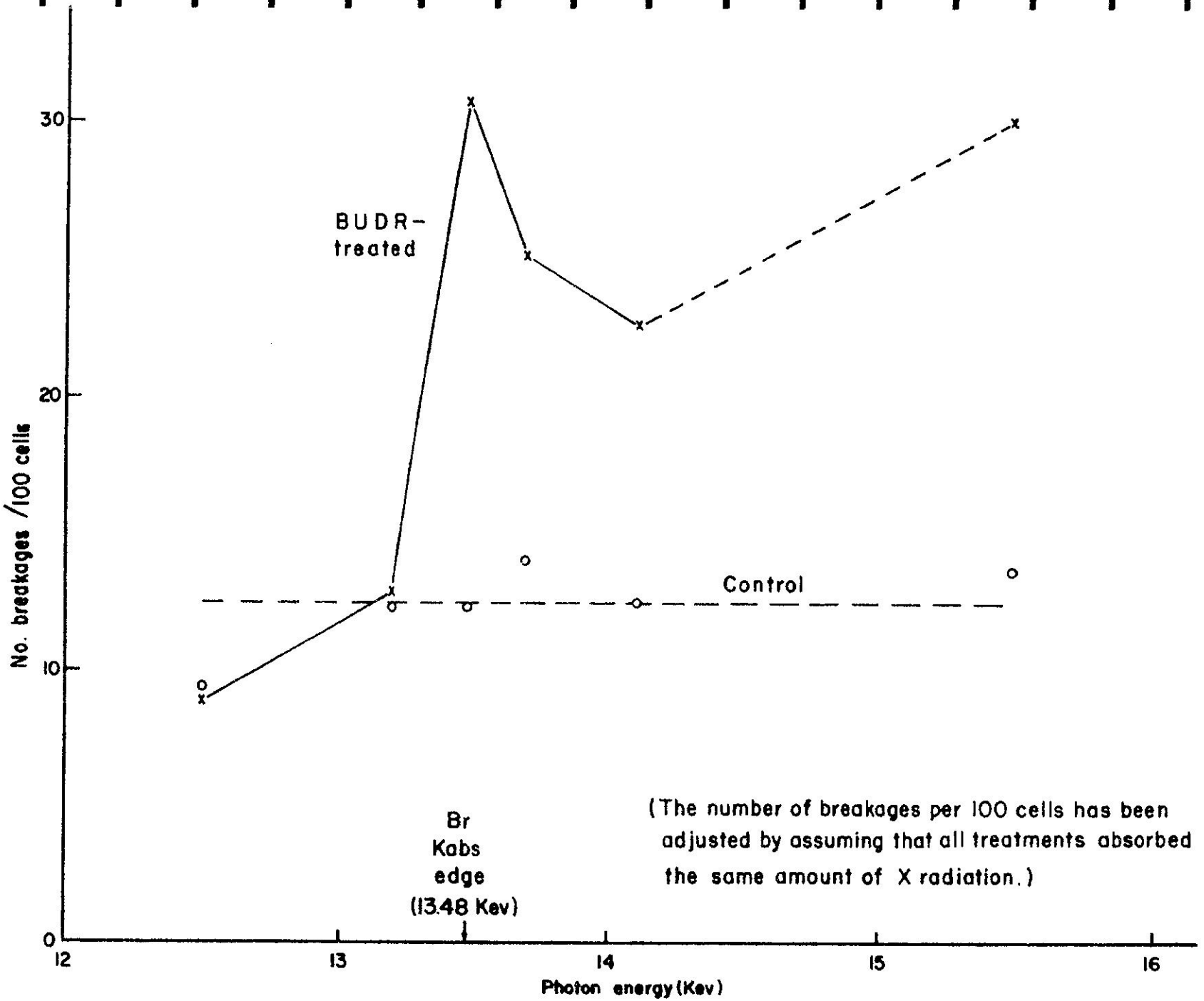

FIG. 2 - NUMBER OF CHROMOSOME BREAKAGES PRODUCED BY MONOCHROMATIC X-RAYS AT VARIOUS PHOTON ENERGIES IN 5-BROMODEOXYURIDINE-TREATED AND CONTROL ROOT TIP CELLS OF ALLIUM CEPA (17)

Evidently, the enhancement in radiation efficiency was present at the photon energies equal to or slightly greater than the K-absorption edge of bromine in the BUDR-treated material while there was no such difference at any of the photon energies in the control material. The utmost increase in efficiency at the K-absorption edge of bromine over that observed below the K-edge energies was about 2.5 to 3-fold, and the increase over that in the control series was about 2.5-fold. The unexpected increase in aberration yield at 15.5 kev in the BUDR-treated series might have resulted from an inadequate sampling. More information will be accumulated to clarify this point.

V. TOTAL ABSORPTION X-RAY CALORIMETER

P. Paraskevoudakis

The calorimeter, constructed at the University of Michigan (19), is used basically to measure the energy content of various monochromatic X-ray beams in the energy region of 5 to 25 kev. The calorimeter was designed for total absorption of monochromatic X-ray beams of photon energy less than 10 kev. A schematic diagram is shown in Fig. (3).

The absorber, or target, of the calorimeter consists of two 0.001" gold plates about 3 cm in diameter, with a heater coil of 0.001" platinum wire sandwiched between them, and two thermistors suitable for operation at liquid nitrogen temperature attached to the gold plates. See Fig. (4). The unbalance or error voltage across a Wheatstone bridge with the two thermistors in opposite arms, is measured with a microvoltmeter (Fig. 5) and recorded as a function of time. A typical plot of data recorded using the calorimeter is shown in Fig. (6).

The rate of change of this voltage, of the order of 1-2 microvolts/sec, is proportional to the rate of change of the resistance of the thermistors, which in turn is proportional to the rate of change of the temperature of the gold target. This rate of change, finally, is proportional to the rate of energy input on the gold target for small temperature changes.

The calorimeter is calibrated by passing known electrical currents, of the order of 100 microamperes, through the platinum resistance wire to provide heating power of the order of 10 microwatts to the absorber. Under these conditions the linearity of the calorimeter has been established. A plot of the rate of change of the error voltage versus electrical power input is a straight line. The power of an X-ray beam is determined from the rate of increase of the unbalance or error voltage produced when the beam is totally

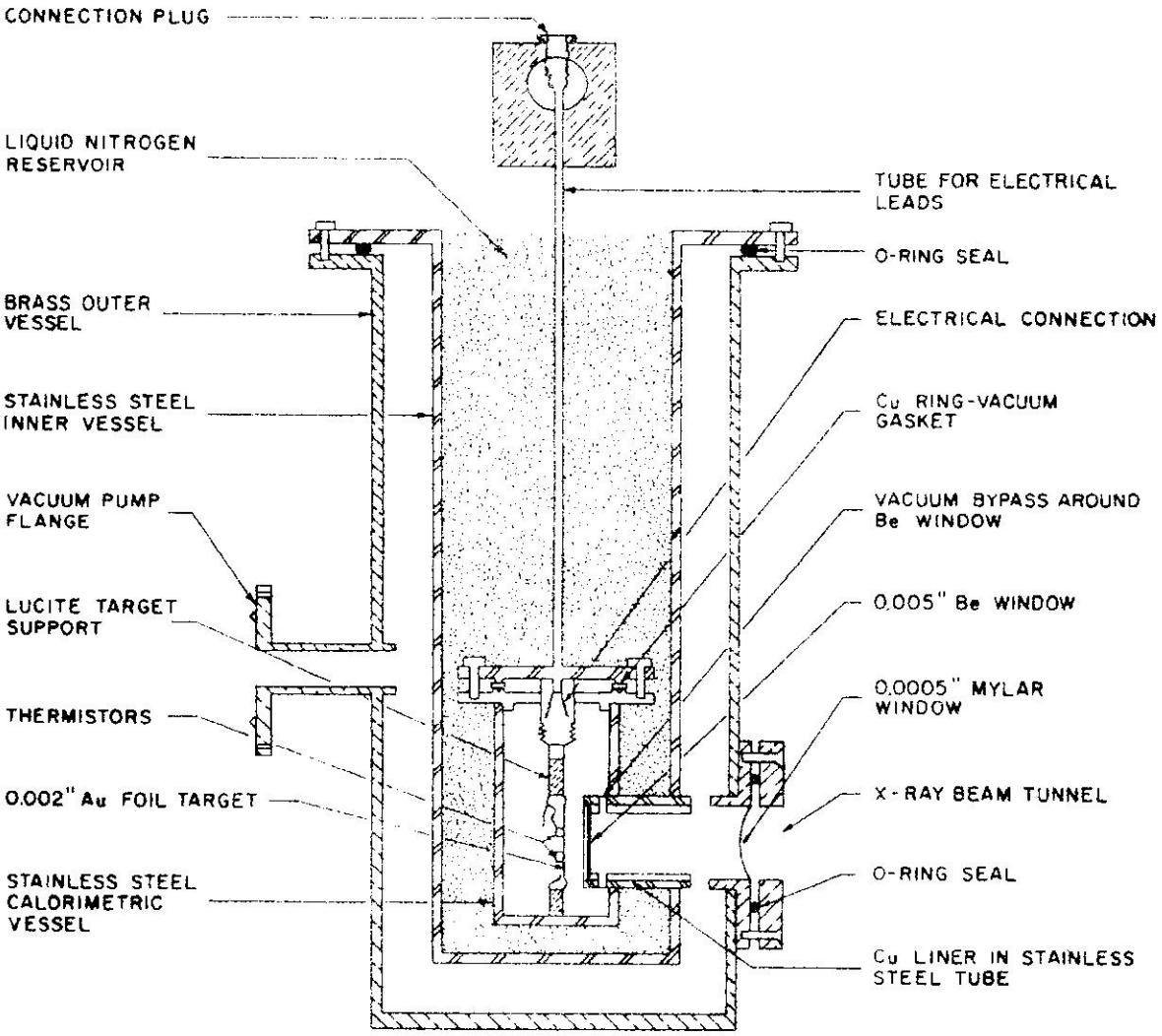

FIG. 3–
SCHEMATIC REPRESENTATION OF X-RAY CALORIMETER

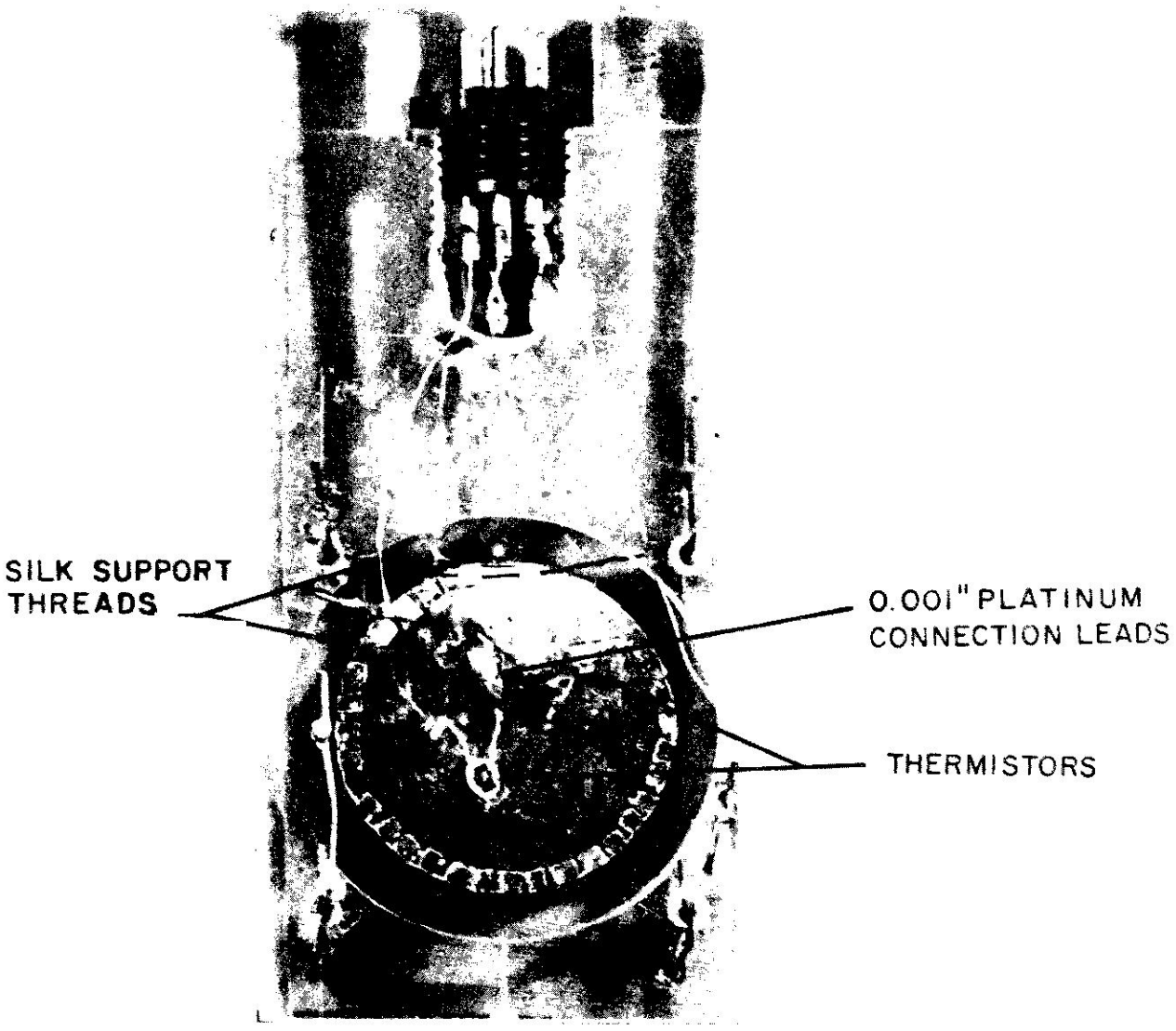

FIG. 4 - GOLD TARGET FOR CALORIMETER.

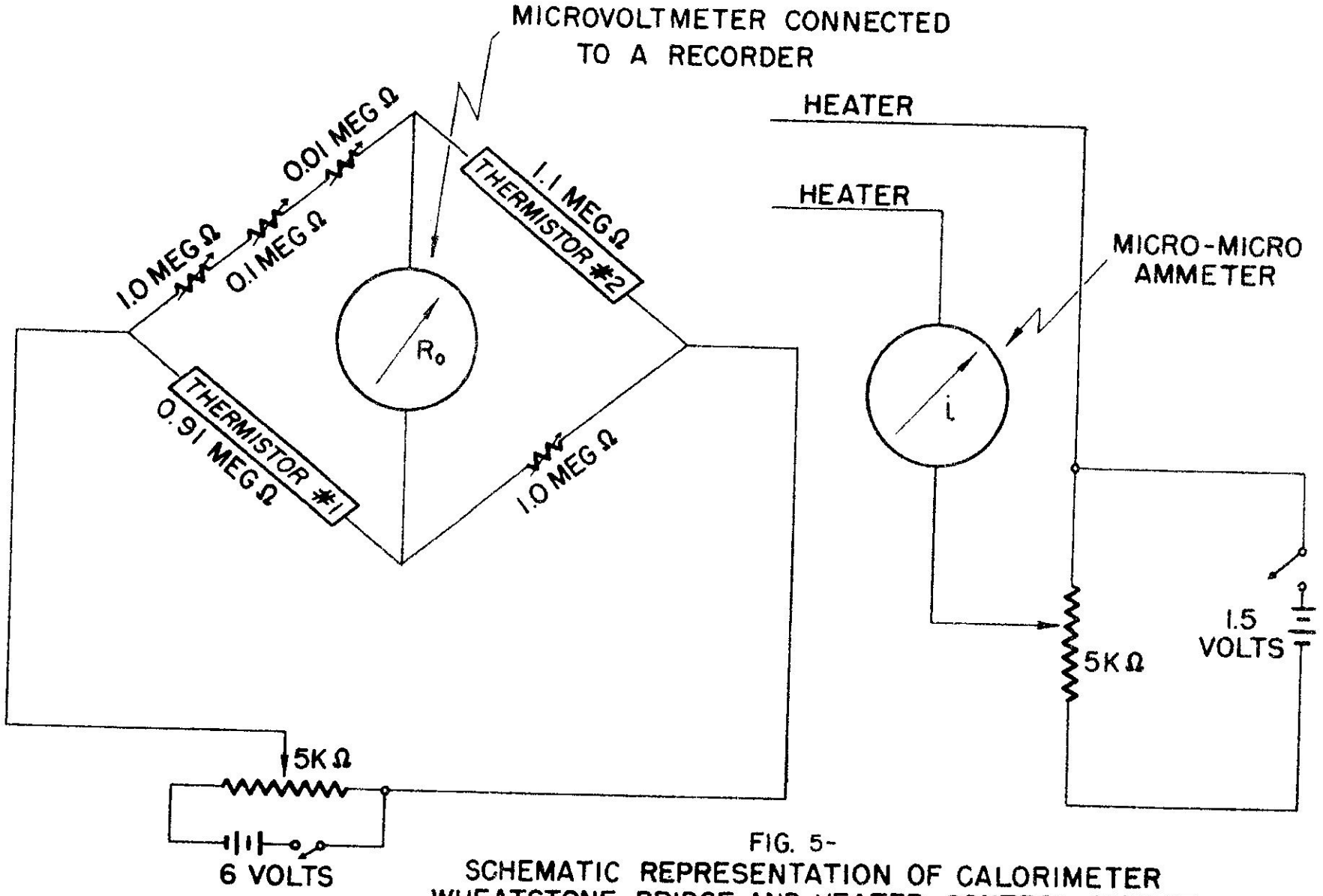

FIG. 5-
SCHEMATIC REPRESENTATION OF CALORIMETER
WHEATSTONE BRIDGE AND HEATER CONTROL CIRCUIT

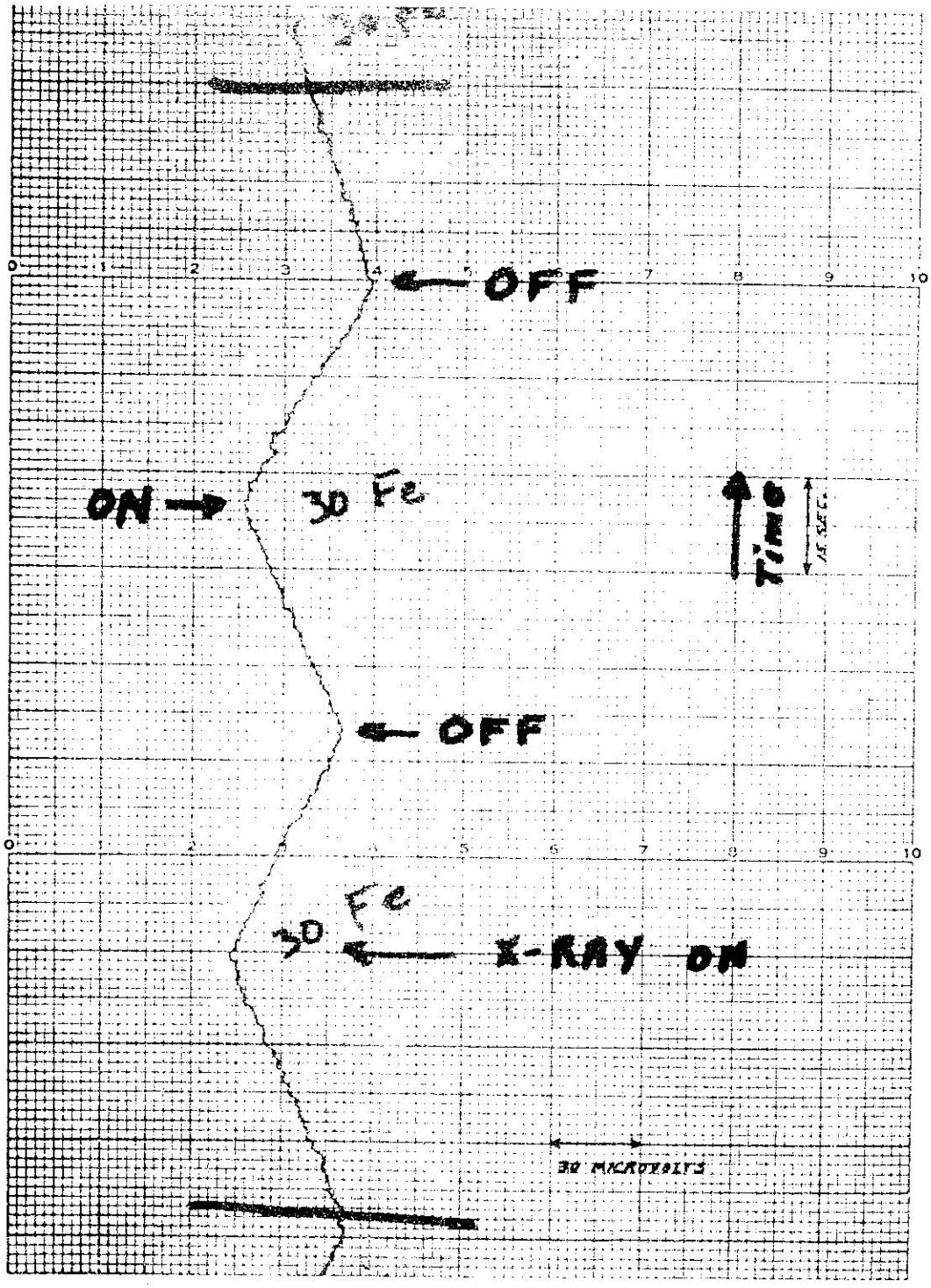

FIG. 6 - TYPICAL PLOT OF CALORIMETER DATA OUTPUT.

absorbed by the gold absorber. The calorimeter chamber is evacuated to 10^{-7} mm of Hg and the calorimeter operates at 77°K.

The calorimeter will be used to measure the energy content of various fluorescent X-ray beams from the XRD-5 X-ray unit in Mayaguez and the XRD-6 X-ray unit in Río Piedras. An attempt will be made to measure the energy content of monochromatic X-ray beams obtained by Bragg reflection. The success of these measurements will depend on the sensitivity of the detecting instrument and the level of background noise. A new microvoltmeter (Astrodata model #121 Z) will be used to measure the error voltage of the bridge. The noise level of this instrument is quite low and it is expected to be sensitive to signals produced by 0.5 microwatt power input. Furthermore, a search is going on for the smallest possible commercially available thermistors with negative temperature coefficient better than 12.5%. If these are obtainable, we can increase the present sensitivity of the instrument, possibly to the levels required for measurements of diffracted X-ray beams.

The calorimeter will serve as a primary standard to calibrate secondary standards such as the Fricke dosimeter and the silver phosphate glass dosimeter for routine measurements of absorbed dose.

The program includes the development of three M. S. theses:

1. Construction of the first gold target, calibration of the calorimeter by electric heat and measurements of X-ray beam power for $h\nu < 10$ kev. (F. Rosa)
2. Calibration of the Fricke dosimeter, i. e., measurement of the G-value as a function of photon energy. (F. Jiménez)
3. Construction of the second target (if necessary more than one target will be made and the work described in (1) above will be repeated for $h\nu > 10$ kev. (J. Aguiar)

VI. HIGH INTENSITY FIELD EMISSION X-RAY SOURCES

F. Vázquez Martínez

OBJECTIVE

The need for new X-ray sources of high output was discussed and the use of a field emission cathode for such a source was proposed in our previous report (PRNC 40). Considerable experience with this technique has now been obtained and substantial progress made.

The primary objective has been to determine the feasiblity of constructing the cathode from sharp edged metal slivers, such as razor blades, and to determine the effect of the various parameters (vacuum, cathode material, cathode-anode spacing, applied voltage) on electron emission and stability.

METHODS

Construction of X-ray Source

Substantial effort went into the initial design and construction of a high vacuum system with adequate provision for introduction of electric power and cooling water and for relative motion between the anode and the cathode. The test unit evolved is shown in the diagram in Fig. (7) and the assembled system shown in the photograph in Fig. (8).

Vacuum

Because high vacuum is needed to operate the X-ray source, considerable effort was devoted to achieving a vacuum of at least 10^{-10} mm of Hg.* Another problem of construction was welding to provide appropriate electrical and water feed-throughs.

Cathode Material

Tungsten and ordinary safety razor blades were tested as cathodes, used singly and in stacks. Tungsten was tested as an emitter material because

*Very careful chemical cleaning of components is required to achieve this vacuum. To detect leaks at 10^{-10} and 10^{-11} mm of Hg, a special leak detector was constructed which operates from the power supply of the ion pump.

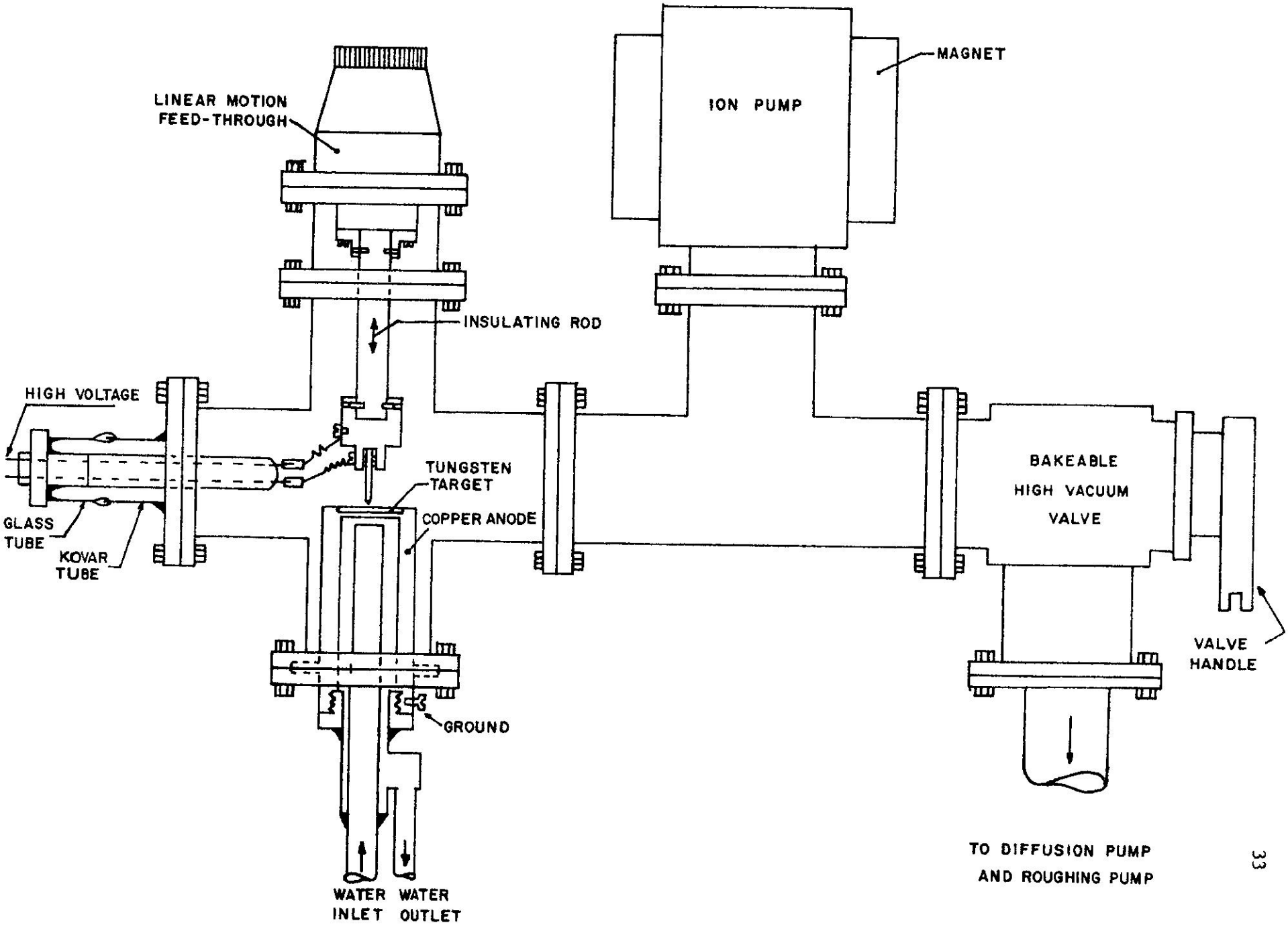

FIG. 7- EXPERIMENTAL HIGH INTENSITY FIELD EMISSION X-RAY SOURCE (schematic diagram)

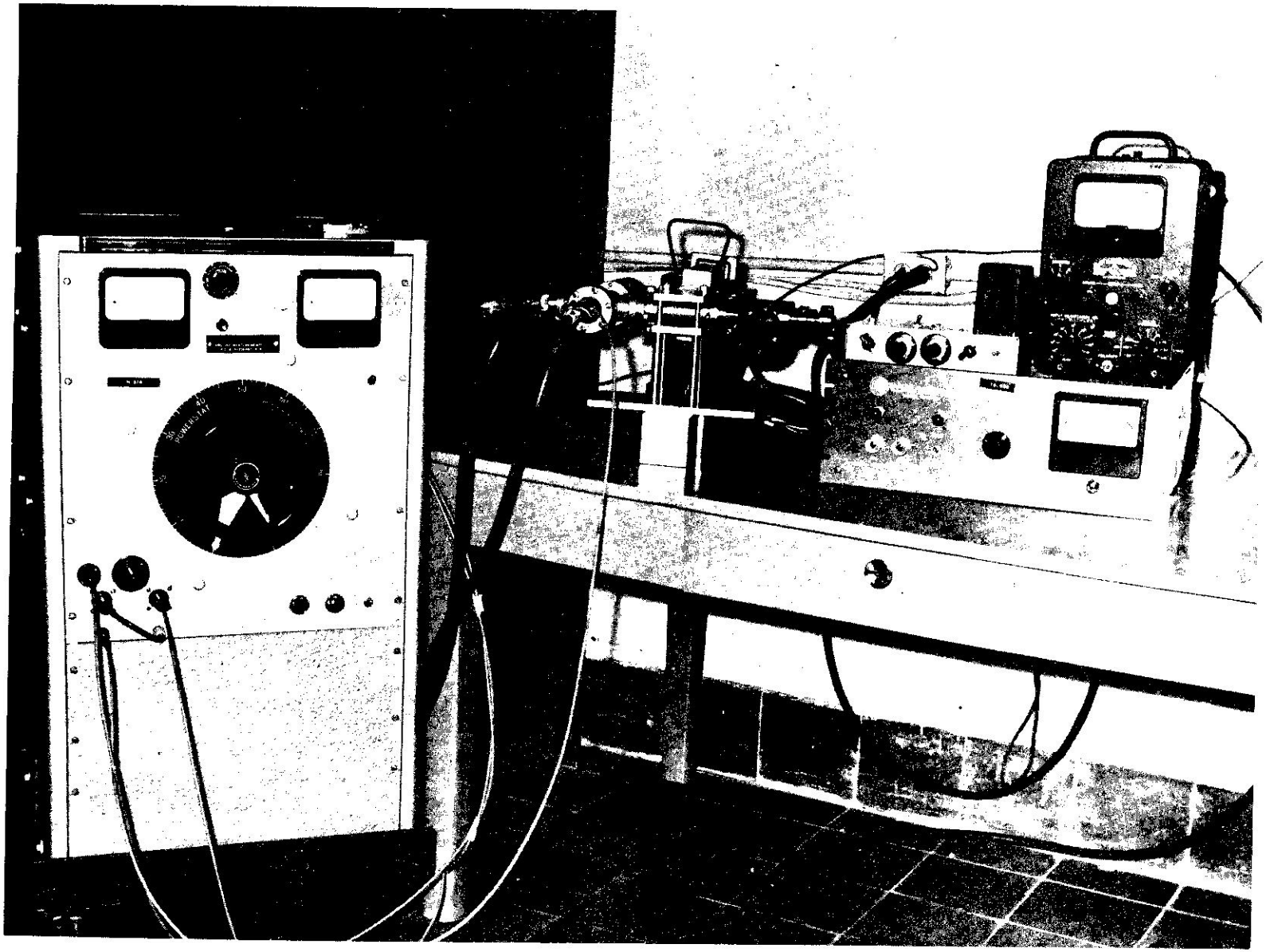

FIG. 8 - HIGH INTENSITY FIELD EMISSION X-RAY SOURCE (assembled system)

of its high melting point, great mechanical strength, and good electrical conductivity. Razor blades required no preparatory sharpening, but the high purity tungsten blades, .007" thick, needed sharpening prior to testing. The sharpening of the edge was done according to the method reported by Dyke and Dolan (20) with appropriate adjustments for this experiment. The tungsten blade was supported in front of a nickel rod (Fig. 9) and immersed at a depth of 15 mm in an electrolyte of 1 N NaOH. The blade was etched for 3 minutes at 10 v. The distance "d" between electrodes, generally about 0.5 mm, is of minor importance.

Testing Procedure

The actual vacuum, ranging from 10^{-9} to 10^{-11} mm of Hg, was achieved by using a roughing and diffusion pump before a high vacuum valve, followed by an ion pump. The fore-vacuum was about 10^{-5} mm of Hg after three hours. With this vacuum, the ion pump was started and the high vacuum valve was closed. In about fourteen hours, the vacuum increased to between 4×10^{-8} and 8×10^{-8} mm of Hg.

Bake-out was done with the ion pump continuing in operation. The outgassing rate was carefully monitored to keep the pressure at or below 10^{-5} mm of Hg. In about three hours the temperature reached 200° and was stabilized at a maximum of 200° in order to prevent damage to the magnet of the ion pump. Bake-outs were usually maintained at this temperature for 7-10 hours, during which the vacuum increased to about 10^{-7} mm of Hg. After cooling, the pressure went down to between 10^{-10} and 10^{-11} mm of Hg.

A separate bake-out was done on the target because of the outgassing necessary for this component. To provide heat, the refrigerating tubes were disconnected, a heater was placed inside the anode, and baking temperatures up to 400°C were reached. Even after bake-out, anode bombardment produced a

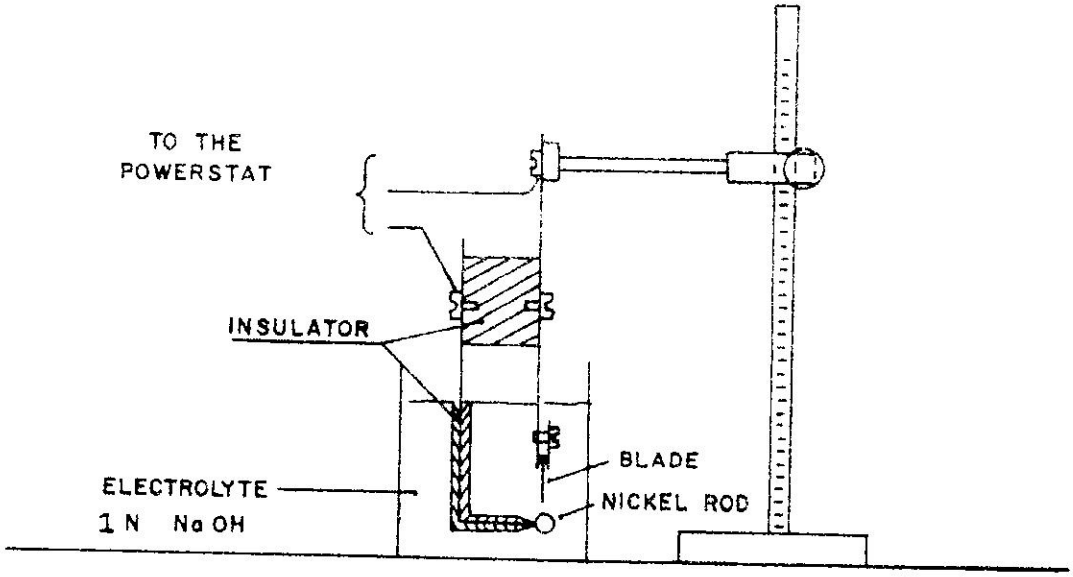

FIG. 9- SHARPENING TUNGSTEN BLADES

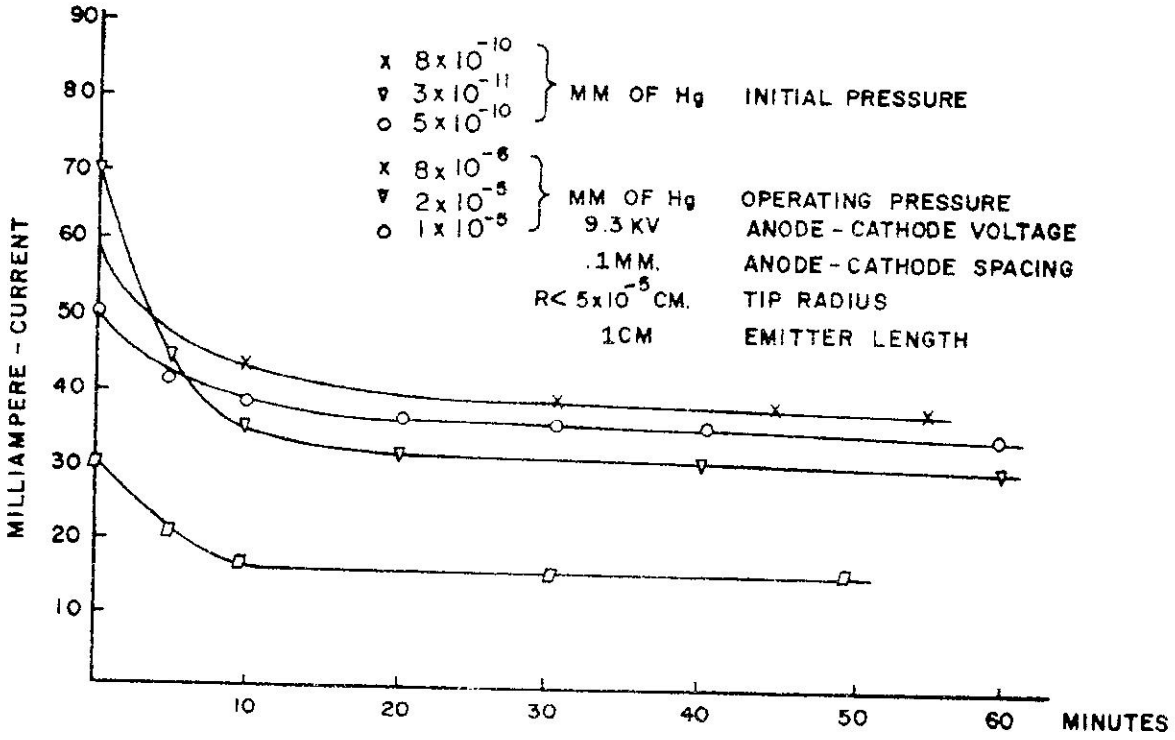

FIG. 10- EMISSION CURVES-TUNGSTEN BLADES.

pressure increase of four to five orders. Therefore, a heating filament is being added for outgassing the anode by bombardment prior to operation of the tube.

RESULTS

Cathode Material

Typical curves for tungsten as an emitter are shown in Fig. (10). With tungsten, currents of 70 ma and higher were obtained at the beginning of the emission. There was a rapid decrease in a few minutes and afterward, an almost flat curve with a little tendency to decrease was observed. This effect was seen in all cases, including those with low initial current.

Typical curves for razor blades as emitters are shown in Fig. (11). Currents as high as 35 ma can be obtained but they decay rapidly to about 3 ma. When the initial current is higher, the final current is lower. Razor blades apparently are very sensitive to ion sputtering and temperature.

For both tungsten and razor blades as emitters, the maximum current per centimeter length of emitter is limited to between 2 and 4 ma under the present conditions. The curves show average values and therefore appear to be smooth, although frequent peak-currents were observed.

Cathode-anode Spacing

The radius of the emitter tip of the tungsten and razor blades tested as cathode material was between 10^{-4} and 10^{-5} cm. With a limit of 10 kv on the applied voltage, a 1 mm cathode-anode spacing is required. On test, milliampere currents began at 8 to 9 kv, and at 10 kv the current density was about 50 amp/cm^2. Considering the exponential increase of the current density with the voltage, it is apparent that very high currents can be obtained with moderate applied voltages.

The close cathode-anode spacing (about 1 mm) presents two main

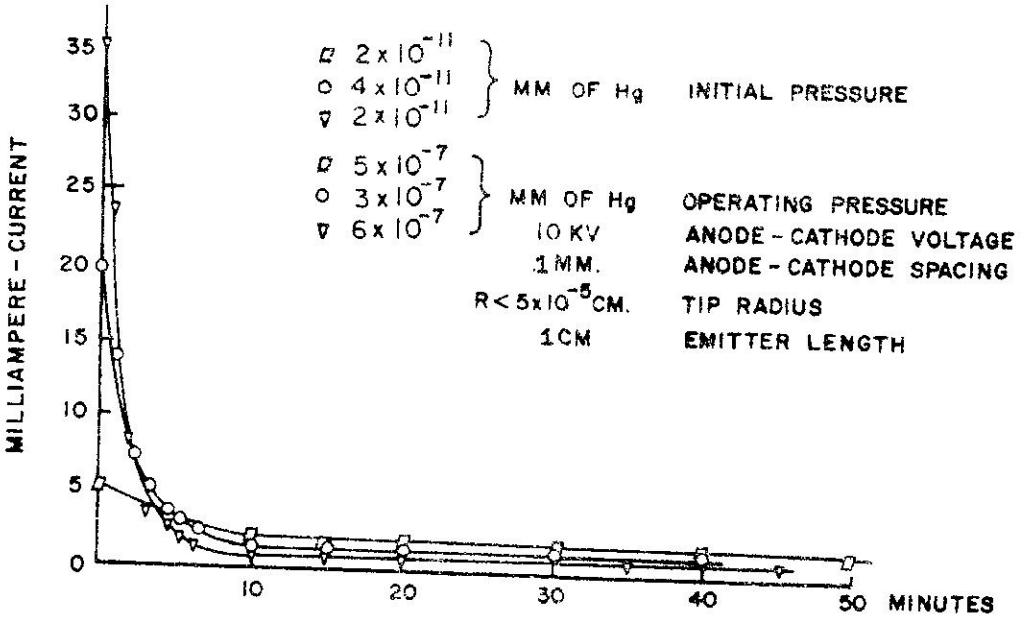

FIG. 11 - EMISSION CURVES - SAFETY RAZOR BLADES.

difficulties. First, it makes the placement of the blades very critical. They must be exactly parallel to the anode to insure a uniform contribution along the full length of the emitter. Second, this spacing, and consequently the emission stability, is strongly affected by very slight changes in water temperature. Emission currents from 5 to 65 ma were obtained by decreasing the water flow from 1 to 1/4 gallon per minute, thus allowing the operating temperature to increase, and cathode-anode spacing to decrease.

CONCLUSION

A stack of ten blades, having a stable current of 40 ma per blade, gives a total current of 400 ma, corresponding to the order of our present needs in intensity. Considering the low voltage (10 kv) of these experiments, we may find that sharp edged metal slivers can be used quite feasibly as X-ray field emission sources. Future experiments in this area will be directed toward improvement of the vacuum during operation by previous electron bombardment of the target, stacking blades, and testing higher voltages.

VII. APPENDIX - X-RAY EFFECTS ON ALKALI HALIDES

B. Cruz Vidal

OBJECTIVE

Our objective is to investigate the mechanism of formation of F centers in alkali halide crystals under X-ray effects. To that end, we want to study the rate of formation of F centers, the energy of formation per F center, the number of incident photons needed to create one F center, and similar questions at discrete energies around the K-absorption edge of the halide ion in alkali halide crystals. Our recent work has confirmed a strong energy dependence of these ratios.

METHOD

X-ray beams from the XRD-5 X-ray unit in Mayaguez were used to excite the fluorescent emission spectra of radiators prepared on aluminum planchets. The fluorescent output consisted primarily of a high intensity $K\alpha$ line and a lower intensity $K\beta$ line. The latter is filtered out using thin filters of an element with a K-absorption edge between the two K emission lines. The filter absorbs preferentially on the high energy side of its K-absorption edge. Finally, we obtain monochromatic X-rays of a much higher intensity than is possible using a crystal monochromator, and the useful beam output has a cross-section of a few square centimeters.

Filters were prepared for the bromine irradiation and their output analyzed for satisfactory performance. The filtered output of the radiators has been measured and the current input on the X-ray tube has been regulated to achieve the same photon flux incident on the sample. We have monochromatic X rays of 11.2 kev (from the $K\alpha$ emission of Se filtered with As_2O_3), 11.9 kev (from Br filtered with Se), 14.1 kev

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This work is being carried out by Baltasar A. Cruz Vidal in partial fulfillment of the requirement for the Ph. D. degree at Harvard University.

(from Sr filtered with RbCl), and 15.7 kev (from Zr filtered with Sr $(NO_3)_2$).

TABLE I

Energy	Radiator	Filter	Current on W tube at 50 kvp[1]
11.2 kev	Se metal	III As_2O_3 29 mg/cm^2	40 ma
11.9 kev	Na Br powder	IV Se powder 22 mg/cm^2	35.5 ma
14.1 kev	$Sr(NO_3)_2$ powder	V RbCl 33 mg/cm^2	38 ma
15.7 kev	Zr metal	VI $Sr(NO_3)_2$ 29 mg/cm^2	27 ma

The photon flux measurements were done using the Fricke dosimetry method (Fe^{++} to Fe^{+++}) (21). The measurements are reproducible and calibration measurements for low energy X-rays are available. However, a significant change in geometry is involved because the dosimeter measures the average photon flux incident on its surface, which is larger than that of the crystal. To avoid the change in geometry, we are now using silver activated glass dosimeters previously used for X-ray work (22). Some calibration experiments can be run using the total absorption X-ray calorimeter described in Section V of this report.

The relative photon intensities are estimated to be the same within a 10% variation. We do not have absolute measurements because the Fricke dosimeter was at the position of the crystal in the liquid nitrogen irradiation. However, the data reported in Fig. (12) a and b was taken at room temperature, where the crystal was located closer to the X-ray window. Intensity measurements using glass dosimetry at that position are in progress.

[1] The current was chosen for equal photon flux on the sample. The actual photon flux measurements were at the position of the crystal for the liquid nitrogen temperature runs. The room temperature runs were done with the sample closer to the source.

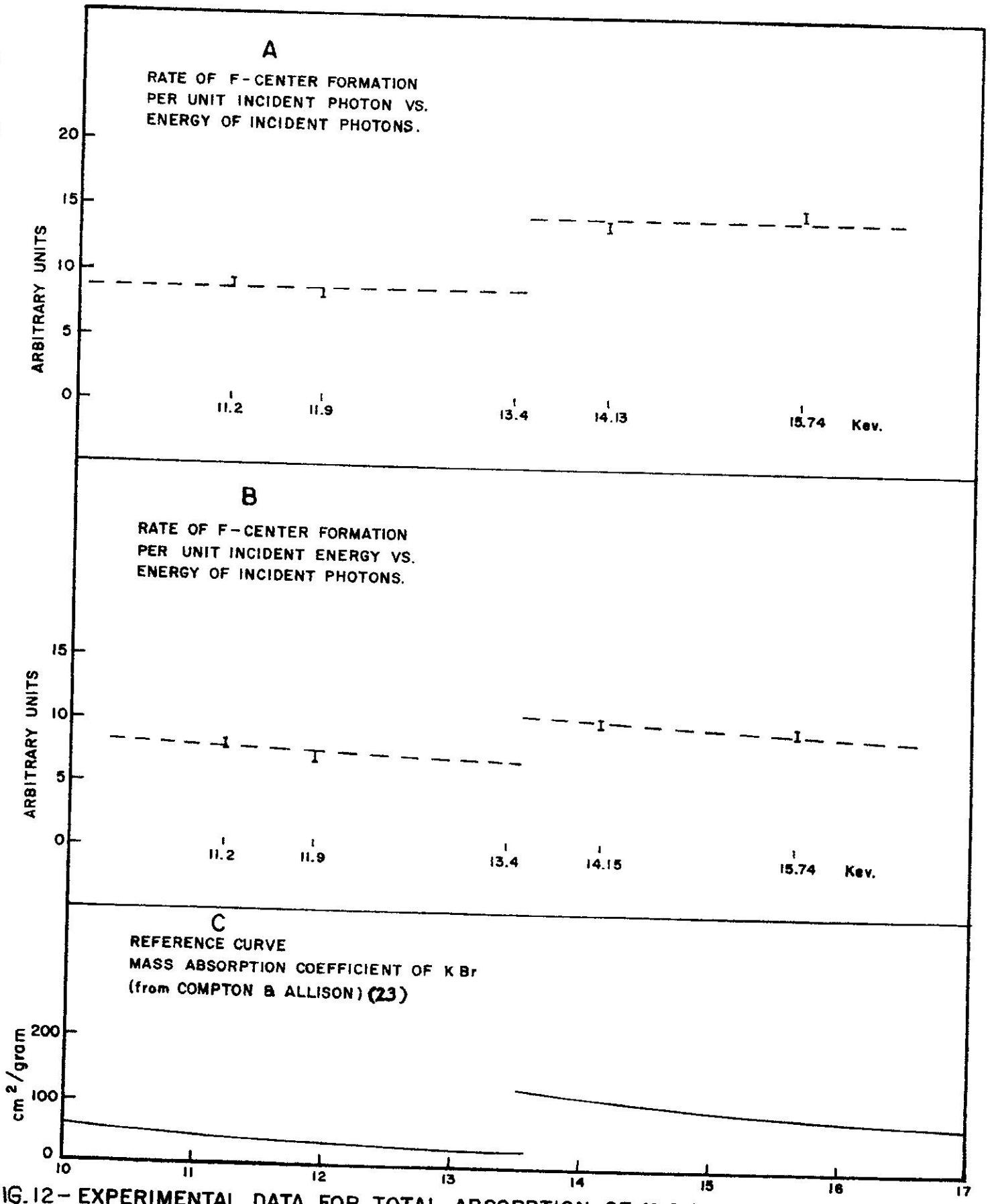

FIG. 12 – EXPERIMENTAL DATA FOR TOTAL ABSORPTION OF INCIDENT MONOCHROMATIC PHOTONS AT ROOM TEMPERATURE. (HARSHAW K Br CRYSTALS 1 to 2mm thick)

Rabin and Klick (22) showed that the lower the temperature, the less dependence there would be on the previous history and impurity concentration of the sample. Accordingly, we have aimed to work at liquid nitrogen temperature. Work at that temperature will be done soon. The necessary equipment, including modification to our spectrophotometer and to our cryostat, has been built.

A new holder for the radiator and a new water cooling shield for the X-ray tube were constructed to give the shortest possible distance between the X-ray tube and the radiator, and between the radiator and the sample. The change in geometry has increased the intensity at the position of the sample.

Irradiations at room temperature were alternated below and above the K-absorption edge. Typically, one crystal was irradiated for about 150 hours, and at least twice on each side of the K-absorption edge. Selenium (11.2 kev) and zirconium (15.7 kev) irradiators were used more frequently because they are metallic and therefore not subject to damage. Three KBr crystals (1 cm x 1 cm x 1 mm to 2 mm thick) were irradiated using this method. The three samples were cleaved from the same Harshaw crystal.

RESULTS

We observed a sharp increase in the rate of F-center formation above the K-absorption edge of the halide, even at room temperature. As the results shown in Fig. (12) a and b indicate, the rate of F-center formation does not change rapidly as the radiation energy changes around values removed from the K absorption. The change in F-center production rate is likewise clearly illustrated. At this time, we don't know whether there is a simple step change in efficiency as the K edge is crossed or whether

there may be larger changes in the vicinity of the K edge. However, for a study of the Varley mechanism hypothesis, the techniques presently used serve quite well. The major requirements are monochromatic X rays and good intensity control.

At equal photon flux, a Varley mechanism would require a nearly constant efficiency of F-center production per incident photon at all energies below the K edge; and a higher, but also constant, efficiency at energies above the K edge. The plot of F-center formation per unit energy input would then show a saw-tooth around the K edge. The experimental data is presented in Fig. (12) a and b. Fig. (12) c is given for reference.

Another energy which we have not yet used is the Kα of rubidium filtered with bromine. At 13.4 kev, it is one point below the K-absorption edge of bromine (13.5 kev), yet extremely close to it.

An observation that could be significant is that the initial fast rise in F-center concentration at room temperature is completed much faster at energies above the K edge than below the K edge. Also, some bleaching is observed, even in darkness. It reaches a saturation after approximately 5 hours and a change of about .01 in optical density. The change is not significant during the time of measurement.

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