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PUERTO RICO NUCLEAR CENTER

RESONANCE IN RADIATION EFFECTS

TECHNICAL REPORT NO. 2

[OPERATED BY UNIVERSITY OF PUERTO RICO UNDER CONTRACT

1NO. AT (40-1)-139 FOR U. $. ATOMIC ENERGY COMMISSION

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RESONANCE IN RADIATION EFFECTS

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Technical Report #2

Work performed at Puerto Rico Nuclear Center

?under U. §. Atonie Energy Commission

Contract AT(40-1)-1833, Project 14

May, 1966

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Abstract of paper presented at the Radiation Research

Society Meeting, May, 1953... :

Abstract of paper presented at Conference of Nuclear

Spectroscopy and Solid State Physics, February, 1964

LITERATURE REPERENCES

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1. RvTRopveTION

?The aim of thie project remains as originally stated: to ansver the

question "What are sone of the unique effects of ionizing radiation on

radiation effects

matter?? To this end, our research program has studied x

tm the 5-20 Key energy range upon biological systens. This energy region

As of considerable importance since it contains the K-absorption edges of

the constituent atone of most living systems.

?The biological systen studied previously has been the enzyme catalase.

Our previous Technical Report (1) dealt with che resonance radiation

effects observed with this metalloensyme and indicated the presence of an

?action spectrun" in which the greatest biological effect (inactivation) was produced at or near the Keabsorption edge of the constituent iron. At Ents potnt of our research (approximately 12 gonths ago), {t was felt that axtaun 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 metal

Accordingly, the original zinc containing enzyme can be converted to esterases containing cobalt, nickel, ttn, mercury, oF molybdenum. A wries of euch enzynes, {dentical tn con position except for the metal, are being {rradiated at wavelengths in- eluding those corresponding to the various K-absorption edges. Observed retonance effects are tested for specific relationship to the metal and for their independence of the renainder of the nolecule. For example, should the cobalt enzyme and the zine enzyme be irradiated at energ:

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

---Page Break---

Kapg of cobalt than at energies corresponding to Kyhe of zinc, and cone

ly for the sinc enzyme, kesulte obtained to date with zine carboxy= Deptidase A indicate that tnaceivation ie encrgy dependent. Purther ?experiments are needed, hovever, to confirm the phenomenon; refineneats:

in dosimetry and nore Intense sourcs

@ required to solve this problen.

The biological studies of resonance effects have been extended {nto genetics by Dr. F. S. Koo. Chronosones in onion root tissue were labeled with « nucleic acid analog, S-bromodexyuridine, and then irradiated at

photon energies at or near the F-

sorption edges of bromine. Preliainary

results indicate that chromosome aberration in the cell nucleus occurs

with highest frequency after {radiation at eners

equal to or greater

than Kgp, Of brosine, Tt {s hoped that these experiments will open new

approaches for probing the nature of radiation-induced mutations.

Since only low-intensity monochronated beans can be obtained froa

Present equipment, much of our effort is directed at overcoming this

Mmitation. Several steps have be

taken: 1) @ new x-ray spectrometer

with higher current capacity has been installed, 2) fluorescent Line

emission produced by the original spectroneter has

yeen utilized, and

3) new field emteston type x-ray devices have been designed and are in

construction. In addition, we plan to survey other biological systens

which, becaut

of thetr larger ?amplification factors", may prove wore

oultable for the study of resonance effects

Since our project is concerned with clarifying the effects of radia-

tion, from the initial physical event co 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

inorgante crystel san excellent subject for study, The literature on

---Page Break---

tthe subject reveals that the monochromatic x-ray technique has not pre-

viously deen utilized. Only recently has similar work been begun in Dr.

Swoluchowski's laboratory at Princeton University. For this reason, we feel that it would be reasonable to devote # nodest fraction of our total ?effort to such studies. ?The bulk of this work {8 being done by a student conducting research tovards hts dissertation requiresente at Harvard University.

Other physical aspects of the project are 1) development of dosinetry techniques vhich will provide accurate measures of absorbed energy in the low-energy x-ray region and 2) completion of a vacuum spectrometer which WiLL produce « high intensity of very lowenergy x-rays (3-10 Kev) suit

able for study of low atonic vetght elenente. The field eat:

entioned earlier ig for use with this spectrometer.

At the pre

nt time, this project ts fully operative in two Labora-

tortes, one {o the Physics eparement, University of Fuerte Rico, Mayaguez,

tthe other in the Bio-Medical Bullding, Puerto Rico Nuclear Center, Rfo

Piedras. The former (e used primarily for the physical studies, the

latter entirely for the biological research.

Report aubsicted by:

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11, RESONANCE RADIATION EFFECTS IN CARBOKYPEPTIDASE A

Carboxypeptidase A is a pancreatic exopeptidase having a solecular

weight of 34,300 and 1 gram aton of zinc per aole of protein (1. ?., 0.2% Zine by weight) (2). This atom of zine is an integral part of the enzy-atic active site, as shown by the fact that its removal by dialysis Kesults in a concomitant loss of enzymatic activity, This sine atom is bound co a mercapto group; as the metal {s renoved, a reactive ~ Sil group ts disclosed (3).

Carboxypeptidase A was obtained from the Horthington Blochentcal Corp. a8 an aqueous suspension of material crystallized by the original mmethod of Anson (4) and recrystallized by chat of Putnam and Neurath (5). He was dtluted to a concentration of approximately 9.3 ag/al (1 x 10°54) {m 10% Lithium chloride solution and then iamediately before use diluted 1:25 vith 0,067H sodium phosphate buffer, pil 7.50. To avoid microbial ?action upon the enzyme protein during the long irradiation periods, the buffer vas sterilized by "Wwillipore? Fileration. The concentration of enzyme in the solutions for irradiation was approximately 4 x 10°7M, «

concentration chosen ag similar to that of catali

¢ used in previous studies by Banons (6) and by Luse (1). In this way, « comparison of Arradtation effects can be sade for these tvo enzymes at the sane concen-tration (and also sintlar pl).

Irradiation of carboxypeptidase A as dilute solution vas carried out

in the cell shown in Figure 1. Sanple conpartnents are of # width equal

to that of the collimated x-ray bean; with appropriate solution depth,

the whole bean Ls incident upon the sample solution. This cell,

improved version of che cell described in our previous report (1), hs

certain advantageous features:

---Page Break---

All dimensions in mm.

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

---Page Break---

Description of Figure I

Ae

Parts C and F are attached to A with five nylon screvs; Pils

Cooling plate with water inlet and outlet (Ivette)

Rear vindow - 0.005" 11m (polyethylene, or other material)

Sanple holder vith irradiation compartment and two control

conpartnents (lucite, polyethylene, teflon, or other natertal)

Front window ~ 0.002" film (polyethylene or other material)

Front plate, vith cut-outs to match sample compartments

Gover for front plate, to provide insulating air-pockets {a

Front of sample conpartuents (these prevent moisture condens

tion on chilled sample holder in path of x-ray bean) = 0.005

film (mylar, polyethylene, or other material)

B, D, and F

fare sealed to plastic with halofluorocarbon grease Kel-? #90, (Minnesota

Mining &

Manufacturing Co.)

---Page Break---

4) Increased chenical reststivity - Plate ¢ which forms the sides of the compartments may be of Ivette, polyethylene, teflon, ete. By

fection of plastic ftlms B and D, conpartnente could be all polyethyl-fene or all teflon, should the solution to be irradiated require thie.

>) Vartable solution thickness = By using different plates ¢ of

varlous thickness, solution thickness may be varied to match the absorp-thon cheractertstics of the incident x-ray bean.

©) Versatility of cells ~ Plate C and its cover plate E nay be re-moved from cooling plate A and replaced vith other sample holders, as for ary F108.

After {eradiation, solutions of carboxypeptidase A were assayed for Fenaining activity by the method of Neurath, Elkins, and Kaufman (7), tn

which the rate of hydrolysis of 0,025 M carbobenzoxyglicyl-Lephenylalanine

1 determined using the Moore and Stein colorimetric ninhydrin method (8).

Tm this procedure, enzyme and substrate (Mann Research Laboratories

material) were reacted at 37°C and pl 7.5; anounts of phenylalanine

formed vere determined after reaction tines of 5, 10, and 15 minutes.

The mean reaction constant, k, was determined from the slope of

Lines

tained ven lonig of 100/(100 = percent hydrolysis) was plotted

Ve. the reaction time. The proteolytic constant, C, was calculated «

Wy (op. of enzyme nitrogen per ml), assuaing 18% N content for carboxy

peptidase A. Remaining percent enzyme activity was extinated as (Cx 100

of trradiated solution) / (average ¢ of in sity controls). The activity

Of auch in situ controls (1. e., kept in the irradiation cell compartments:

* We found that slight anounte of ultraviolet light-absorbing material a leached from mylar filme during 20-50 hour contact vith the 0.8N sulfuric acid solvent of the dosineter solution. This causes erroneously high entinates of dose fron absorbance neasurenents at 224 mu.

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weed to that of other portions of the non-irradiated enzyme solu-

tion stored in the refrigerator.

The experimentally determined values of collimated x-ray bean inten sity as measured by the ferrous dosineter are presented in Table 1.

Reproducibility is poor, probebly due to long irradiation periods and anall increases in optical absorbance over that of the control samples. Te may be concluded that the bean intensity ie approximately 2 x 10lt photons/hr/al of dosineter in the 7.5 to 9.7 Kev region. Thte value te im general agreenent with previous data obtained with the KRD-5 spectro-

1

meter at Mayaguer.! The sharp decreate in intensity at 11.69 Kev is

unexpected, Possible causes, such ai

elated with the target position, or target danage, are being looked into. Since the spectrometer system is sharply collimated, snall changes in the xray source position will have a aarked effect on {ntensity at the tes sample position,

The x-irradiation of carboxypeptidase A with collinate

beans hae

been carried out at three photon energies: 7.6 Kev, 8.68 Kev (the Keline

of zine), and 9.69 Kev (the K-absorption edge of zinc). Experimental

retulte are as given in Table 2, vhere enzyme activities are expressed

?as the proteolytic constants C. Percent of renaining enzyne activity is estimated from the ratio: (¢ of irradiated solution)/(C of in situ control). Fercent of inactivation, of course, equals 100% less percent

Fenaining activity. Such preliminary experimental data may de used an a

1 Koray tntengity te a function of che applied current (na), the applied voltage (as V8), the atonic number of the target. (2), and general cube Seonetry. The XRD-6 apectroneter in capable of higher intensity becaus ?Of ite higher current ratings, but in the pri chromium tar= get tube 9 used instead of « tungsten targe to avoid line eniesion near the zine K-absorption edge. This difference in target ma= terial reduces intensity by nearly one-third (Zo¢/2, * 26/74).

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Table 1. Measurement of Intensity of Monochromated X-Ray

Beam, Using Ferrous Dosimeter

Dosinetry techniques vere as described in our previous Technical

Report (1). The x-ray tube was operated at 18 Kup and 75 na; its output

?was collimated vith two soller elite and LiF diffraction crystal

Concentration of ferric ion formed, C,

= Increase in absorbance /1000E,

noles/al

where E has the value 4565 at 224 mu and 2196 at 304 qu, subject to ten

perature corrections, as given by Scharf and Lae (9).

Intensity of absorbed radiation, as photons per hr per 1 nl of dosi-

eter

concentration of ferric (on formed y 4.46 x 1021

energy per photon, in Kev

We ??targy Wave tae Ta Ferrie ton intensity,

cu per? Tren. length A, over? cone. ax au photgns

erystal) photon period of A _ controls moles beter

Kev Hours a x10 yo

48.83" 7.47 27.0 228 0.037 oat 18

304 009 oa 0.9

47.83" 7.60 22.0 226 16 43

304 028 1s 3.5

iste goa 2s 26 046 1.0 2

304 025 Lt 2.6

4nst® 868 18.5 228 0320.70 Le

306 2016 63 La

37.17 9,69 20.0224 1844.0 9.2

30% 056 2.6 5.0

37.17" 9.692.028 926 5.4

306 2038 17 3.6

30.577 11.69 37.5 226 ors 0.61 0.62

308 OL 150 49

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10

basis of plots of insctivation vs. x-ray dose (?survivel curves"). Esti-

rates of absorbed dose are based on beam intensity data, for which the

averaged value of 2 x 10"! photons/hr/ml of solution vas utilized. This

best ave

fe value of intensity ie subject to correction with further

dosimetry. The survival curves, plotted in Figure 2, must be considered

fs of a preliminary nature, subject to future reviston.

---Page Break---

yorqw0> yo 9 /torqw0s TAT

skqddy soy z0uyy sakzue repso 28273 s29yn teTshtosphy oaesa9qne YO? - 7

Jo eBuex oq3 uy poansvon ?(3x02 999) 4 tequeaeu0> uoya9¥er uve ox] PsuTEIO3ep 279M

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eT UE aden

jequaazed eFql

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saayaomey _£ayay328 ?uros ?p2ay Jo Yor3009 Jo. pfo038 50% nox ee (qea8ks9

?aus] Suyuyeme: xy wyauezeue) 2}34qos301g aw ?1023009 swox0ud/a 451) 92" Kesey

30 Kayay22y ?03 er

WorarTpHU=x aFIMNOAylOUOK YaFH ¥ esePTadedhcoqzeg Jo VORIPATIONU *Z STqEL

| 8 ob 8 8 8 8 ee le lll lll

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2

Figure 2-Course of Carboxypeptidase A Inactivation at Various

Photon Energies

9.69 Kev

76 Kev

&

8.68 Kev

Percent Remaining Activity

&

°

50, 100 Hours of Irradiation

a oT a

° 1 2

Dose /mil. as photons x 10!

(assuming = 2x10")

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B

EI, PRODUCTION OF P-CENTERS IN ALKALI HALIDE CRYSTALS (NACL, KBE).

4) Introduction and Theoretical Sackground

coneides

In the Last few yea

ble effort has

8 made to de-

termine the nature of defects in fonte erystels and to reach an understand=

Ang of the mechanian by which such defects are introduced under the influ:

fence of fonizing radtation. The interest in this re

arch stems from the

fact that the typical alkalt halide crystal is a renarkable aediua for the

study of radiation damage effects auch as Lattice imperfections. These

crystals have a very simple structure and can easily be obtained in « high

te of purity and perfection, A wealth of physical data can be obtained;

fanong the feasible measurements are many related to the crystalline taper-

fections, the existing dislocations, the density of electrons and holes

and thetr mobility, the effects of impurity atoms, and the presence of

vacant Lattice sites,

Our present research deals vith the formation of color centers

4m alkali halides by x-rays in the energy region of the Keabsorption edges

of the conseieuent atoms (ef, Table 3). xetrradtation effects have been obtained by other workers using the whole output spectrum of the x-ray tube; our study utilizes monochromatic x-ray beans. Experiments have

centered upon tavestigation of the P-center, vhich consists of

trapped in a heloge

ton vacancy, Such Fecenters are temperature

(4K eo 300%)

at Liquid helium temperature, they are associated with «

?neutral interstitial halogen atom (i-center). At such low temperatures, other workers have found that the rate of formation of P-centers te a Dulk property independent of tonte diffusion and of the presence of

Aelocations, The results at lov temperature support Varley's mechan:

for F-center formation (10) in vhich an initial multiple ionization of

---Page Break---

Blesent.

Sodiua

Chtorine

Potassion

Bromine

Todine

?

KeEdges of Elements in Alkali Halides

u

?

1»

35

33

Kabe Ke Ke

Kev Kew Kev

to 1.04 Lor

2.83 2.82 2.82

3.61 3.31 3.59

13.48 1 13.29

33.16 28.51 32.29

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1s

the halogen ion results in a positive halogen ion that is then ejected

from ite lattice position.

F Auger process is Likely to take part in

the mult{ple ionization. For this reason, x-irradiation near the X, Ly

and Neabsorption edges of the halide i of special interest. Here, such

questions arise as:

14) What is the x-ray photon energy dependence of the

formation race of F-centers, especially around the

K, L, My... absorption edges? (This question ts

relevant because of the {aportance of the postulated

Auger process.)

b) What is the time for recoabination of electrons

the suleiply ionized halogens?

©) What {6 the time required for the halogen to migrate

4 given distance?

4) Wat L6 the energy nece:

sary to create one F-center?

at room temperature, che unsolved problens are even greater. As the

tenperature rises, thermal diffusion as well as the presence of lattice

vacancies, (mpurities and dislocations becone incr

Angly Amportane.

eure hi

Furthermore, the intensity of irradiation at room tenpe! some

8 yet unexplained effect. All these effects tend to detract from the

feasibility of Varle;

native processes.

To obtain information on the density of F-centers, use (e made

of the equation of Snakula (11):

Ne 1.29% 10!7 1 2

Perr se

---Page Break---

Here Ws the nuaber of Focenters per em, £ 18 the oscillator

strength, m the index of refraction, o g the optical absorption coeffictent

at the maximus of the Foband in em?, and W the width in electron volte of

the

and at half maximum, The number <q {8 obtained from the relation

for transmitted intensity at thickness d An terms of the incident inten-

sity To, viz, 1 © loe?m!, for Light of a frequency equal to that of the

?aaximua absorption. At a given temperature neither f nor W changes as the

density of color centers increas

8. Under these circumstances N ie pro-

portional ton ge Ae

function of irradiation tine 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 erystels 1 to 2 am thick

and

roximately 1.5 cmon each side, For vork at room temperature, they

fare mounted on a holder designed to fit in the sample slide of our Beckaan

?stagle-bean DU spectrophotometer. The crystal {s located at one of tvo

openings in the holder. The other opening i left unoccupied so as to

Provide the air reference standard for the relative measurements of

optical density. For low temperature work ve have designed a sasple

holder to be mounted in vacuum inside the cryostat.

Irradiation of a KBr crystel has been carried out for 200 hours

at 11.2 Kev (selenium radiator) a

for an additional 125 hours at 14,2

Kev (strontium radiator), with the sane intensity and at room temperature.

For details of radiators and f1lters, see Tables & and 5,

Present intensity seacurements have used the ferrous oulfate

dosimeter as vell as the proportional counter of the spectrometer (for

measuring relative intensities and while monitoring intensities). In the

---Page Break---

Relative N, density of F- centers

?

1 Fime of irodition

Figure 3- Typical density of F-centers against time of irradiation

?at room temperature.

Typical volues ore t, 10 hours, ty

17 hours for a K=Br erystal irradiated

at 1.2 Kev, 4 x 10° photons ?emt see

---Page Break---

Le 4,

Characteriaticn of Fluorescent Radiators

for Alkali Halide crystal Irradiation

Ky» Energy (Kev)

Chemteal Porm

Physical rora

2 element

35 Bromine

38 Strontium

39 yeerdom

40 zirconium

27 cobaie

2 Mt

34 Selenium

47 stiver

48 cadmium

49° indium

72 Hafatun

76 osmium

78 Platiqus

73 Gold

11.923

14.166

14.957

15.774

6.930

2477

a2

22.162

23.172

26,207

Lacye EOOERY (Kev)

7.098

8.910

9.4an

om

Ne or

SF (03)

¥9(009)3

2 0

co

Nt

se

as

ca

an

Fine powder

Metallic sheet

cast Am AL

planchee

Metallic sheet

Metallic sheet

Metal, foe

powder

Metallic sheet

eee

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Table 5. characteristics of X-nay Pilters Used vith

Fluorescent radiators!

Essay (EG) Saeray (RaW) hanical PRT ayaa

Radiator ?Kc Kg, Filter used ?Rabe form (np/eay

1

1 $$

Se UL22t 12.495 as 11.863 40303 29

Br 11.923 13.290 se 12.652 ge Pr

Sr 16.166 15.836 ab 14,323 mbet 3

¥ 14.957 16.736 sr 16.105 $0803). 29,

ze 15.77% 17.666 se 16,106 "

eee

Toe appropriate filter for # given radiator was deterained by using

the emission Line and absorption edge energies compiled by Fine ead

Hendes (12). The absorption edge of the filter must be between the K,.

and Ke Lines of the radiator. The desired thickness of the filtering

by means of the following equation:

wher

Pr density of the ftltering element (g/cm?)

= thickness (cm)

jorption coefficient (cx? /g)

---Page Break---

2

future, our prinary energy measurenent standard vill be a calorimeter

obtained from the Michigan Memorial ~ Phoenix Project research group in

Resonance in Radiation Effects, It consie

of an evacuated steel vessel

with 4 mylar window and « berylliun window shielding © totally absorbent
(for the energies of interest) gold foil target, Two thermistors are
attached to the gold foi target for measurenent of the energy absorbed
and 1 has a chin heater cot] for caltbration, the caloriaeter measures
Ancident povers in the range from 4 £0 30 alero-watts,

The curve of density

inst 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
coeffietent) of radiation at different energies. Irradiation of KBr crystals
vunder the sane conditions (11.2 Kev, room tenperature) shoved that the change
of slope occurred after 37 hours of irradtation at # beam intensity of 6 x
10° photone/en?/sec. We have concentrated attention on the slope and occur=

Fence of the straight part of the density curve because the initial fast

rise in Po

enter concentration is dependent on the history of the crystal.

At low temperatures this initial rive does not appear, Likevise, the

effects of impurities, lattice imperfections, and diffusion are aiataal.

?The subsequent Iinear increase in the

center density at room temperature

arises solely from lattice displacement caused by the incident radiation.

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1, RESONANCE RADIATION EFFECTS IN PRODUCING CHROMOSOME ABERRATIONS

Preliminary Report

by

F. 8. Koo

Puerto Rico Nuclear Center, Mayaguez, Puerto Rico

First results indicate that the x-radiation effect on a given biow

logical system can be increased if the photons are delivered at the Ke

edge absorption eneray of a particular constituent atom of the eysten.

Evidence supporting the hypothe:

of radiation dependence was first ob-

tained during studies of the enzyme catalase which contains four atoms of

fron tn [ts porphyrin ring structure, Emons (6), Pa

evoudekis (13),

and Use (1) independently showed the enhanced inactivation of the metal-

loenzyne catalase by monochromatic x-rays at wavelengths (or photon energy) near the Keabsorption edge of iron

The choice of an enzyme that contains a heavy element has been the key factor in the tests of the resonance radiation effect hypothesis

vanced by Gonberg. A critical test for the genetic system may be {rra tation at resonant energies of DNA labeled with halogenated thymidine analogs.

A first attempt h

1s been nade by investigating S-bromodeoxyuridine-

labeled chromosones in Allium cope root tips. Roots from germinated seeds

were first treated with BUDR solution at a concentration of 1S up/al. for 15 hours, and then irradiated vith monochromatic x-rays for } hours. The Deas intensity was approximately 5.9 x 10'° photons per en? per hour. {x photon energies were applied to the whole series of sanples, vhich were then lefe to recover for 24 hours before being fixed for cytological study.

---Page Break---

2

In summarizing the data, which are now available at four photon energies, the chromosomal aberrations at metaphase in forms of chromatid and chromosome breakages, fragrents, interchanges, ete., were scored and ?expressed as the nunber of chromosomal breakages per cell. The results are presented in Table 6 and Figure 4.

---Page Break---

Table 6. Chronosomal Breakages Produced by Monochromatic X-Rays at Various Photon Energy Levels in BUDR-Labeled Chronosones of Alliua cope Root Tip Cells

ae

Photon er No. of celle No. of breaks No. of breake

applied studied observes per cell

2s 85 7 08

13.2 92 a5 16

3.43 we 36 29

3.7 a o> now being

ea we an ae Htudied

15.5 68 as 22

Although the data obtained at the pre:

nt stage of re

rch are

Father Limited, there are strong indications that the photon energies at

or near the

jorption edge of bromine cause a clear-cut incre

tn

the production of breakages in BUDR-labeled chronosones in Allium cop

Detailed reports on methods of matertal handling, chenical treatnent,

irradiation, cytological studies, etc., will be compiled and published

?pon completion of the whole study.

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mu

o3|

2

2

No. of chromosomal breakoges per c

Kabs edge Bromine

128 135 13s 155

Photon Energy (Kev)

Figure 4~ Resonance radiation effect of monochromatic x-rays

?on BUDR-labeled chromosomes in Allium cepa.

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Y. HIGH LVTENSLTY FIELD EMESSION X-RAY SOURCES

Te appeared that one basic experimental difficulty which aust still

be faced in spite of the prosress nade in the course of the last year ts

the question of low bean intensitics at the required energy. The follow

ing crude approximation say serve to illustrate the problen, We have

found hy

the typical intensity output of a tungsten x-ray tube is of the order of 10"? ev/en®-hour. For crystal structure research, his requires trrad(etion periods of about 50 hours to 160 hours during which the relative effect of intensity and the rate of color center formation ie studied, Using a crystal monochromator, even at optimum geouetrical alignment, the energy output after the second collinating soller slic is of the order of only 10§* to 10'S ev/en*-hour, {. ?., four or five orders of magnitude le

han for the tungsten tube. With che irradiator tech= ?nique, on the other hand, output intensity is of the order of 10!7-10!8 ev/en®-hour, that is to say, one tenth to one hundredth of the total bees output. In view of the mich higher intensities obtainable from irr.

Alators, they have been used in the preliminary crystal studies. How fever, ve still need the continuous adjustability of wave length afforded by the crystal systen.

Presently available intensities result (n long periods of irradiae

thon vhich give rise to munerous experimental difficulties, with regard to both the alkali halide and the biological irradiations. A consider~ ?able improvenent tn experinent control and more reliable data could de obtained L? the irradiation pertods were shorter. These considerations hhave Led to « study of x-ray sources other than the available comercia Xray tubes in the hope of achleving a higher intensity output. The

chee

design of a high intensity x-ray source rais

ie problens:

---Page Break---

ion from the source

4) How to achieve high electron exi:

b) How co focus the emleced electron beam on the target; and

©) How can the cathode

ind target design eliminate the high x-ray

dispersion found in comercial tubes.

?The solution of the first problen lies in the principle of field

emission, The use of a pointed cathode--entailing a very high ffeld (n=

tensity per unit are:

lowers the potential barrier to electron enission

?and produces electron current densities vbich are significantly higher than those obtained with hot filament cathodes. In field entaaton, che electrons leave the cold metal under the action of a strong electric field intensity. The entssion nechanism, which could not be explained

by classical physics,

has been well described in terns of ave mechanies:

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 enission according to Somerfeld

land Bethe (14) is seo

J (electrons fence) = © NCTE) DCF, B, 8) a8

where N(Z,E) measures the relative ounber of electrons at « given absolute

temperature T whose kinetic energy, based on the conponent of velocity

nome! to the surface, hy

Somerfeld end bth, hes the form

sonata [is epeann]

strong electric field F, the potential barrier becomes thin and ts r

duced in hetght. Electrons impinging on this finite barrier from the

inside of the metal have a certain probability of penetrating it and

---Page Break---

2

ap

ies

ring outside the metal. This probability is expressed by the trans-

on coef Etctent

caeacer [sari 6-9 apn]

Here g a the electron vork function in ev, ? e the electric fe

fo

volt/em, Eis in ev and f(y) ie 4 dimenstonte

elliptic function of he

variable y = 3.79 x 10-4 rl/2/(g-) introduced by Nordheim (15). ¢ te

constant that has the fora 47m kr/b3, where m ie the mass of the elec

tron and h is Planck's constant.

?A plot of this forma (Figure 5) indicates the dri

ithe teen

ein

current de

nity with respect to the applied f1eld for a needle-shaped tungsten emitter at different teaperatures (16). Some significant properties of field emission cathodes are

*) Current densities of up to 10%amp/en?, exceeding the Romperature-Limited currents of conventional pure tungaten cathodes by a factor of one million.

b) No energy other than that stored in the field is required for ontesion.

©) The enttted current de

ity depends exponentially on the applied electric f1eld and hence can

controlled,

In view of these advantages, particularly the possibility of high current density and consequently, high x-ray {atensity, we decided to Anvestigate # field emission source for our x-ray spectrometer. At the present time, we are experimenting with comercial razor blades to be used as the cathodes. nee

of thetr sherp @:

At de relatively

ey Co create high field intensity by placing the anode close to the edge. The experinental x-ray tube and the field eal

on source now

Ddeing tested ai

shown tn Pig. 6.

---Page Break---

SQUARE CENTIMETER)

?CURRENT DENSITY (AMPERES PER 3

10" 7

APPLIED FIELD (VOLTS FER CENTIMETER)

Fie. 8

From Scie America, an. 1968(06)

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B3OYNOS NOISSIN3 G73l4

ONISN 38NL AVY-X WLINBWIY3dX3-9 914

anne Not

aw enemy os

1991-49189 ?O-- _

beamn von Jo]

Shy |

whos L) ; |

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3a

The iret

yurenents of the radius tip of comerctel atainless

steel razor blades iodicate that radii of the order of 10"Sem are avatl-

able. Ae the work function of iron ts quite close to that of tungsten,

razor blades should vork well as fleld enizcers. In fact, some work ba

already been done using iron needler instead of the wore conventional

fe, We WAIL have to establish the influence of the

other elenents present in steel razor blades

Tn an x-ray tube using a {leld-eataeion electron source, the

main caus

of instability and short Life are surface contamination and

sputtering. The latter occurs vhen the cathode 1 bonbarded by the

res{dusl gas tons in vacuums as good a 10°20 am, of ig. Helin gas 18

fone of the major probleas. Hovever, ?eproving the vacuum and the clean-

Line!

of the eystem my result in very good stability.

The f1eld emtesion electron source further offers the possi-

bility of an extended ?area? source. The high gradient 1s eatablished

at the potat -or edge- of a sharp source. A sharp source 18 about 1 x

10-4 = 1 x 1075 em tn diameter. Anothe

sharp source 1 x 10-1 em from

the first 18, for field purposes, far avay. Thus t should be posetble

fo set up # group of points, or # parallel set of bla:

+ to get the

?equivalent of an ?area? source.

Tt ts trve that initially, individual sharper pointe will emit

fret, possibly reducing the voltage at other points, Ina short time,
however, Judicious ?melting? can increase the tip radiue and reduce the
sradient to the point where other points begin to emit simultaneously.
We plan to test Ehis concept soon,

our objective here is the creation of a eimple, rugged high-

output x-ray source, Ia our total prograa, though, ve are not overlooking

---Page Break---
at

the hot cathode sources. A conventional hot filament source te being
fade for the vacuum spectrometer now under construction in our shops and

in which ve plen to use the field eaission source, if succesaful. Also,

we have been following the development of the Large filament-area, electron-focussing type sources aa used by Dr. Burton Menke of Pamona College in California and Dr. William Gross of the Radiological Research Laboratory, Columbia University.

---Page Break---

?VE. INSTALLATION OF NEW FACILITIES

1) KeRay Spectrometer, GE Model 100-6

To expedite the biological phase of thts program, an x-ray \ctrometer vas installed in the Rfo Pledras Laboratory of PRNC for use by Dr, Luse, This spectrometer, model X8D-6 of the General Electric Co. (see Pig. 7), bas higher current ratings than the spectrometer used in previous studies, so chat # more intense beam of x-rays for a given target is available vithout sacrifice of beam purity.

?The x-ray tube, type EA=75, can be operated at up to 100 ma

current in full wave rectified node of operation, a value twice the rating

of tub

An our XRD-S spectrometer.

?A high degree of beam resolution has been obtained in this nev

spectrometer by application of the system used in the original spectrometer,

vize: a) collimation with a medium resolution soller slit of the bean

emitted from the x-ray tub

d) diffraction from a crystal of the colli-

?mated beam; and c) further collimation with # high resolution soller slit

of the diffracted beam before it impinges on the sample to be irradiated.

With auch 2 resolving system, 85% of the energent beam energy ie vithin

+30 e.v, of nominal energy, i.e., lees than + 1% beam eneray spread tn

the 5000-18000

¥. range. Por details of measurement, see Project
Report 1 (1). The intensity distribution of the emergent x-ray beam
produced by the XRD-6 equtpuent has been measured; typical values are
siven in Table 7.

>) Low-Temperature Irradiation Chasber

Tee production by x-radiation of F-centers in alkali halide

crystals can be

aed quantitatively only at low tesperature, vhere

effects due to impurities, crys

1 dislocation and theraal diffusion

?are absent. For this reason, @ low temperature irradiation chasber was

---Page Break---

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%

Table 7, Intensity Profile of Colliaated X-Ray 3eam

Photon intensity, Percent of

Position bighest value

1 -

2 33.8

3 ae

4 96.6

3 88.0

6 92.7

7 93.0

8 95.1

: 10070

10 96.3

u 75.6

n 345

rf 26.3

1? +

13 :

The intensity of x-ray photons vas measured by moving the SPC-6

detector! in 0.86 um intervals horizontally across the face of the bean?

?4 0.1" (0.254 om width) slit was used in front of the detector window.

?The x-ray tube was operated at 18 KVP, and 45 ma, Collimation

fs described for tests of energy reeolution, The intensity profile

was made with the Lip analyzer crystal set at 37.17" for selection of

9.69 Kev radiation, This is the energy of the K-absorption edge for zinc.

Width of beam utilized in radiations 46 0.92 mm, t.e., distance

from position 2 through 13,

T the detector is a proportional type counter tube, xenon-filled, with a

0.010 in. berylisum window; ite efficiency ta 80% for the 7.1 Kev energy

photons here utilize:

2 vor detatie,

Pig. 11-6 and Fig. 11-5 of our previous report (1).

---Page Break---

constructed by modification of # two-Liter experimental cryostat (manufac

tured by Superior Atr Products

orp.) In this cryostat (Fig. 8), the sample

4s held in contact with a metal rod which is in turn in contact with a

Tvoir containing liquid nitrogen or Ifquid heliws, The metal rod can
be encircled with resistance heater colle, so that St is possible to vary
the sample temperature from that of the reservoir Liguid (4*K for helium,
90°K for aitrogen) to room temperature (300°K) or above. Such variation
of temperature is essential in measurements of the temperature at which
Pocentere appear and of the range of their tomperature stability.

?The crystal Ae trradiated through # port covered with « mylar
{lm window, other of the four exyoetat ports may be fitted with quarte
windows for subsequent optical measurements. The sample chanber beneath
the cold Liquid reservoir 4 evacuated to about 10° torr by a diffusion
Pump with mechanical forepump (Vactronie Laboratory Equipment Co.). The

seme vacuum system provides heat insulation to the cold reservoir.

Although this irradiation chamber 1s

featly tn use only for

alkali halide crystals, it offers the potential for irradiation of éry

enzyme films at low comperature in future research.

©) Devices for P-center Measurement

Production of F-centers in alkali heli.

crystals via x-red{ation

As measured in the low temperature chanber described

ove. To accomplish

Ehis, the spectrophotometer (Beckman model DU) us

18 for optical absorption

leterminations was modified by substituting for the ulval 1.3 em thick

sample carrer compartment # comartuent of sufficient size to accomodate

the Lower portion of the cryostat. (Cf. Pig. 8). This Light-tight com

partaent, 16 x 12 x 12 om (Lx Wx i), Le fitted between the monochrosstor

hnousing and the phototube housing; within s© the cryostat may

poastiones

50 that the spectrophotoneter light beam prsses through two port windows

---Page Break---

38

land the sample crystal before reaching the photomultiplier. The spectro

Photoneter is couipped with a SERA attachment and a chart strip recorder

Which permit automatic plotting of trenen{tted energy av a function of

vevelength

Present investigators have utilized pot

stun bromide crystals which

have P-center adsorption peaks at 6200 A. For this wavelength, the aylar

film windows covering the cryostat provide satisfactory Light transnt

on,

that in, the sane poct used for x-radintton may be ased for optical measure-

ments, However, for studies utilizing sodius chloride, with Pecenter peak

at 2409 A, optical measurements must be made through quartz windows which

Eransait in the ultravioler region. Several devices have been designed

#0 4s to permit x-irradiation through mylar vindows and optical neasure-

sents through quartz windows. The firet of these is a mirror system by

wnich the ineident spectrophotoneter beam enters # port other than the

Arradiation port, is bent to pass through the erystal, and then is bent

?agein to energe from che port opposite to its point of entry. A second device would permit turning the sample crystal 90° from the irradiation POrE s0 as to face the optical measurement port. The third device is

eaigned to rotate an irradiation port vhich has two halves, one mylar,

fone quarts, from the ay!

wr half (for x-irea

ton) to the quarts half

(for absorption neasurenents). A prototype of the First of the

devics

has been constructed and

ed. The other devices may prove more simple

and hence wore reliable.

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VIL. APPENDIX

Resonance Radiation Effects of Low Energy Monochromatic

X-Rays on the Metalloenzyne Catalase!

Henry J. Gonberg and Robert A. luse

Puerto Rico Nuclear Center, Mayagues, Puerto Rico

To answer the question "what are the unique effects of ionizing radiation on matter?", our present research program has studied x-Lrradiation effects im the 5-20 Kev energy range upon blologteal systene. This energy region is of considerable ieportance since it contains the Ke absorption edges of the constituent atons of most Living systems. X-rad{ation of these energies {8 produced frow incident radiation of much

nipher energies (as codale-50 ganas, 250 ev x-rays) by degradation

ehrough Compton scatter. Biological systens chosen for study were tho

which are coaposed primarily of Light elenents with but traces of medium atomic weight elenents. The netalloenzyne catalase has been studied extensively to date by three investigators. For this enzyme, which contains

four atone of iron in (es porphyrin ring structure, the question was ?Does

radiation absorbed by the tron atone 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 catal

solecule?" Experinents vere desi gnt

to show or disprove the presence of

a true action spectrus of radiation in the kilovolt region. The presence

of auch a spectrum has been shown and indicates untque effects of such ra~

dation, entirely divorced fron the general ?indirect? effects of radiation

(Wich may

simulated chenically under conditions of far less energy ab-

jorption per aolecule). (Work supported by U. S. Atomte

srey Comiasion.)

Vaatract of i

May, 1963.

1r presented at the Radiation Research Society Meeting,

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Focenter Production with Monochrosatic X-Rays!

F. Vazquer Mactinee

Puerto Rico Nuclear Center, Mayaguez, Puerto Rico

Our present studies are concerned with the rate of F-center produc

jon as a function of the energy of incident x-ray photons. Sodium

chloride crystals of 10x 10 x .S mm, vere (rradiated at room temperature

Fluorescent x-rays from three élfferent targets were used to produce K-

Lines at 7.477 ev (Ki), 11.221 ev (Se), and 23.172 ev (C4). A mterocalo-

cimeter vas ve

fas primary standard to ensure Line intensities. The x-

vray tat

was operated at 50 RV and various milllanpere current settings

fo obtain the sane bean intensity from all sources. Saskula's formula

sed to determine Pecenter concentration fro éata on optical absorp-

thon obtained with a Becknan DU spectrophotoneter. At lov x-ray doses the

process of fitling the normal vacancies of the crystal vith electrons pre-

dominates and no proportionality vas observed between absorbance and

energy. Irradiation times extended two days beyond the point where the

region of Linear xelationshtp between F-

F production and energy wae

reached. At higher doses, where the creation of new vacancies by the

(neident photonn crises, the nuaber of Pocenters produced 1s independent

of the photon enersy in the energy range unéer consideration, This

supports Varley's mechanies (Phys. Chen. Solids, 23, 985 (1962)), 0

Plaining chlorine ion dtsplacenent by means of an Auger process of culti-

ple fontzation. Calculations confire this hypothesis for x-

well above the Keabsorption edge of chlorine.

Uivstzact of paper prevented at Conference of Ruclear Spectroscopy and

Solid State Physice, February, 1964,

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Figure No.

3?

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Table No

1

LAST oF TABLES

Page

X-Badlation aon fesse WL

KeRdges of Elenents in Alkali Halides 1?

Guuractertotics of Fluorescent Radiators for Altalt

Wal ide Crystel Irradiation . Tesssesecetesseesses 18

Characteristics of X-Ray Filtere Used vith Fluorescent

Radiators : a 9

Ghronosontl Breakages Produced by Monochromatic X-Rays

?at Various Photon Energy Levels in BUDA+Labeled Chrono-

tones of Alliue cepa Root Tip Celle ? 2B

Intensity Profile of Collinated X-Ray Bean ?

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