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PUERTO RICO NUCLEAR CENTER

RESONANT ACTION OF Low

. CHROMOSOMES INCOR ?4S

SOCHROMATIC X-RAYS ON

MODEOXYURIDINE

OPERATED BY UNIVERSY OF PUERTO RICO UNDER CONTRACY

NO. AT 30-1893 FOR U. 5, ATOMIC. ENGRGY COMMISSION

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RESONANT ACTION OF LOW ENERGY

MONOCHROMATIC X-RAYS ON CHROMOSOMES

INCORPORATED WITH 5-BROMODEOXYURIDINE,

FP. K. S. Koo and H. J. Gonberg

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Effects program. It was carried out at the Puerto Rico Nuclear

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Resonant action of low enerey monochromatic X-rays on chromosones incorporated with 5-bromodeoxyuridine

by

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Defining radiation as electromagnetic waves or photons, it has been shown that radiation effects are often a function of the energy or wavelength of the incident radiation, one of the best known examples is the induction of mutations and chronosome aberrations by ultraviolet Light. It has been well demonstrated that UV light of about 2600 & wavelength is most effective in producing genetic changes and also that the wavelength corresponds to the peak of the Uv Light absorption spectrum for nucleic acid.* However, the relative efficiency of the UV quantum at 2600 & in producing genetic changes, based on mutations or chromosome aberrations produced per unit energy absorbed, does not appear to change. ?The change in effectiveness would thus appear to be due to the increased absorption of the 2600 & photons rather than the greater efficiency of the absorbec photon or energy.

Higher energy radiation such as X-rays may also be character

Astically absorbed as determined by the constituent atons of the

system being irradiated. The interaction between photons and

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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 enercies 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 on the energy of the incident radiation. Any change in effect with energy my be due either to change in absorption, such as occurs with Uv Light, or due to a change in efficiency (effect per unit energy absorbed) as a function of energy.

Previous attempts to elucidate the energy or wavelength

dependence of X-radiation effect in biological systens have yielded inconclusive and often contradictory results.? Although the results of catcheside and Lea? showed a definite increase in the production of chromosome aberrations in Tradescantia poller tubes at a wavelength of 4.1 & all previous studies in this area aii not unequivocally demonstrate any energy dependence of X-irradiation effect as the yield which represented the efficiency was not expressed in terms of the effect produced per unit of enersy absorbed. rt should be noted that most of these atudies were carried out using radiation sources with rather broad bands of emission

energies, or with characteristic emission source with

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energies well above the K-absorption edge energies of the constituent elements. To help resolve the question of resonance phenonena in radiation effects, a program employing monochromatic adjustable X-ray sources (Bragg type spectrometers) 2s been in operation for some tine.*

pmphasis has been placed on distinguishing carefully between

any changes in radiation effect due to changes in the absorp-
tion coefficient of the system under study, and the efficiency
of the absorbed radiation in producing its effect.

in a study of radiation inactivation of catalase, Brmons®

and Paraskevoudakis® at the University of Michigan, and Lus
at the Puerto Rico Nuclear Center, found that the efficiency
itself changed with change of incident enersy, In three
separate studies, the catalase was irradiated at energie:
below, at, and above the K-absorption edge of ion which is
contained in the porphyrin ving atructare. A sharp, signifi-
cant increase in the Zestruction of the ability of catalase
to react with hydrogen peroxide, on a 'per unit energy
absorbed! basis was observed in each case, as the incident
photon energy crossed the iron K-absorption edge

Since the genetic effect is one of the most important
consequences observed in Living systens following exposure

it is of considerable interest to study the

actions of monochromatic X-rays on genetic material in the

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energy range of the K-absorption edge of their constituent elenents. such exploration may lead to a better under~ standing of the nature of radiation-induced mutations and chromosome aberrations.

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

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 BUDA). ?The chronosones thus treated contain 5-bronouracil 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 thi

BUDR-incorporation in the onion root chromosomes in view of

the success of other incorporation experiments. It has

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been shown that the halogenated deoxyuridines including

BUDR can be incorporated into DNA during replication in

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microorganisms, human cells, and plant speci

evidence of BUDR-incorporation into the ONA of Allium cepa

also

root cells n reported recently by Futik and

?This communication 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.

Material and Method - The onion seeds (variety Yellow Burmuda from Burpee) were germinated on wet filter paper in petri dishes at 25°C. Roots reaching 6-8 mm long were treated with BUDR solution at a concentration of 15 ug/ml for 15 hours. For irradiation the BUDR-treated roote and the control were washed and arranged in an exposure area of 6 x 9 mn at the center of the Plexiglas sample holder. The area on two sides was delimited by two stripe of Plexiglas to form a trough
0 that the roots could be arranged between the two strips.
?The exposure azea and the surroundings were first padded

with wet filter paper and then the treated and control roots were arranged in two separate rows (upper and lower) with tips opposite each other. The samples were again covered

with wet filter paper and finally to keep the moisture in,

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the whole holder was wrapped in Saran Wrap. The exposure

area was aligned with the beam delivered from « General

Electric X-ray Diffraction Unit xRD-5 operated at 25 Kv and

25 ma. The irradiation system employed a conbination of collimators and a LiF diffraction crystal to produce a beam

of monochromatic X-rays with a high purity of energy (+ 50 ev) .t% The samples were irradiated for three hours at a beam intensity Of approximately 5.9 x 10° photons per square centimeter per hour, and then returned to petri dishes for recovery for 21 hours at 25°C followed with 0.2°/o colchicine solution treatment for 3 hours before being fixed in Carnoy's solution. six

Photon energies, namely, 12-5, 13.2, 13-48, 13.7, 14.1 and

15:5 Kev, with one energy level of irradiation per day, were applied. For cytological examination, the material was treated with 4°70 pectinase for 2 hours and the root tips, approximtely 1.5 mm long, were squashed in a combination of aceto-orcein

and -carmine staining, Roots treated with BUDR but not irradiated were also prepared as controls of the effect of

BUDR alone.

rations observed at metaphase in the root tip cells of Allium copa in both treated and control series include chromatid and chromosome breaks, free acentric fragments,

interchanges, and others. In sunmarizing the data, all

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aberrations involving breakages are grouped together as

chromosome breakages and are presented in wuble 1. The

cchanges was extrexsly infrequent. There

were only two cbserved in some 240 aberrations sco:

tn four out of txelve series, the nunber of cells examined

was less than 100, For comparison, aberrations in al?

are expressed in terme of the nusber of breakages per

100 cells. zn the FUDR-treated material (see Table 1, column

the number of breakages at the photon energy 12.5 Kev

was 2.9 per 100 cells but it inereased with increasing photon

energies. The amount of breakage arose sharply to a maximum of 27.9 breaks per 100 celle, showing a 3-fold increase, at the K-absorption edge energy of bromine (13.6 Kev), and then decreased slowly as the photon energies were further raised

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 mterial which was irradiated

at the same time with th

seme series of photon energies as the BUDR-treated material. The variation in aberration yield from treatment to treatment was relatively small (see Table 1, column 9).

As the essence of this investigation the efficiency of production of chronosone aberrations is elucidated as the

photon energy of an effectively monochconatic beam of X-rays

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is varied. The variation is over a spectrum region containing

the K-absorption edge of the target ator: bromine in the chrono-

somes, This variation, first of all, would change the spatial

Aisteibution of the energy absorbed by the chromosomes, when ?the impinging photon enerey 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 chronosone. When the impinging photon energy is at or above that of the target atom, ita absorption coefficient increases by a factor of about 7.5. However, the coefficients for the other atoms are essentially unchanged. Thus, there is 2 signi¢icant 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 effecte ave to energy absorbed by the non-target stons remain

essentially unchanged. Howey

, the large relative increase

in the energy ebsorbea in the target atum will show whether

the energy absorbed en that site is of vnusual sign!

or efficiency.

go determine if there is a discrete photon energy of X-

rays that is capable of producing genetic damage in excess

of that produced by photons with energies slightly higher

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and slightly lower, the mass absorption coefficient of the

gross target material must be computed, For the calcula-

tion the chromosome as an entity was chosen instead of the

whole cell 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°/o DNA, 46°/o histone, 8.5°/o residual protein, and 1.5°/o RNA.*# other components such as ca and Mg in an unknown trace quantity were not, considered in the calculation, It was algo assumed that the

chromosomes contain water in the same amount by weight

all the macromolecules combined.*? The AT content in the DNA of Allium cepa is 64°/o.** The replacement of thymine by bromouracii during one replication of DNA in the BUDR-treated chromosomes was assuned to be about 20°/c. Based fon the above-mentioned information, the chromosomes were estimated to contain approximately 0.18°/o of bromine.

The ma:

absorption coefficient was calculated in terns of

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on®/gram which did not represent the actual situation of the exposure area and the bulk sass of the root tips exposed. Nevertheless, these values calculated for the different energy levels are relative and therefore they are valid for reference.

the mass absorption coefficients for bromine, and for the control chromosomes and those treated with BUDR are plotted as a function of pheton energy in Figure 1. For bromine the coefficient curve showed a sharp discontinuity at 13.48 Kev corresponding to the bromine K-absorption edge (see A). This fluctuation represents an approximate 7.5-fold increase in photon absorption at its K-absorption edge. However, the coefficient curve for the chromosomes containing approximately 9.18°/. bromine exhsbited only @ small fluctua~

tion at the bromine K-absorption edge {see c). Here les

than 10°/e increase is. photen absorption is noticed. As expected the curve for the control chromosomes showed no fluctuation (see 8). 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

vels .

photons absorbed by the chromosomes at atl enercy

in Table 1 columns 6 and 10 are listed for the BUDR-treated

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Photon anergy {Kev}

Pigure 1. Mass absorption coe ata for the element Pr

chronosones (2) x the chronosones not

with BUDR ?2), ane taose treate! «ith

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and control roots, respectively; the adjusted nunber of break ages per 100 celis assuming that 211 the chromosomes at all energy levels absorbed the sane amount of X-radiation.

These adjusted nunbers aze plotted as a function of photon energy in Pigure 2, 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 pupR-treated material while there was no such difference at any of the photon energies in the control mterial. The utmost increase in efficiency at the K-absorption edge of bromine cver that observed below the K-edge energies vas about 2.5 to }-fold and 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.

in the physical measurement *% there were no intensity

peaks or sharp fluctuation in the intensity within the wav

Lengths employed. The maximum intensity differences between the two extreme wavelengths under observation is estimted to be about 3°/°. Also there is little change of the reflectivity power of the LiF crystal in this range. Thus no farther

adjustment was made.

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BUDR is known to be a mutagen,*® chromosome breaker,27* *°

and radiosensitizer.2®? °° when BUDR was used alone in this

experiment it waa found to have some effect on inducing the

chromosome aberrations. In a total of 216 cells examined 5 breakages were observed which approximated 2.3 breakaces per 100 cells. However, the combined effact of BUDR with radiation was enhanced when the BUDR-treated material was irradiated at or above the F-absorption edge energies of bromine and at

13.48 Key it waa found about twice as much as the sun of the separate effects of these two agents. although this result appears to be consistent with the findings by Koo*® on chromo-some aberrations induced by SUDR and gamma rays in Zebrina endula, most likely the enhanced effect is attritutable to higher efficiency cf photon energies at and above the K-absorption edge rather than to radiosensitizing effect of

WUDR. In view of these facts, it becomes difficult to interpret other results obtained with radiations of much

higher energies ae to the mechanisme involved. szybaleki®®

postulated that the ineorsoration of the halogen atoms into DNA presumably creates a strong electrostatic repulsion between the negitively chazyed Halogen atom and the oroximate phosphate ?group. As a consequence, the phosphate-ester bond becones strained and nore vulnerable to radiation. In his postulate

the radiosensitizing effect of bromine is clearly implied.

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However, the information available fram this investigation

suggests that purt of the enhanced combined effect observed

in BUDR-treated material irradiated with x-roys or ganma

rays of higher energies my have resulted from the interac-

tion between bromine end photons with energies at or slightly

above the K-absorption edge of bromine. In other words, some

effect enhancement my have resulted from higher »:

ciency

Of photon at certain energy levels. ?The shotons of these

energy levels in hard X-reys and camma rays are presumed:

derived from degradation through Compton effect.

It ie of special interest to know how the monoctironatic

X-ray photons act initially on the target atone. ?The msde

of energy absorption for a given atom changes as the wave-

length change:

For the longer wavelength, absorption is

in the 1 and higher electron shells with ejection of one

fast electron. At the shorter wavelength, absorption is in

the K shell. Tt has been shown that this can result in very

high ionization involving removal of over half a dozen electrons

Presunably the initial event vas the ejection of the K electron

of the bromine atoms. ?This was accompanied by renoval of many electrons from the other shells of the atoms. As a result a high local density of bombarding particles and un-neutraliced electric charge on the stripped atone were produced. This high

concentration of energy and force would rause local disruption

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of chemical bonds which in turn could be manifested into chrono. some breakages. Therefore it is believed the chromosome aber- rations might have resulted prominently from the action of photons on bromine atoms at K-absorption edge energy.

According to Lea®® chromosome aberrations produced by Xarays are through the actions of the tail portion of the electron path. Therefore in addition to the mode of action jast mentioned above, the densely-ionizing tails of electrons ejected from the bronine atoms might have also engaged in ?the production of chromosome aberrations

Since the roots were treated with BUDR for 15 hours it ig believed the chromosomes were labeled with bromine unifiliarly only. Also the chromosomes studied at metaphase were those which at the time of irradiation had gone through

one division following incorporation of BUDR. Based on the semi-conservative mode) of the DNA replication and chromosome duplication these chromosomes arrived at the metaphase for ?scoring should have only one chromatid labeled in each chromosome. Therefore a certain number of chromatid aberrations were expected if the action of photons was mainly confined to the immediate vicinity of the target atoms. The whole chromosome aberrations then may be considered as resulting from either the manifestation of the initial actions

of x-rays or the actions of the densely-tonizing electron

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tails. However, this interpretation can be applied very well only to the BUDR-treated material. Further investigations are needed to clarify this point,

Summary - Experiment with nL1ium sepa chronosomes in roots treated with 5-bronodeoxyuridine have shown an increase in breakages by monochromatic X-rays at the energy levels equal to or slightly oreater than the x-absorption edse of bromine (13.49 Kev). ?This is attributed to the increase of efficiency in photons at these particular energy levels.
3m contrast, there has been no evidence of such resonance

radiation effect in the control irradiated with the eame

series of photon energies as the BUDR-treated material.

The implication of radiosensitizing effect of BUDR and

Probable modes of action of low energy X-rays were discussed

briefly in the light of the present findings.

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ime authors are grateful to Dr. F. Vazquez Martiner

for setting up the irradiation system and determining the

dosage and other physical measurenents, and to Mrs. Baith

of

R. de Irizarry for technical assistance on some phai

the investigation.

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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 cytogenctics) as reference for our calculation, The percentages for the constituent macromolecules in the onion chromosomes have been arbi-

trarily set by considering these findings.

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No information is available on the water content of

the chromosomes. Here we have considered water as the

integrated part of the chromoro:

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in a chronosone water weighs the sane as al) maero-

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