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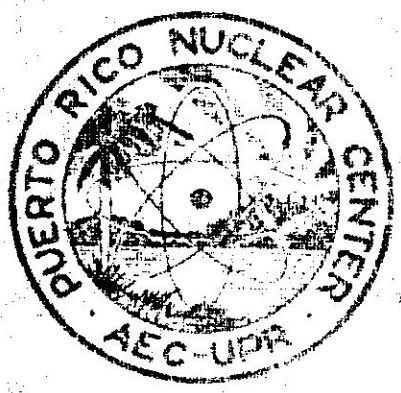

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RESONANT ACTION OF LOW ENERGY MONOCHROMATIC X-RAYS ON CHROMOSOMES INCORPORATED WITH 5-BROMODEOXYURIDINE

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This investigation is a part of the Resonance in Radiation Effects program. It was carried out at the Puerto Rico Nuclear Center, Mayaguez, Puerto Rico, operated by the University of Puerto Rico under contract No. AT (40-1)-1833 (Project 14) for U. S. Atomic Energy Commission.

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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 chromosome aberrations by ultraviolet light. It has been well demonstrated that UV light of about 2600 Å 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.[1] However, the relative efficiency of the UV quantum at 2600 Å 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 Å photons rather than the greater efficiency of the absorbed photon or energy.

Higher energy radiation such as X-rays may also 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 on the energy of the incident radiation. Any change in effect with energy may 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 systems have yielded inconclusive and often contradictory results.[2] Although the results of Catcheside and Lea[3] showed a definite increase in the production of chromosome aberrations in Tradescantia pollen tubes at a wavelength of 4.1 Å all previous studies in this area did 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 energy absorbed. It should be noted that most of these studies were carried out using radiation sources with rather broad bands of emission energies, or with characteristic emission source with

energies well above the K-absorption edge energies of the constituent elements. To help resolve the question of resonance phenomena in radiation effects, a program employing monochromatic adjustable X-ray sources (Bragg type spectrometers) has been in operation for some time.[4] Emphasis has been placed on distinguishing carefully between any changes in radiation effect due to changes in the absorption 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, Emmons[5] and Paraskevoudakis[6] at the University of Michigan, and Luse[7] at the Puerto Rico Nuclear Center, found that the efficiency itself changed with change of incident energy. In three separate studies, the catalase was irradiated at energies below, at, and above the K-absorption edge of iron which is contained in the porphyrin ring structure. A sharp, significant increase in the destruction 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 systems following exposure to X-rays, it is of considerable interest to study the actions of monochromatic X-rays on genetic material in the

energy range of the K-absorption edge of their constituent elements. Such exploration may lead to a better understanding of the nature of radiation-induced mutations and chromosome aberrations.

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 the investigation, heavier elements such as bromine and iodine in the form of halogenated thymidine analogues may be introduced into DNA and chromosomes. These heavier target atoms can be conveniently irradiated with the 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.[8] The evidence of BUDR-incorporation into the DNA of Allium cepa root cells has also been reported recently by Fučík and Kára.[9]

This communication summarizes the results[10] 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 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 the Plexiglas sample holder. The area on two sides was delimited by two strips of Plexiglas to form a trough so that the roots could be arranged between the two strips. The exposure area 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,

the whole holder was 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 (± 50 ev).[11] The samples were irradiated for three hours at a beam intensity of approximately 5.9×10^{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°/o pectinase for 2 hours and the root tips, approximately 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.

Experimental results and discussions - 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 1. The occurrence of interchanges was extremely infrequent. There were only two 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 1, 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 arose 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 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 material 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 1, column 9).

As the essence of this investigation the efficiency of production of chromosome aberrations is elucidated as the photon energy of an effectively monochromatic beam of X-rays

Table 1. Chromosome breakages produced by monochromatic X-rays at various photon energies in 5-bromodeoxyuridine-treated and control root tip cells of *Allium cepa*.*

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

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

+The number of breakages per 100 cells is adjusted by assuming that all the treatments absorbed the same amount of X-radiation.

is varied. The variation is over a spectrum region containing the K-absorption edge of the target atom bromine in the chromosomes. This variation, first of all, would change 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 chromosome. When the impinging photon energy is at or above that of the target 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 the energy absorbed on that site is of unusual significance or efficiency.

To 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

and slightly lower, the mass absorption coefficient of the gross target material must be computed. For the calculation 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.[12] 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.[13] The AT content in the DNA of Allium cepa is 64°/o.[14] The replacement of thymine by bromouracil during one replication of DNA in the BUDR-treated chromosomes was assumed to be about 20°/o. Based on the above-mentioned information, the chromosomes were estimated to contain approximately 0.18°/o of bromine. The mass absorption coefficient was calculated in terms of

cm^2/gram which did not represent the actual situation of the exposure area and the bulk mass 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 photon 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 0.18% bromine exhibited only a small fluctuation at the bromine K-absorption edge (see C). Here less than 10% increase in photon absorption is noticed. 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 1 columns 6 and 10 are listed for the BUDR-treated

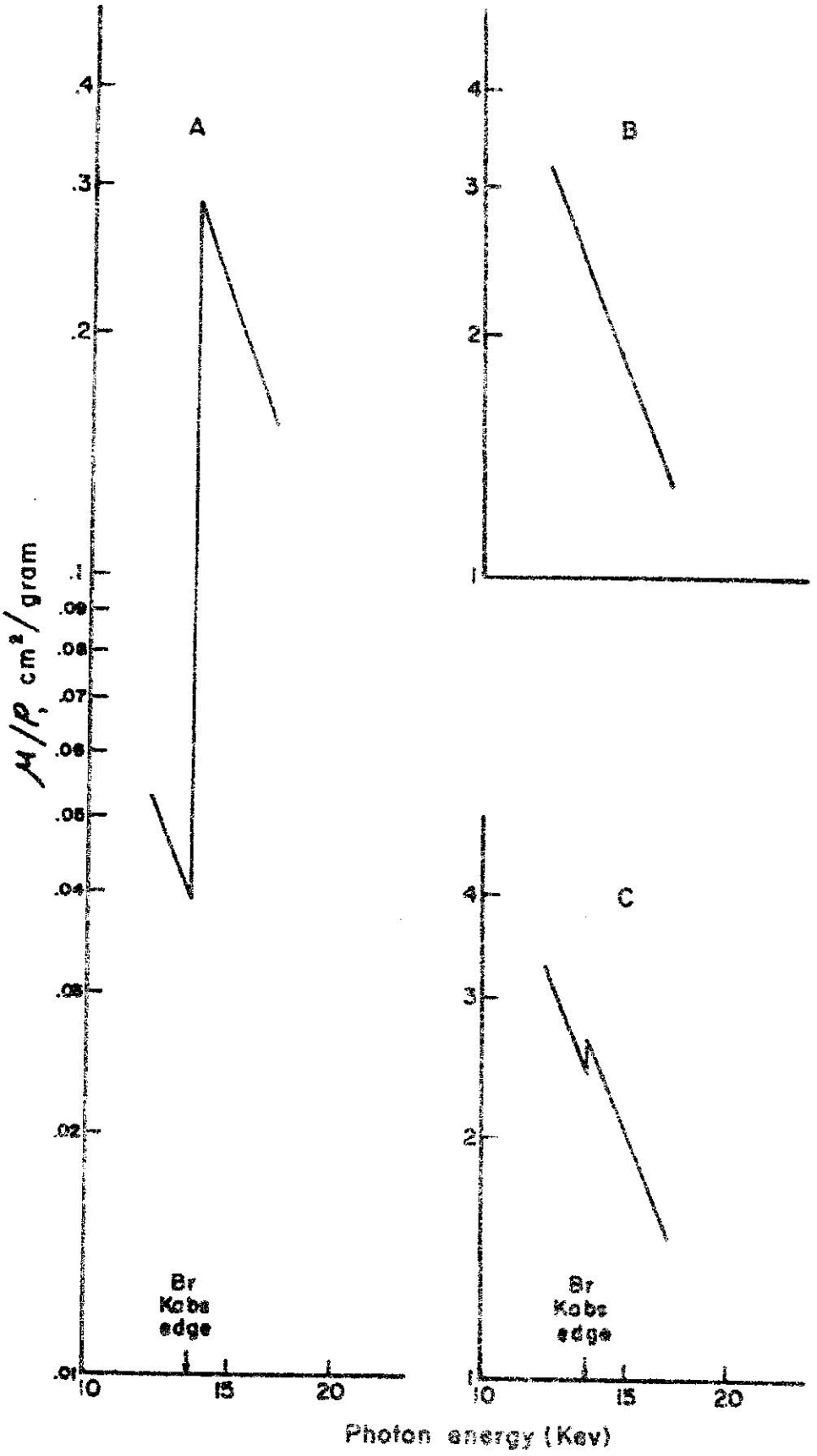

Figure 1. Mass absorption coefficients for the element Br in chromosomes (A), and for the chromosomes not treated with BUDR (B), and those treated with BUDR (C).

and control roots, respectively; the adjusted number of breakages per 100 cells 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 Figure 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 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 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 [15] there were no intensity peaks or sharp fluctuation in the intensity within the wavelengths employed. The maximum intensity differences between the two extreme wavelengths under observation is estimated to be about 3%. Also there is little change of the reflectivity power of the LiF crystal in this range. Thus no further adjustment was made.

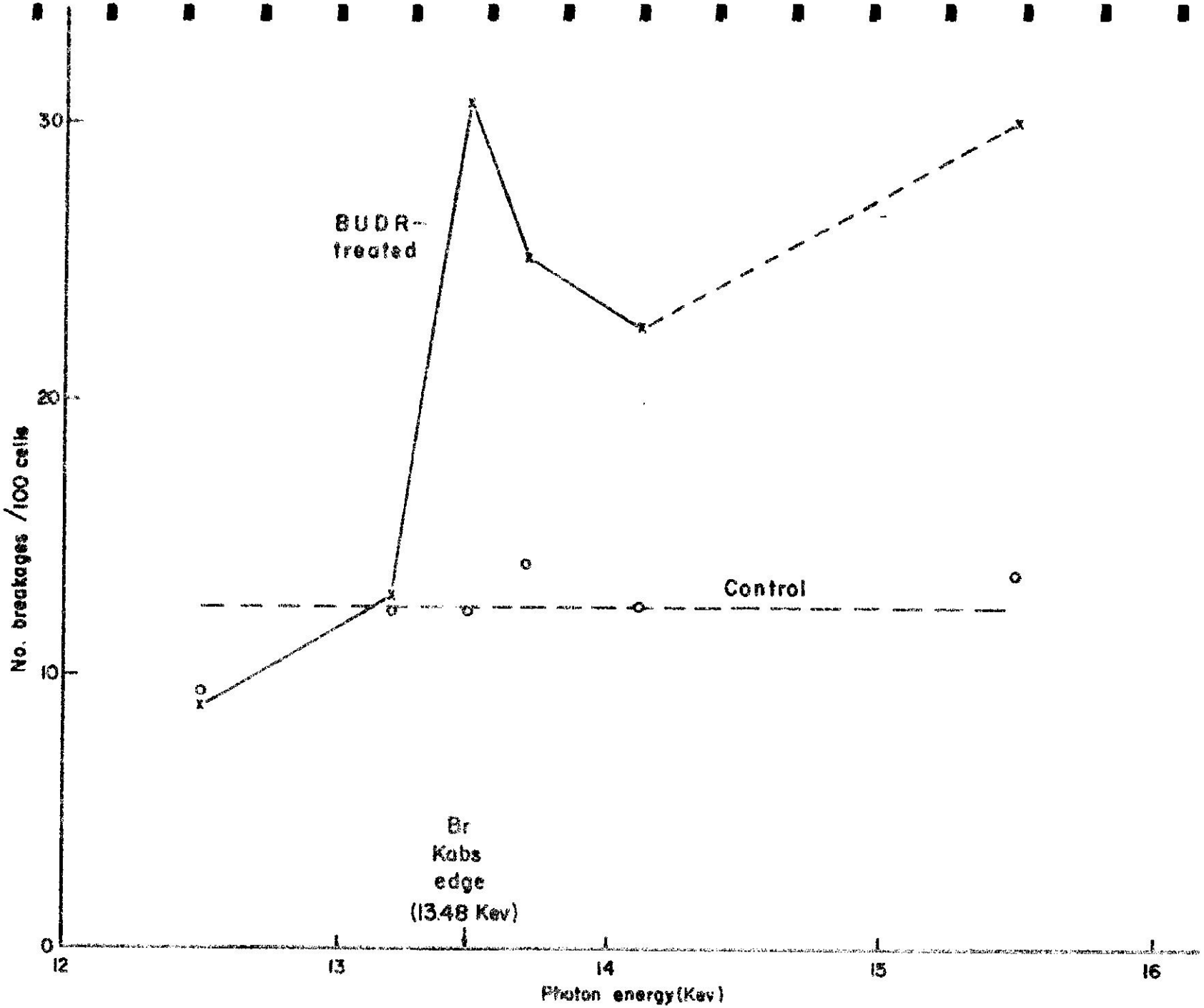

Figure 2. Number of chromosome breakages produced by monochromatic X-rays at various photon energies in 5-bromodeoxyuridine-treated and control root tip cells of Allium cepa. The number of breakages per 100 cells has been adjusted by assuming that all treatments absorbed the same amount of X-radiation.

BUDR is known to be a mutagen,[16] chromosome breaker,[17, 18] and radiosensitizer.[18, 19] When BUDR was used alone in this experiment it was found to have some effect on inducing the chromosome aberrations. In a total of 218 cells examined 5 breakages were observed which approximated 2.3 breakages per 100 cells. However, the combined effect of BUDR with radiation was enhanced when the BUDR-treated material was irradiated at or above the K-absorption edge energies of bromine and at 13.48 Kev it was found about twice as much as the sum of the separate effects of these two agents. Although this result appears to be consistent with the findings by Koo[18] on chromosome aberrations induced by BUDR and gamma rays in Zebrina pendula, most likely the enhanced effect is attributable to higher efficiency of photon energies at and above the K-absorption edge rather than to radiosensitizing effect of BUDR. In view of these facts, it becomes difficult to interpret other results obtained with radiations of much higher energies as to the mechanisms involved. Szybalski[20] postulated that the incorporation of the halogen atoms into DNA presumably creates a strong electrostatic repulsion between the negatively charged halogen atom and the proximate phosphate group. As a consequence, the phosphate-ester bond becomes strained and more vulnerable to radiation. In his postulate the radiosensitizing effect of bromine is clearly implied.

However, the information available from this investigation suggests that part of the enhanced combined effect observed in BUDR-treated material irradiated with X-rays or gamma rays of higher energies may have resulted from the interaction between bromine and photons with energies at or slightly above the K-absorption edge of bromine. In other words, some effect enhancement may have resulted from higher efficiency of photon at certain energy levels. The photons of these energy levels in hard X-rays and gamma rays are presumably derived from degradation through Compton effect.

It is of special interest to know how the monochromatic X-ray photons act initially on the target atoms. The mode of energy absorption for a given atom changes as the wavelength changes. For the longer wavelength, absorption is in the L and higher electron shells with ejection of one fast electron. At the shorter wavelength, absorption is in the K shell. It has been shown that this can result in very high ionization involving removal of over half a dozen electrons.[21] Presumably the initial event was the ejection of the K electron of the bromine atoms. This was accompanied by removal of many electrons from the other shells of the atoms. As a result a high local density of bombarding particles and un-neutralized electric charge on the stripped atoms were produced. This high concentration of energy and force would cause local disruption

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

According to Lea[22] chromosome aberrations produced by X-rays are through the actions of the tail portion of the electron path. Therefore in addition to the mode of action just mentioned above, the densely-ionizing tails of electrons ejected from the bromine atoms might have also engaged in the production of chromosome aberrations.

Since the roots were treated with BUDR for 15 hours it is 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 model 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-ionizing electron

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 Allium cepa chromosomes in roots treated with 5-bromodeoxyuridine have shown an increase in breakages by monochromatic X-rays at the energy levels equal to or slightly greater than the K-absorption edge of bromine (13.48 Kev). This is attributed to the increase of efficiency in photons at these particular energy levels. In contrast, there has been no evidence of such resonance radiation effect in the control irradiated with the same 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.

The authors are grateful to Dr. F. Vazquez Martinez for setting up the irradiation system and determining the dosage and other physical measurements, and to Mrs. Edith R. de Irizarry for technical assistance on some phases of the investigation.

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12 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.

13 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.

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