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RESONANCE IN RADIATION EFFECTS

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INTRODUCTION

Radiation effects in chemical and biological systems are often correlated with such quantities as energy lost per unit path length of ionizing particles, ionization density, dose rate, temperature, etc. There has been little work, particularly in the soft X-ray region, on the relationship between the energy of the radiation and the effects observed. It is of interest, therefore, to determine whether preferential or "resonance" effects exist in radiation damage, and whether any correlation exists between the absorption spectrum of the system and the effects observed.

It appears reasonable to think that preferential absorption of photons in the electron shells of specific important atoms in a complex molecule may result in "radiation damage" in excess of the proportional amount of energy absorbed. As an example, specific metals, such as iron or manganese, exist in only trace concentrations in certain biological systems such as cytochrome, catalase, or chlorophyll, but their action is vital to the behavior of these systems. Preferential absorption of radiation by such atoms could lead to more significant "damage" effects than would the absorption of an equivalent or greater amount of energy elsewhere in the complex molecule.

It was the purpose of this study to explore any existing relationship between radiation at specific energies in the X-ray region and some definable "radiation effect." Specifically, various compounds were irradiated with monochromatic X-rays in the energy range of from 5 to 35 kev and the extent of "radiation damage" was determined.

"RADIATION DAMAGE"

Unfortunately, the term "radiation damage" has been used frequently without precise definition. Such precise definition is necessary since the extent of damage observed will depend upon the particular analytical damage-detection method chosen and will not necessarily take into account all radiation-induced changes which have occurred in the system.

For the systems described in this report, radiation damage was defined in terms of a property of the system which, it was thought, might depend upon the energy of the impinging radiation. In interpreting data, however, it was necessary to account for the fact that the extent of absorption of X-radiation by a sample is a function of energy. For example, if the system under study contained a large fraction of atoms of an element whose K absorption edge was in the energy region investigated the mass absorption coefficient for the sample would be appreciably greater at energies just above the K absorption edge than at energies just below this K edge. Since the amount of radiation absorbed will

be a function of energy, it was necessary to evaluate the data in terms of "damage per photon absorbed or per unit energy absorbed" as a function of "energy of the incident radiation."

Observed variations in damage per photon absorbed must be due to some phenomena directly connected with the effectiveness of the absorbed energy in creating damage, since variations in the absorption coefficient are taken into account.

GENERAL EXPERIMENTAL TECHNIQUES

The monochromatic radiation source utilized in all these studies was a General Electric, Model XRD-5, X-ray unit. In some studies essentially monochromatic X-rays (see the Appendix) were obtained using a sodium chloride crystal as a diffracting medium. In other studies, nearly monochromatic X-radiation was obtained utilizing the fluorescent emission spectrum of various targets, about 85% of the radiation being due to K_α fluorescence of the target radiator, the remainder, K_β. The most recent studies utilized radiation that is more truly monochromatic consisting of approximately 99 to 99.5% K_α radiation obtained by filtering out most of the K_β radiation emitted by a fluorescent radiator.

The energy dose rates imparted to samples were determined with either a Fricke-ferrous sulfate dosimeter, a Victoreen Model 651 ion-chamber rate meter, or the SPG No. 1 X-ray counter associated with the General Electric XRD-5 X-ray unit. The SPG-1 counter operates in the proportional region, has a 65-cm argon gas filling, and a beryllium window 0.03 in. thick. These counters were "calibrated" by comparing their efficiencies with that of the Fricke dosimeter (a G value of 13.5 was used[1]). A comparison of the efficiencies of these various dosimeters is given in Table I. Even though the detection efficiencies are vastly different, the ratios of efficiencies appear to be reasonably consistent and apparently exhibit no large energy dependence. As is evident from the differences in detection efficiencies, it is not possible to ascertain the absolute energy dose imparted to a sample. In a continuing effort to determine this quantity, a total absorption thermal dosimeter is now being constructed. It will consist of a gold plate which will intercept the X-ray beam. The radiation, upon conversion to heat energy, will be detected by thermistors, thus providing a direct measure of the total energy of the X-ray beam.*

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[1]Cottin, M., and Lefort, M. J., "Etalonnage Absolu Du Dosimetre Au Sulfate Ferreaux, Rayons X mous de 10 et 8 Kev," J. Chim. Phys., 53, 267 (1956).

*At the time of writing, initial tests at 25 microwatts were successful.

TABLE I

COMPARISON OF EFFICIENCIES OF RADIATION DOSIMETERS AT VARIOUS ENERGIES

Target and Energy of Fluorescent K_α Radiation, kev		Energy Dose Rate*: ergs/cm²/hr			Ratio $\frac{B}{A}$	Ratio $\frac{C}{A}$
		(A) Fricke	(B) X-ray counter	(C) Ion Chamber		
Zn	8.64	5.67×10^5		2.31×10^5		0.407
Cu	8.05	4.91×10^5		2.32×10^5		0.472
Ni	7.47	5.68×10^5	5.47×10^4	2.42×10^5	0.096	0.426
Co	6.93	3.03×10^5		1.16×10^5		0.383
Fe	6.40	4.29×10^5	4.16×10^4	2.55×10^5	0.097	0.595
Mn	5.89	2.47×10^5	2.44×10^4	1.15×10^5	0.099	0.466
Cr	5.40	1.28×10^5	1.33×10^4	4.20×10^4	0.104	0.328

*All determined at, or corrected to, X-ray tube operation at 50 pkv and 40 ma.

I. STUDIES OF THE EFFECTS OF MONOCHROMATIC RADIATION ON SOLID SOLUTIONS CONTAINING ORGANIC HALIDES*

In an attempt to avoid possible ion-diffusion reactions resulting from the radiation, a series of experiments were performed using solid solutions containing organic halides.*

The solid solutions were prepared in the form of thin plastic films 0.25 to 0.38 ± 0.005 mm thick and containing the halide. These films consisted of three basic components:

1. An organic halide, or mixture of two organic halides. Included in the studies were: CHI_3, CBr_4, C_2HBr_5, and C_2Cl_6.
2. A dye-forming substance. That used was p,p;p"-Methylidynetris-(N,N'-dimethylaniline) (MTD) which upon reaction with halogen becomes crystal violet (CV).
3. The film-forming material was polystyrene. It was chosen because it exhibits much greater radiation resistance to free radical production than do the organic halides. A polystyrene and MTD film is far more resistant to radiation, as measured by color change, than is a film with MTD and halide.

In preparing the films, usually 2 gm of polystyrene, 0.1 gm MTD, and 3.3 gm of the organic halides were dissolved in 100 ml of carbon disulfide. This mixture was then poured on an area about 15 x 15 cm^2 and allowed to dry.

Small portions of the film were irradiated with diffracted X-radiation of a specified energy. The extent of absorption of X-rays was determined with the SPG-2 kyrpton-filled or the SPG-1 argon-filled proportional counter associated with the instrument.

Damage was defined in terms of the extent of production of crystal violet and thus was an indication of the extent of production of halogen atoms. The amount of crystal violet in a sample was ascertained by determining the optical density of the film at 615 mμ. An unirradiated piece of the film was used in determining the "background" optical density of the film. The extent of absorption of crystal violet was determined by preparing solutions containing known amounts of MTD and I_2.

*More extensive data and information can be found in the Ph.D. thesis of Julian L. Garsou filed in 1959 with the Faculty of Sciences, the University of Leige, Belgium.

RESULTS

Because of the complexity of these systems, it was somewhat difficult to obtain reproducibility. Table II contains a summary of these initial results. The reason for these variations in damage is not apparent, especially since it was thought that all large sources of error were eliminated. It is possible, however, that the irreproducibility might be due in part to some unknown variable in the films.

On the basis of experience gained in the manufacture of these previous film samples, a new series of films was prepared. The preparation of these films appears to some extent to be an art in itself. These new films appeared to be much better both in quality and uniformity.

One such film, consisting of 0.1 gm MTD, 2 gm polystyrene, 0.3 gm CHI_3, and 3.32 gm CBr_4, was cut into small portions and irradiated at various energies. Usually three separate portions of this one film were irradiated at each energy. The results in the energy range 24 to 35 kev are given in Fig. 1. Again, there appears to be some variation in the results. These data seem to indicate that slightly higher extents of damage occur in the region of the iodine K_α energy, and slightly lower extents of damage in the energy region just above the iodine K absorption edge. However, whether or not these effects are real (in view of the variations observed at any given energy) is open to question.

It appears as though these data of Fig. 1 could be approximated by the solid line drawn on the graph. Such decrease in damage with increase in energy is also observed in the liquid n-butyl bromide—DPPH system described in this report. It is suggested there that this effect could be due to dosimetry energy dependencies.

Portions of this same film (CHI_3 + CBr_4) were also irradiated in the region 11 to 15 kev. These results are given in Fig. 2 and indicated by open circles. Again there are variations in damage observed at given energies.

In an attempt to minimize further any possible inhomogeneities in the film itself, a single piece of film prepared in this same manner was subjected to irradiation in such a manner that the diffraction unit oscillated through a small angle and thus irradiated the film over a linear distance of about 5 mm. This linear distance was related to the energy of the impinging X-rays. These data, obtained by determining the optical density of this film at every 0.25- or 0.5-mm interval, are plotted as closed circles in Fig. 2. Here small, but perhaps significant, sensitivities to damage are observed in the energy region of the K_α, K_β, and K absorption edge of bromine.

Because of the uncertainties in these data it is difficult to conclude whether any abnormal effects did occur in the K_α, K_β, or K absorption energy regions of these halogens.

TABLE II

NUMBER OF HALOGEN ATOMS PRODUCED PER X-RAY PHOTON ABSORBED FOR VARIOUS X-RAY ENERGIES

X-Ray Energy, kev (b,c)	Organic Halide Film Constituents[a]			
	3.32 gm CBr_4	0.3 gm CHI_3	3.32 gm CBr_4, 0.3 gm CHI_3	3.32 gm CBr_4, 0.3 gm CHI_3, 0.24 gm C_2Cl_6
38.5	0.4×10^3	0.9×10^3	-	0.3×10^3
34.1	1.2	0.8	0.6×10^3	0.5
33.1	1.0	2.3	0.6	0.6
33.0	1.1	3.1	0.6	0.5
32.2	1.1	2.0	1.1	0.6
30.5	1.4	-	1.3	-
30.2	1.6	3.2	1.2	-
29.4	2.5	-	1.2	-
28.6	1.7	1.7	1.9	1.2
28.3	2.1	2.0	1.6	0.7
26.4	3.2	4.9	10.7	1.2
25.6	2.6	-	-	-
24.8	3.5	-	-	-
24.3	2.4	14	1.8	-
23.0	2.0	7.9	-	-
22.1	2.3	10.9	-	-
20.9	1.8	-	-	-
18.0	1.5	-	-	-
15.3	1.5	-	-	-
13.8	1.4	3.3	0.8	0.9
13.5	0.8	3.0	1.1	1.1
13.4	1.5	2.1	1.1	1.6
13.3	1.3	1.3	1.8	1.5
13.3	-	1.2	-	-
13.2	-	6.6	-	-
13.2	-	1.3	-	-
13.2	-	2.0	-	-
12.6	-	0.7	-	-
11.9	1.7	2.6	1.4	2.6
11.9	2.1	2.1	1.7	1.7
11.2	2.6	2.7	-	2.1

[a]The films contained, in addition, 0.1 gm MTD and 2 gm polystyrene.

[b]To obtain a high photon flux, a large slit width was used. In each irradiation 90% of the energy imparted to the sample was in the approximate range 0.95 E to 1.05 E, where E is the stated X-ray energy (see Appendix).

[c]The characteristic energies, in kev, associated with the halogens are:

Iodine: $K_{\alpha 1} = 28.6$, $K_{\alpha 2} = 28.3$, $K_{\beta 1} = 32.3$, $K_{\beta 2} = 33.0$, $K_{abs} = 33.2$

Bromine: $K_{\alpha 1} = 11.9$, $K_{\alpha 2} = 11.9$, $K_{\beta 1} = 13.3$, $K_{\beta 2} = 13.4$, $K_{abs} = 13.5$

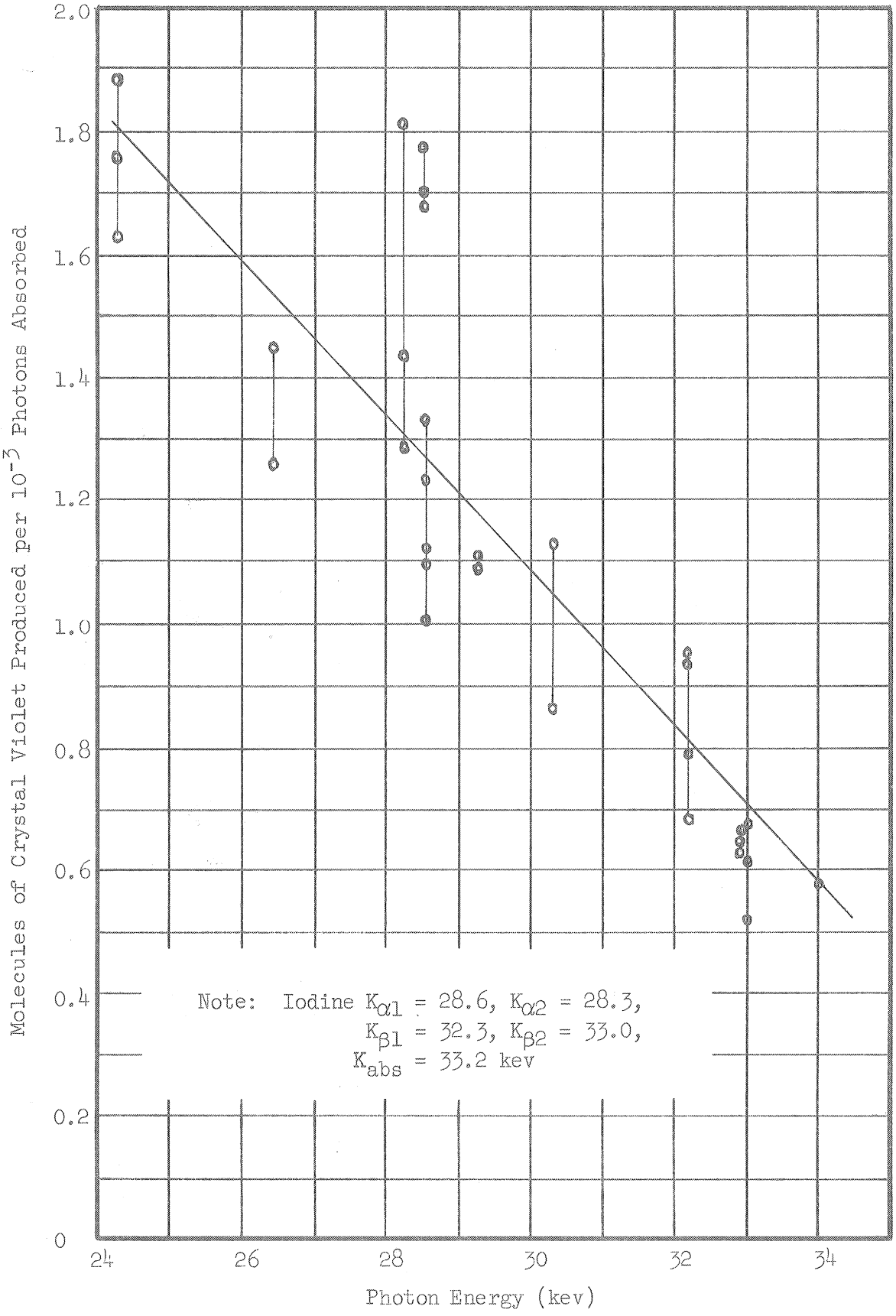

Fig. 1. Reproducibility of results as a function of energy for irradiations of portions of a film consisting of PS-MTD-CHI_3-CBr_4.

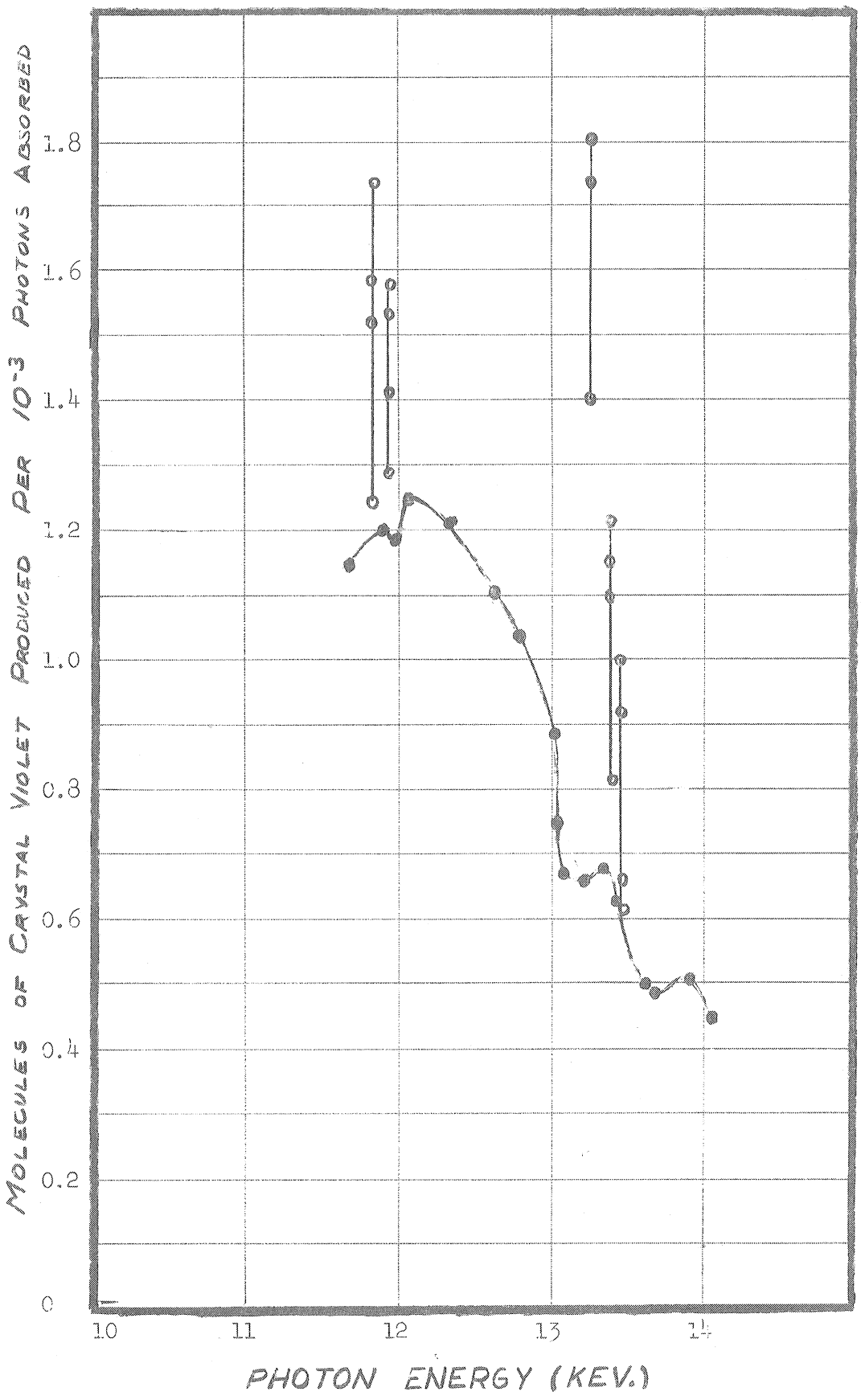

Fig. 2. Damage response as a function of energy for film consisting of PS-MTD-CHI_3-CBr_4. (a) Open circles: reproducibility of results for static irradiations of portions of a film. (b) Closed circles: response of a single portion of film to an oscillating irradiation.

If there are abnormal effects in these energy regions, such phenomena would be unique and unexplainable in terms of current theories. To investigate whether such effects do indeed occur, it was decided to center attention on systems that, perhaps, would tend to be more reproducible.

The remainder of this report describes the results obtained in studies of the catalase system, the n-butyl bromide system, and a system consisting of an organic mercury compound. In the process of investigating these systems, various techniques, a few of which are somewhat novel, have been perfected. To date, the most complete results have been obtained for the catalase system. These data, which are presented in the next section, also suggest, but with a higher degree of confidence, that abnormally large effects occur in the energy region of the iron K_α, K_β, and K absorption edge.

II. STUDIES OF THE EFFECTS OF MONOCHROMATIC RADIATION ON THE CATALASE SYSTEM*

INTRODUCTION

Catalase is a high-molecular-weight (225,000) enzyme whose most important property is the ability to catalyze the decomposition of hydrogen peroxide. The catalase molecule belongs to a general class of biologically important molecules whose function is attributed primarily to the trace quantity of a metal present in the molecule. Four iron atoms are incorporated in each molecule, a concentration of 0.09% iron by weight. The ability to cause destruction of hydrogen peroxide is attributed to this iron content.

It was the initial purpose of this study to attempt to determine if this assumption is correct. Specifically, it was decided to study the extent of damage to this molecule per proton absorbed in the energy region of the iron K absorption edge. If the iron is intimately associated with the biological activity of catalase, it is conceivable that a larger extent of damage per proton absorbed would occur at energies immediately above the iron K edge because a larger fraction of the energy absorbed per molecule is deposited in the iron atoms.

Radiation damage to this molecule has been defined in this experiment as the loss of catalytic activity with respect to hydrogen peroxide decomposition. Thus if the assumption is correct, the experimental results would serve as an indication of the extent of "damage" to those iron atoms or those portions of the molecule encompassing iron atoms which are "activity sites."

The amount of catalase in a sample, as well as the amount of catalase which did not undergo "radiation damage," was determined by measurement of the post-irradiation capability to cause catalytic decomposition of hydrogen peroxide. The catalase was dissolved in 1/15th molar phosphate buffer of pH 6.8. Measured amounts of hydrogen peroxide were then added and the transmittance of the mixture recorded automatically (at 2120Å) for a period of about one minute following the addition of the peroxide. Since the initial rate of decomposition of hydrogen peroxide by catalase is a first-order reaction,[2] it was possible to relate the concentration of catalase to these observed initial first-order rates. Catalase concentrations were measured by this method; the uncertainty in the measured values were ± 10% of the value.

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*More complete descriptions of experimental techniques and data can be found in the Ph.D. thesis of Ardath H. Emmons, filed with The University of Michigan, 1960, and available through University Microfilms, Ann Arbor, Michigan.

[2]Dixon, M., and Webb, E. C., Enzymes, New York: Academic Press, Inc., 1958.

The experiments were essentially of two types: those involving solutions of catalase and those involving samples of dry catalase.

CATALASE SOLUTIONS—DIFFRACTED X-RADIATION

Constant Absorbed Dose Irradiations.—A stock solution 10^{-7} molar in catalase and 1/15 molar in phosphate buffer was prepared. A lucite holder was constructed with two sample positions; one intercepted the radiation beam and the other was shielded from scattered X-rays. Samples of 0.18 ml were contained in these positions. The holder was cooled by circulating 5°C water and the whole assembly painted black to minimize enzyme activity loss induced by light.

With the X-ray tube operating at 30 pkv, samples were subjected to a selected X-ray energy (as obtained by NaCl diffraction). The irradiation at each energy was for a period such that 18×10^{11} X-ray photons were absorbed per cm^3. Photon detection was effected by means of the X-ray counter.

The dose rate was maintained at $9.5 \pm 1.0 \times 10^{10}$ photons incident per hour by adjustment of the X-ray tube current. Three 0.050-ml aliquots of the irradiated and of the control samples were then analyzed for catalytic activity. These data are plotted in Fig. 3 where each point represents the average of the measurements on the three aliquots of a given irradiation.

These data show an increase in effect as the photon energy crosses the iron K-absorption edge (7.11 kev). For comparison, a normalized mass absorption curve for iron is indicated by the broken curve in Fig. 3. Referring to Fig. 4, it is noted that, because of the very low iron concentration, the total mass absorption of the molecule and therefore of the solution is a reasonably smooth function in the energy region 5-12 kev. Hence, in terms of damage per photon to the catalase solution as a whole, no abnormal effect would be expected in this energy range. However, a larger fraction of the photons absorbed by the solution would be absorbed by the iron atoms for photon energies just above the iron K edge than for those just below this edge. Therefore, if damage per photon absorbed is measured in terms of the iron component of the molecule, a discontinuity could exist. A possible conclusion, therefore, is that damage to catalase is, as suggested, associated with the iron atoms in the molecule, and that this damage can be affected by selection of irradiation energy.

Possible Effect of Second-Order Radiation.—This increase in damage in the vicinity of 7 kev could perhaps be ascribed to effects of contaminating second-order diffraction lines. The $L_{\alpha 1}$ line of tungsten, the target material in the X-ray tube, is at 11.28 kev. Therefore, this could contribute a second-order line at 5.64 kev. Perhaps there exists a dependence of damage on energy that does not exhibit any discontinuities. If the extent of damage increases as the energy increases, an 11.28-kev X-ray would be more effective than a 5.64-kev X-ray, although the efficiency ratio is not known. On this basis, then, one would expect an increase in damage at the 5.64-kev crystal setting due to the 11.28-kev contribution.

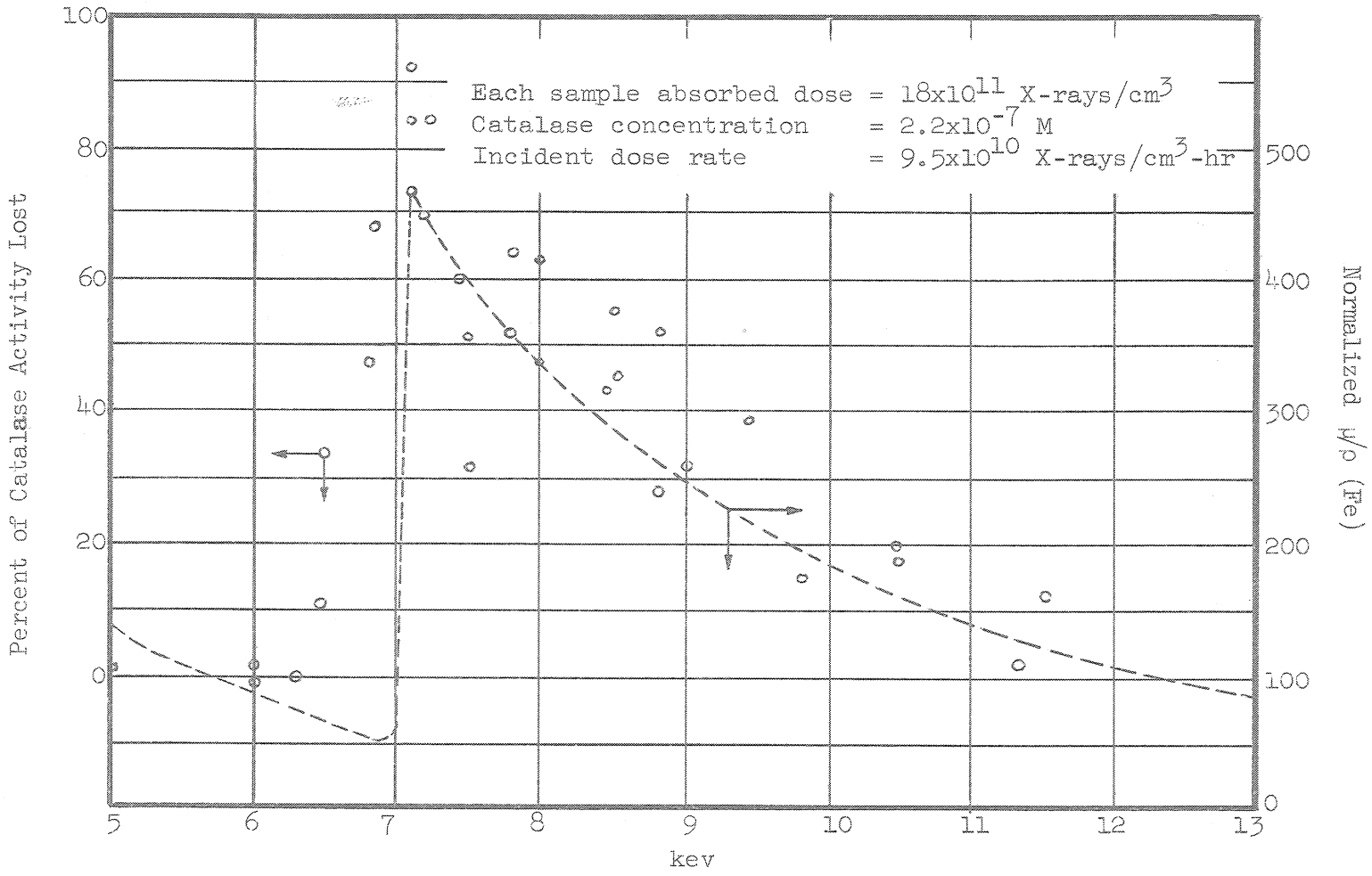

Fig. 3. Catalase solution, loss of catalytic activity when irradiated with selected energies of X-radiation. Normalized iron-mass absorption curve given by broken curve.

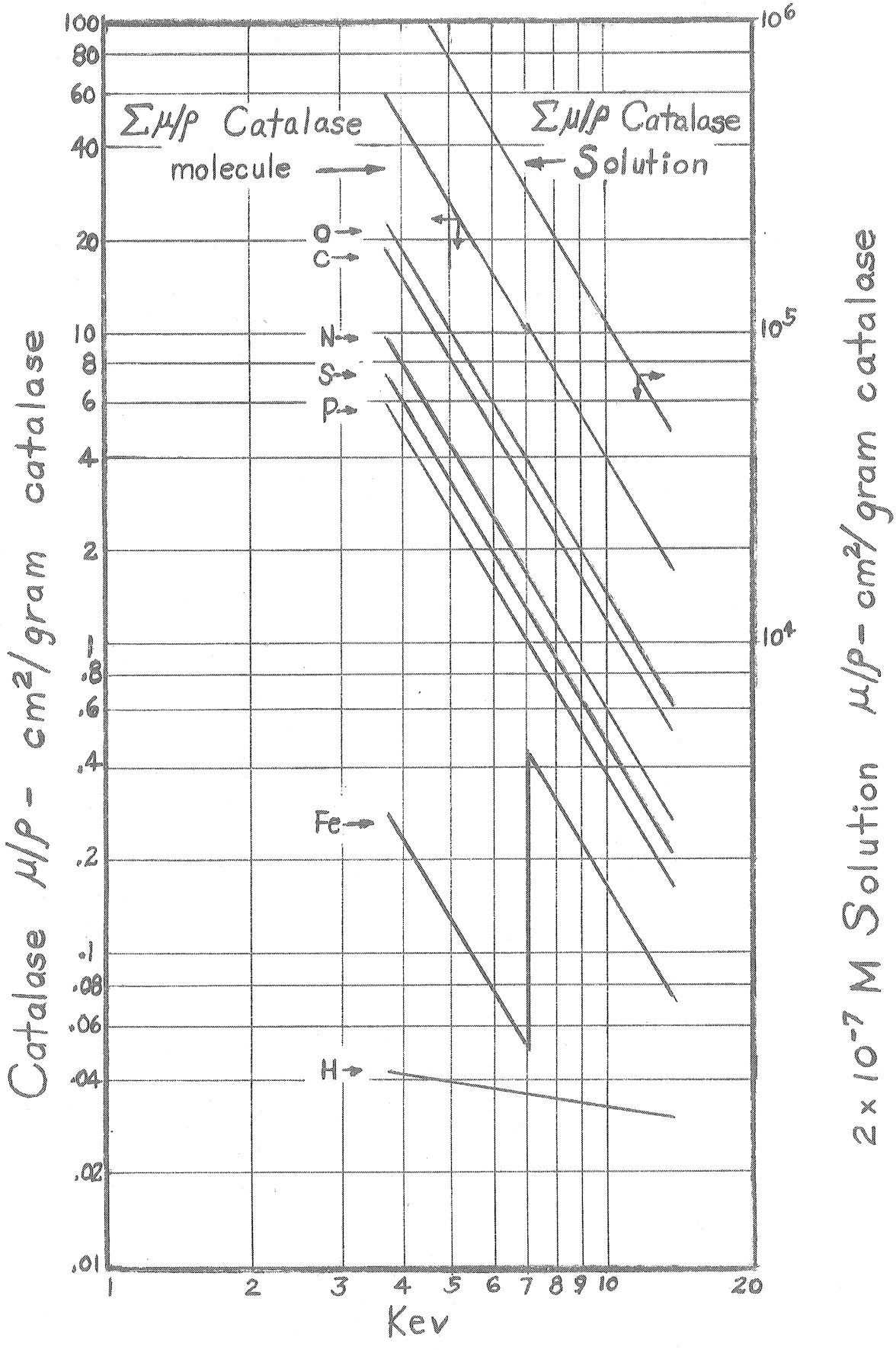

Fig. 4. Weighted and total mass absorption coefficient for catalase and catalase solution.

This possibility is, however, somewhat difficult to accept for three reasons:

First, the actual ratio of damage at 11.2 kev compared with 5.6 kev would have to be very large. According to the data of Fig. 3, this is not so. Second, the discontinuity in Fig. 3 appears to be nearer to 7 kev, even considering the large uncertainties in the measurements. Third, the observed data correlate reasonably well with the mass absorption curve for iron.

DAMAGE OF CATALASE SOLUTIONS AT 6.9 AND 7.3 KEV AS A FUNCTION OF PHOTONS ABSORBED

To minimize any possible effects due to second-order radiation, two additional sets of experiments were conducted at 6.9 and 7.3 kev. In these runs the excitation potential on the X-ray tube was reduced to 15 pkv, thus reducing any second-order contributions.

The damage results at these two energies as a function of total dose are presented in Fig. 5. The characteristic initial increase[3] in catalase activity displayed by irradiated catalase solution is also observed here.*

It is possible to compare qualitatively the data of Figs. 3 and 5. The data of Fig. 3 were obtained at a constant absorbed dose of 18×10^{11} photons per cm^3. If one assumes that no dose-rate dependence exists, the data of Fig. 5 may be interpolated at the level where 18×10^{11} photons were absorbed per cm^3.

The data for the 7.3-kev irradiations have been extrapolated (dashed curve) in terms of an exponential tailing, as is characteristic of such irradiations. Therefore, at the 18×10^{11} absorbed photon level, almost 100% of the catalase activity was lost when irradiation was with 7.3-kev photons and about -10% lost when the irradiation was with 6.9-kev photons. Qualitatively, then, these data of Fig. 5 are in agreement with the data of Fig. 3.

The data of Fig. 5, however, also provide a direct correlation of damage efficiencies at the two energies. Neglecting the characteristic initial increase in activity, it is noted that the linear portions of these two curves, when extrapolated, have the same intercept, indicating a consistent ratio of efficiencies and the lack of a "multi-hit" effect. The damage efficiencies may therefore be obtained from the ratio of the slopes. Such a comparison indicates

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[3] Forssberg, A., "Action of X-rays on Catalase and Its Biological Significance," Arkid. Kemi. Miner. Geol., 21A, 1 (1945).

*It was noted that this activation could be duplicated by adding micromole quantities of very dilute hydrogen peroxide to the catalase solutions prior to the analysis in which, as mentioned, macro amounts of hydrogen peroxide were used.

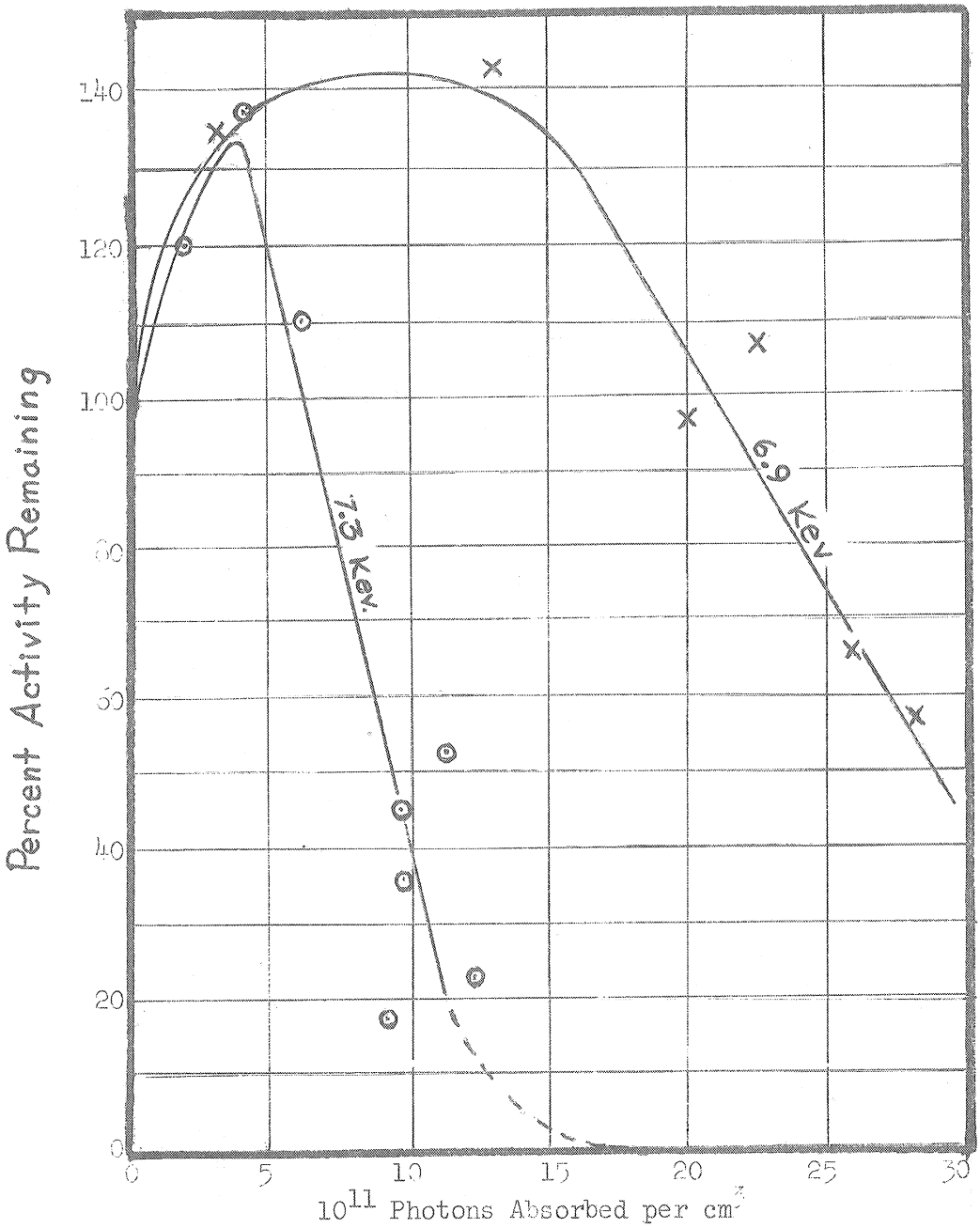

Fig. 5. Destruction of catalase solution with monochromatic X-rays of 6.9 and 7.3 kev. Catalase concentration: 2.2×10^{-7} M, incident dose rate: 2.5×10^{10} X-rays per cm^3 per hour, radiation obtained by NaCl crystal diffraction.

radiation at 7.3 kev is about 2.9 times as effective per photon in producing damage as is radiation at 6.9 kev.

X-RAY FLUORESCENT IRRADIATION OF CATALASE SOLUTIONS

To obtain higher dose rates and thus shorter irradiation times with the existing X-ray equipment, a series of irradiations was undertaken utilizing the fluorescent radiation emitted by a target. Three different targets were used. Since the K_β radiation of the target, which constitutes about 15% of the total radiation, was not filtered out in these experiments, these irradiations were only approximately monochromatic.

The three target radiators used and the energies of the emitted fluorescent radiation are given below:

Target Element	K_α (kev) ~ 85% of Total	K_β (kev) ~ 15% of Total
Iron	6.40	7.06
Nickel	7.47	8.26
Manganese	5.89	6.49

(Note: Iron K absorption edge is at 7.11 kev.)

The results of these irradiations, again showing the characteristic initial activation, are given in Fig. 6.

The extent of damage produced per photon for the catalase solutions irradiated with nickel and manganese fluorescent radiation appear, qualitatively, as would be expected on the basis of data of Figs. 3 and 5. The nickel radiation (which is of greater energy than the iron K absorption edge) is more effective than manganese radiation (energy less than iron K edge) in producing damage.

Possibility of Sharp Resonance Absorptions.—The results using iron fluorescent radiation are, however, completely unexpected. On the basis of the iron K absorption edge model indicated by the previous data, it would be expected that the iron radiation, being of an energy lower than the iron K edge, would exhibit a damage efficiency less than that of nickel radiation and of the order of that observed for manganese radiation.

This high efficiency of iron fluorescent radiation in producing damage in catalase solutions is inexplicable in terms of existing theories. Perhaps some type of resonance radiation absorption may occur when the iron-containing catalase is irradiated with iron fluorescent emission radiation.

Because of the nature of these results with iron fluorescent radiation, it is important to investigate further this possibility of increased damage due to resonance radiation absorption. It is planned to repeat this particular set of

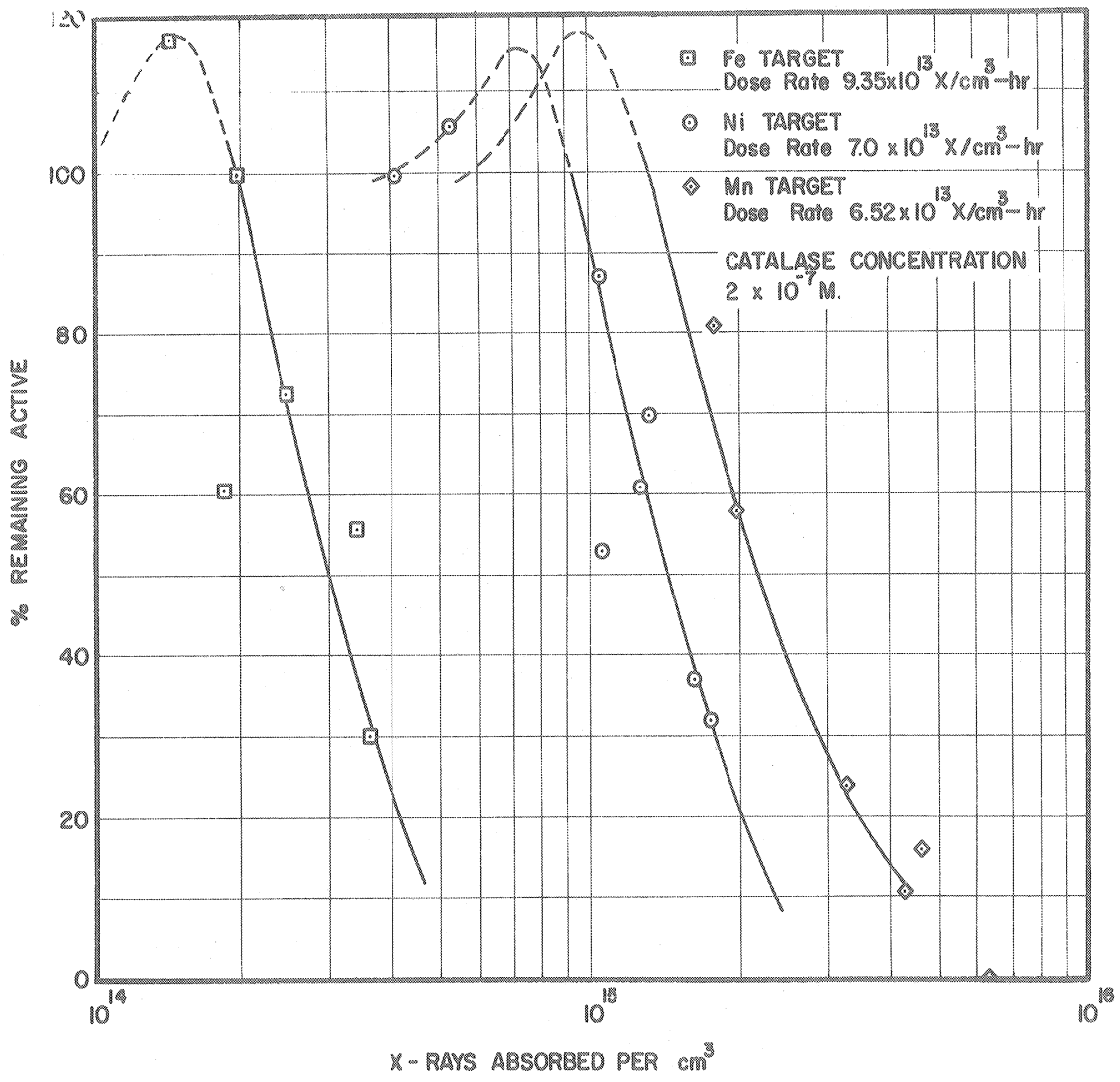

Fig. 6. Destruction of catalase solutions by target X-ray fluorescent radiation.

experiments as well as investigate the effects of other fluorescent radiation on catalase solutions.

COMPARISON OF RESULTS—CATALASE SOLUTIONS

The data of Fig. 6, for convenience, have been plotted as a function of the logarithm of the number of photons absorbed. If plotted on a linear scale (Fig. 7), variations exist larger than that apparent in Fig. 6. However, as was the case with the data of Fig. 5, these three sets of Fig. 7 could be approximated by a straight line. The relative slopes then serve as an indication of the relative efficiencies of the fluorescent radiation in producing damage. These damage per photon efficiency ratios are approximately:

$$\frac{\text{Nickel}}{\text{Manganese}} = 1.7 \; ; \qquad \frac{\text{Iron}}{\text{Manganese}} = 6.6 \; ; \qquad \frac{\text{Iron}}{\text{Nickel}} = 3.9 \; .$$

This nickel-manganese ratio, being an indication of the ratio of efficiency of damage of photons of energy above the iron K absorption edge to photons of energy less than this K edge, is in reasonable accord with the 7.3-kev—6.9-kev data (efficiency ratio = 2.9) of Fig. 5.

Again the ratios involving the iron fluorescent radiation suggest the presence of some unexpected phenomenon. Since both the iron and manganese radiation consist of photons whose energy is less than that of the iron K absorption edge, one would expect comparable efficiencies in producing damage. As stated above, the observed ratio of iron-manganese is about 6.6.

X-RAY FLUORESCENT IRRADIATIONS OF DRY CATALASE

In an attempt to minimize possible effects due to free radicals produced in the aqueous solvent, as well as to increase the catalase target concentration, a series of irradiations was performed using dry catalase as the target material.

Irradiation was by means of target fluorescent emission. A Fricke-ferrous sulfate dosimeter was used for absorbed dose and dose-rate calibration. After irradiation, the sample was dissolved in 50 ml of 1/15th molar phosphate buffer.

Chromium, nickel, iron, and manganese target radiators were used in these experiments. The chromium radiation consists of about 85% K_α radiation of energy 5.41 kev and about 15% K_β radiation of energy 5.94 kev. The radiation energies for the other three targets are listed on page 17. Dose-rate data for these irradiations, obtained using a Fricke dosimeter, are listed in Table III.

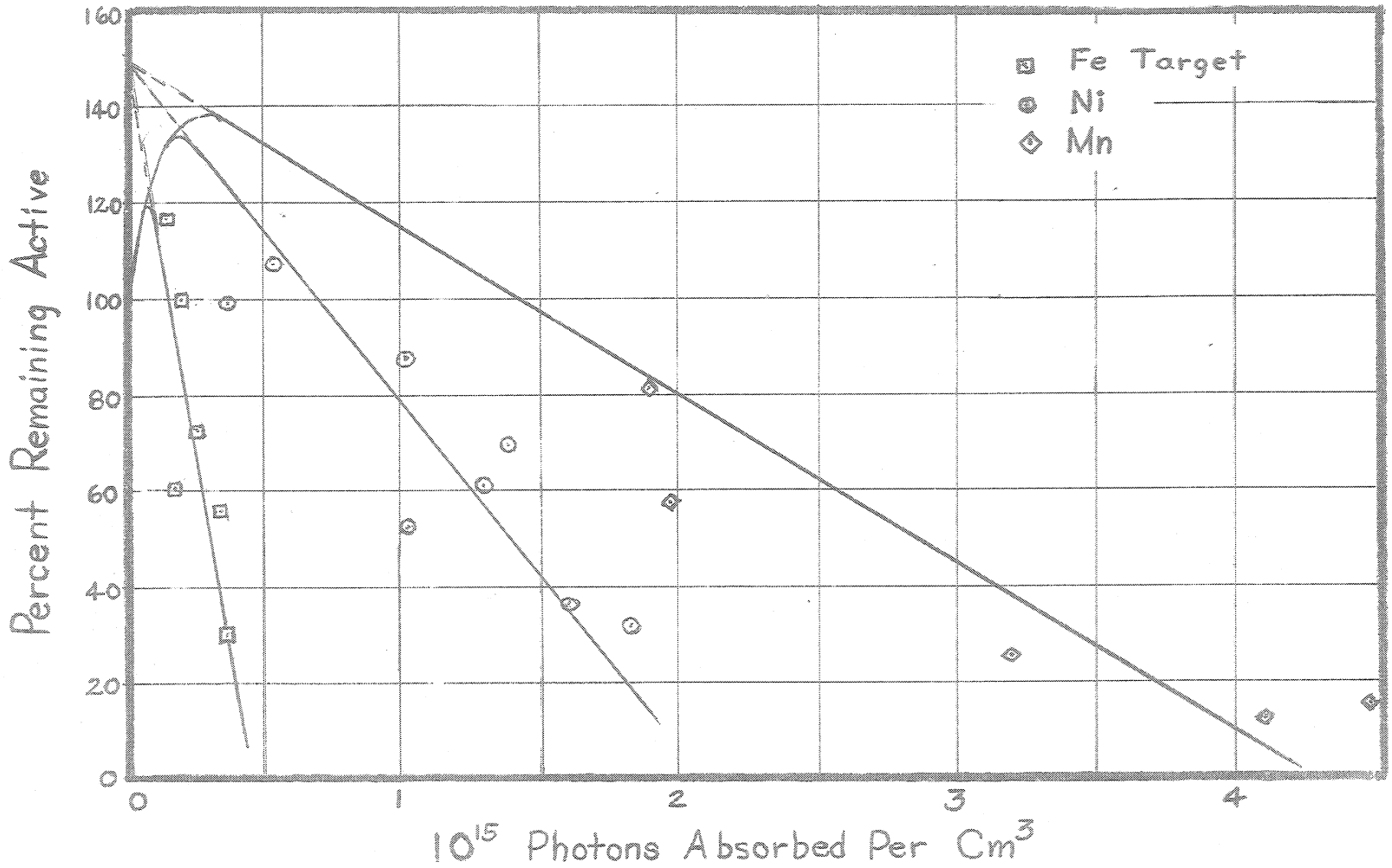

Figure 7. Destruction of Catalase Solutions by X-ray Fluorescent Radiation. Same data as Figure 6.

TABLE III

DOSE-RATE DATA, DRY CATALASE IRRADIATIONS

Target Fluorescent Radiator	Average Dose Rate	
	Ergs Absorbed per gm-hour	Photons Absorbed per gm-hour
Nickel	3.8×10^6	3.1×10^{14}
Iron	4.0×10^6	3.8×10^{14}
Manganese	2.6×10^6	2.7×10^{14}
Chromium	1.8×10^6	2.0×10^{14}

Chromium and Nickel Irradiations.—Chromium fluorescence is of energy less than the iron K absorption edge whereas the nickel is of energy above the iron edge. If these data are examined in terms of the logarithm of the percent remaining activity as a function of energy absorbed (Fig. 8), the damage response appears to be a linear function and it is seen that apparently the nickel and chromium fluorescent radiation are equally effective per unit energy absorbed in damaging catalase. Thus it appears that the damage response is not a function of energy, at least for the photon energies emitted by these two radiators.

Manganese Irradiations.—The irradiations performed using manganese fluorescent radiation are depicted in Fig. 9 by the same two-slope curve ascribed to the iron-target irradiations. It is open to question whether such a two-slope representation for the manganese data is valid, especially since only one datum point exists with which to identify the initial "induction period." It is possible, therefore, that these manganese irradiations result in a damage per unit energy absorbed similar to that observed with the nickel and chromium radiation. Further experiments will provide a more exact representation.

Iron Fluorescent Irradiation of Dry Catalase.—The two-slope representation for the iron fluorescent irradiations (Fig. 9), however, appears to be real. As was the case in the irradiations of catalase solutions, here again the iron K_α and K_β radiation appear to produce completely inexplicable effects.

Radiation induction periods, as apparently present in this instance, have been observed by various experimenters working with other systems. This induction dose of radiation prior to the appearance of damage was ascribed to a multi-hit effect. In view of the lack of knowledge of the mechanism resulting in radiation damage to catalase, it is difficult to postulate a mechanism based on a multi-hit phenomenon.

Perhaps even more significant is the steep response curve following the induction region in these iron fluorescence radiations. It is particularly difficult to explain why, in a system for which a given group of energies (nickel

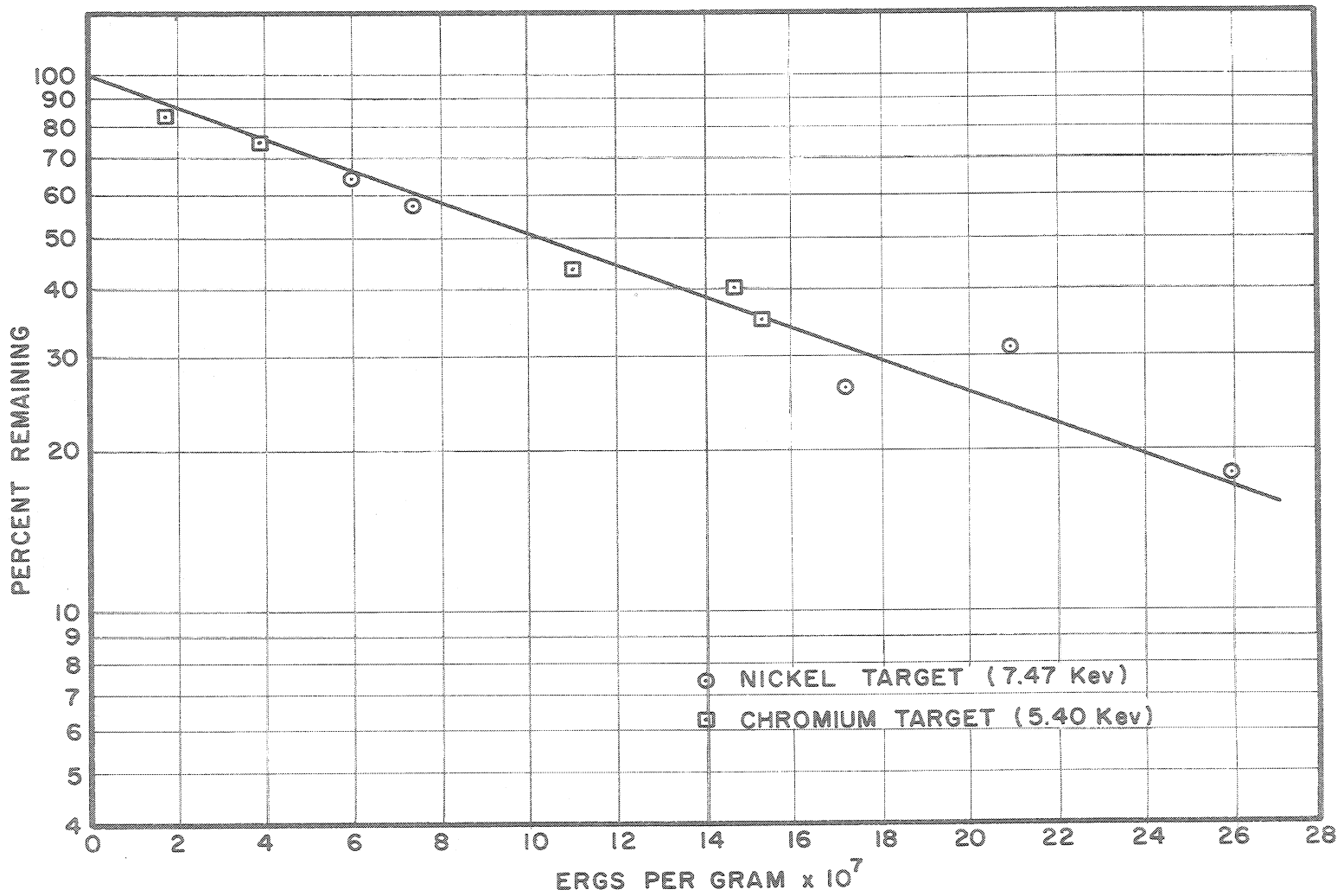

Fig. 8. Destruction of dry catalase by X-ray fluorescent radiation from nickel and chromium targets.

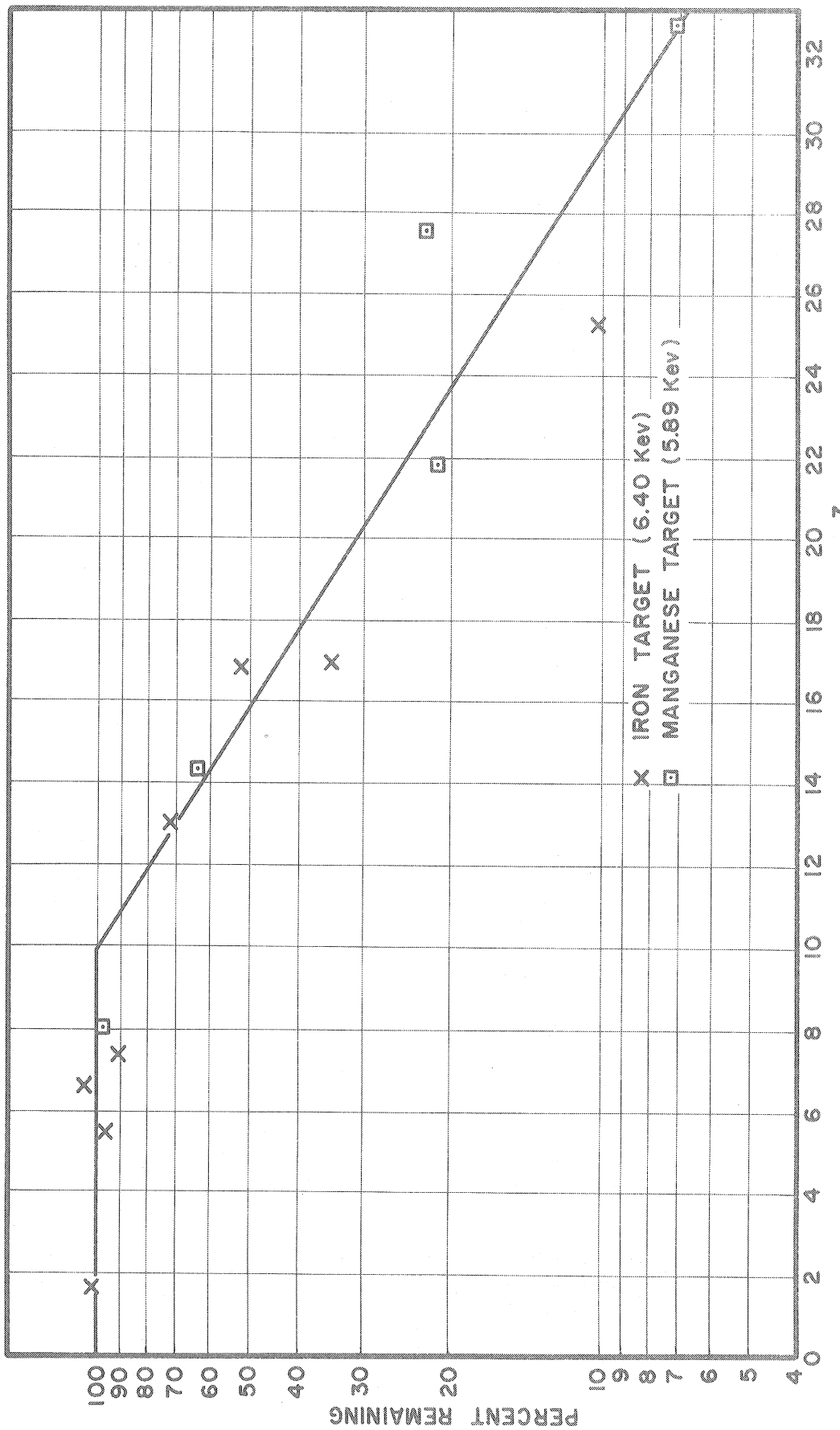

Fig. 9. Destruction of dry catalase by X-ray fluorescent radiation from iron and manganese targets.

and chromium radiation) shows a one-hit response, iron fluorescent energies show a multi-hit response with what becomes a much higher (steeper slope) ultimate sensitivity.

DISCUSSION OF RESULTS—CATALASE SYSTEM

In attempting to interpret fully the significance of these and other results, cognizance must be taken of the number of molecules damaged per photon and the existence of any dose-rate dependency.

Molecules Damaged per Photon.—The determination of the number of molecules damaged per photon will depend upon the accuracy of the dosimetry measurements. As evident from Table I, an approximately tenfold difference in sensitivity existed between the Fricke-ferrous sulfate and the SPG-1 dosimetry measurements. Therefore any calculations of the number of catalase molecules destroyed per photon would be only approximations of the true value. When the total absorption thermal dosimeter, mentioned earlier, is completed, it should be possible to determine accurate values of these photon damage efficiencies. On the basis of the present dosimetry measurements, values of these efficiencies have been calculated for the 50% damage level (Table IV).

In the irradiations of catalase solutions, it can be calculated from the mass absorption coefficients (see Fig. 4) that only one photon is absorbed by an iron atom for approximately every 10^6 photons absorbed by the solution. Therefore one would not expect any observable difference in damage efficiency as the iron K absorption edge was crossed unless the photon damage efficiency was of the order of 10^{-6} molecules damaged per photon absorbed. The lowest damage efficiency for solutions, as shown in Table IV, is 0.028. Therefore on the basis of conventional interpretations of photon damage efficiencies, most of the catalase molecules are being damaged as a result of secondary radiation-induced processes. Thus, the discontinuity at the iron K absorption edge, and, more important, the apparent greater efficiency of iron fluorescent radiation in producing damage in catalase solutions become even more puzzling.

In the irradiation of dry catalase, referring to the mass absorption data of Fig. 4, it can be calculated that, at energies below the iron K absorption edge, one photon is absorbed by an iron atom for every 190 photons absorbed by the molecule. Above the iron K edge the relative ratio is 1 in 24. Here again the data for the nickel and chromium irradiations given in Table IV indicate that most of the molecules are damaged as a result of secondary energy-transfer processes. For the iron irradiations, if one examines only the steep portion of the curve in Fig. 9, it can be calculated that at the 50% damage level 235 molecules are destroyed per photon absorbed. If one includes in the calculation the photons absorbed during the induction period, the value is 88 molecules destroyed per photon absorbed. In either case these data regarding the irradiation of dry catalase are of the same order of magnitude in terms of photon damage efficiencies. Therefore because of the large magnitude of these efficien-

TABLE IV

NUMBER OF MOLECULES DAMAGED PER PHOTON
ABSORBED IN VARIOUS CATALASE IRRADIATIONS*

Catalase System	Molecules Damaged Per Photon Absorbed	Dosimeter Used	Molecules Damaged Per Photon Absorbed in Terms of Ferrous Sulfate Dosimetry
Solution, 6.9 kev Fig. 5	23	SPG-1 X-ray Counter	~ 2.3
Solution, 7.3 kev Fig. 5	70	SPG-1 X-ray Counter	~ 7.0
Solution, Iron Radiation, Fig. 6	0.20	Fricke	0.20
Solution, Nickel Radiation, Fig. 6	0.046	Fricke	0.046
Solution, Manganese Radiation, Fig. 6	0.028	Fricke	0.028
Dry, Nickel Radiation, Fig. 8	160	Fricke	160
Dry, Chromium Radiation, Fig. 8	114	Fricke	114

*Calculated at the 50% damage level.

cies it again appears puzzling that two types of response should be observed for the irradiation of dry catalase.

Dose-Rate Dependence.—Associated with these observations are the possibilities of variations in response due to variations in dose rate. A series of samples of catalase solution were irradiated with cobalt-60 gamma radiation. The dose required to damage 50% of the catalase proved to be a function of the dose rate. The extent of damage, however, was found to be relatively independent of concentration. These data are presented in Table V and Fig. 10.

It is quite possible that the difference in damage efficiencies for the irradiation of catalase solutions at 6.9 and 7.3 kev as compared with the fluorescent irradiations (see Table IV) could be due to a dose-rate dependency.

If we assume that the irradiations at 6.9 kev are similar in effect to the irradiations using a manganese target and that the 7.3-kev and nickel-target irradiations are also similar, we may then calculate the ratios stated in Table VI.

Three observations may be made in regard to these data. First, it is probably not vastly significant that the values in the last column are not in more perfect agreement; a variety of experimental data are involved in calculating these ratios. Second the dose-rate dependence in this X-ray energy region is much smaller than when irradiation is by means of cobalt-60 gamma radiation (see Fig. 10). Third, the observation of the much greater damage response of catalase solutions to iron fluorescent radiation cannot be ascribed to the small dose-rate differences between the iron, manganese, and nickel irradiations (data given in Fig. 6).

These irradiations of catalase solutions utilizing iron fluorescent radiation were at a slightly higher dose rate than those utilizing the manganese or nickel radiation. Therefore, on the basis of a dose-rate dependence, it would be expected that the iron irradiations would require the absorption of a slightly larger number of photons to damage 50% of the molecules, an effect opposite to that observed.

Although no dose-rate-dependence studies were made on dry catalase, it is conceivable that only a small dependence would also exist.

In summary, it appears that the peculiar damage response of dry catalase or catalase solution to iron fluorescent radiation is not a result of a dose-rate effect. These abnormal effects in response, as well as the apparent iron K absorption edge effect in catalase solutions, appear even more puzzling since apparently most of the catalase molecules are damaged as a result of some secondary process.

Further studies will be performed in an effort to determine, if possible, the nature of the response of catalase to radiation. One aspect of these studies that has been annoying, as well as of concern, is the time required for a

TABLE V

DESTRUCTION OF CATALASE SOLUTIONS AS A FUNCTION OF DOSE RATE

(Cobalt-60 Gamma Source)

Dose Rate, rep/hr	Concentration, mgm/L	Rep Dose to 50% Destruction	Ergs/gram 50% Destruction
650	43.6	1,000	1×10^5
7,800	16.8	7,800	7.8×10^5
86,000	57.6	60,000	6×10^6
131,000	100	85,000	8.5×10^6
131,000	50	75,000	7.5×10^6
1,100,000	18.8	196,000	1.96×10^7

TABLE VI

RATIO: DOSE RATES AND PHOTON DAMAGE EFFICIENCIES, CATALASE SOLUTIONS

	Ratio: Dose Rates	Ratio: Photons Absorbed to Produce 50% Damage
Manganese 6.9 kev	2700	80
Nickel 7.3 kev	2800	150

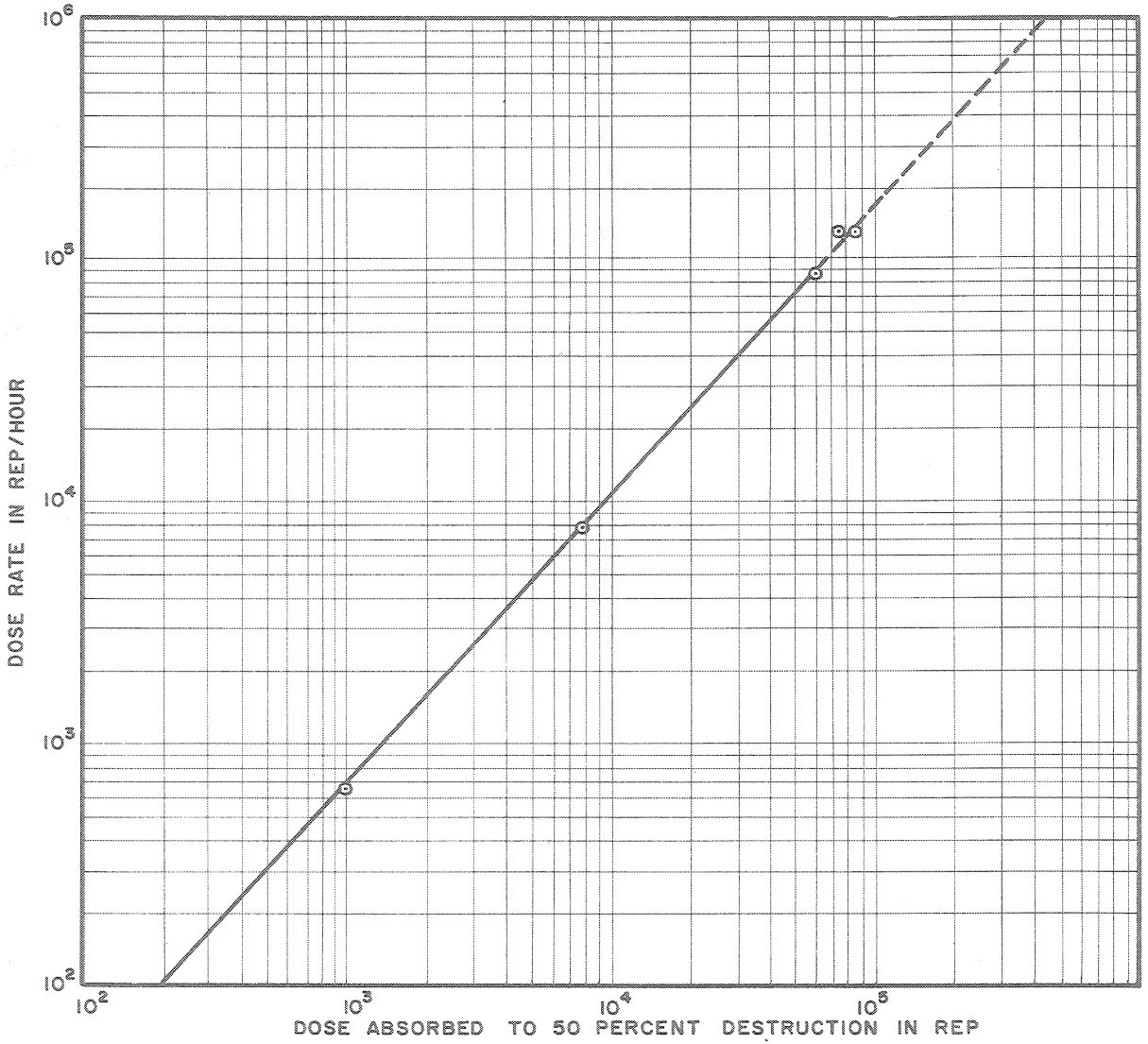

Fig. 10. Destruction of catalase solutions as a function of dose rate—cobalt-60 gamma source.

single irradiation. Irradiation times of from 10 to 50 hours were not uncommon. Because catalase solutions were found to be easily "damaged" by light and heat (see Figs. 11 and 12), it was necessary to observe extreme caution in the handling of the samples. Control samples were used in the irradiations, thus hopefully compensating for any nonirradiation effects, such as the time of standing. The solutions were maintained at 5°C and shielded from light during the irradiation. The samples of dry catalase were irradiated at approximately room temperature but, again, control samples were used.

Ideally, to minimize any possible nonradiation effects, it would be desirable to irradiate the samples for much shorter periods. This would be possible if higher dose rates could be obtained. Unfortunately, higher dose rates are not obtainable with the X-ray unit used in this study. It would be desirable, therefore, to use a new X-ray unit capable of a higher emission flux. We are currently looking into the possibility of such an acquisition.

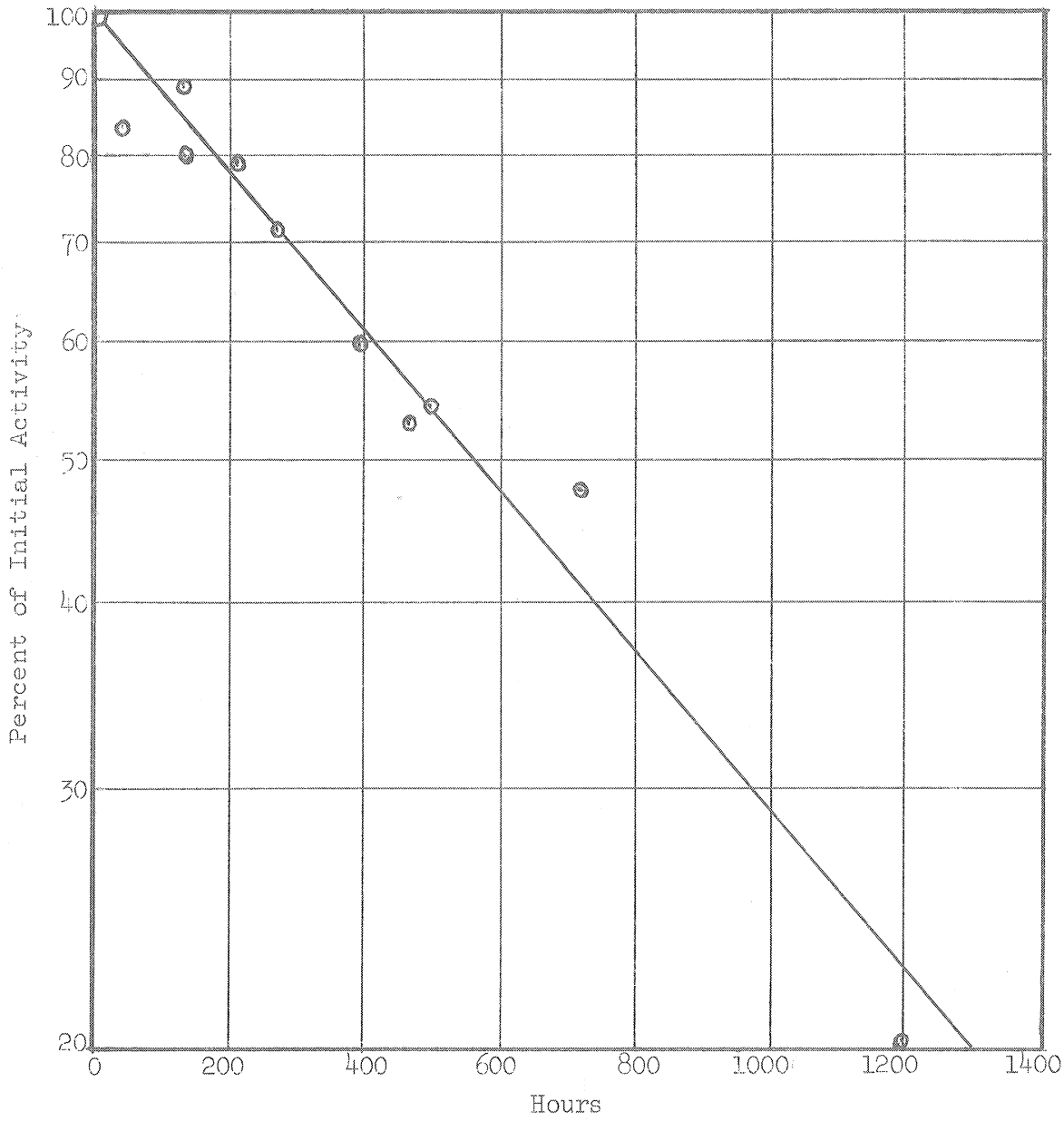

Fig. 11. Catalase solution loss of enzymatic activity at 5°C and shielded from light. Concentration = 2.2×10^{-7} M.

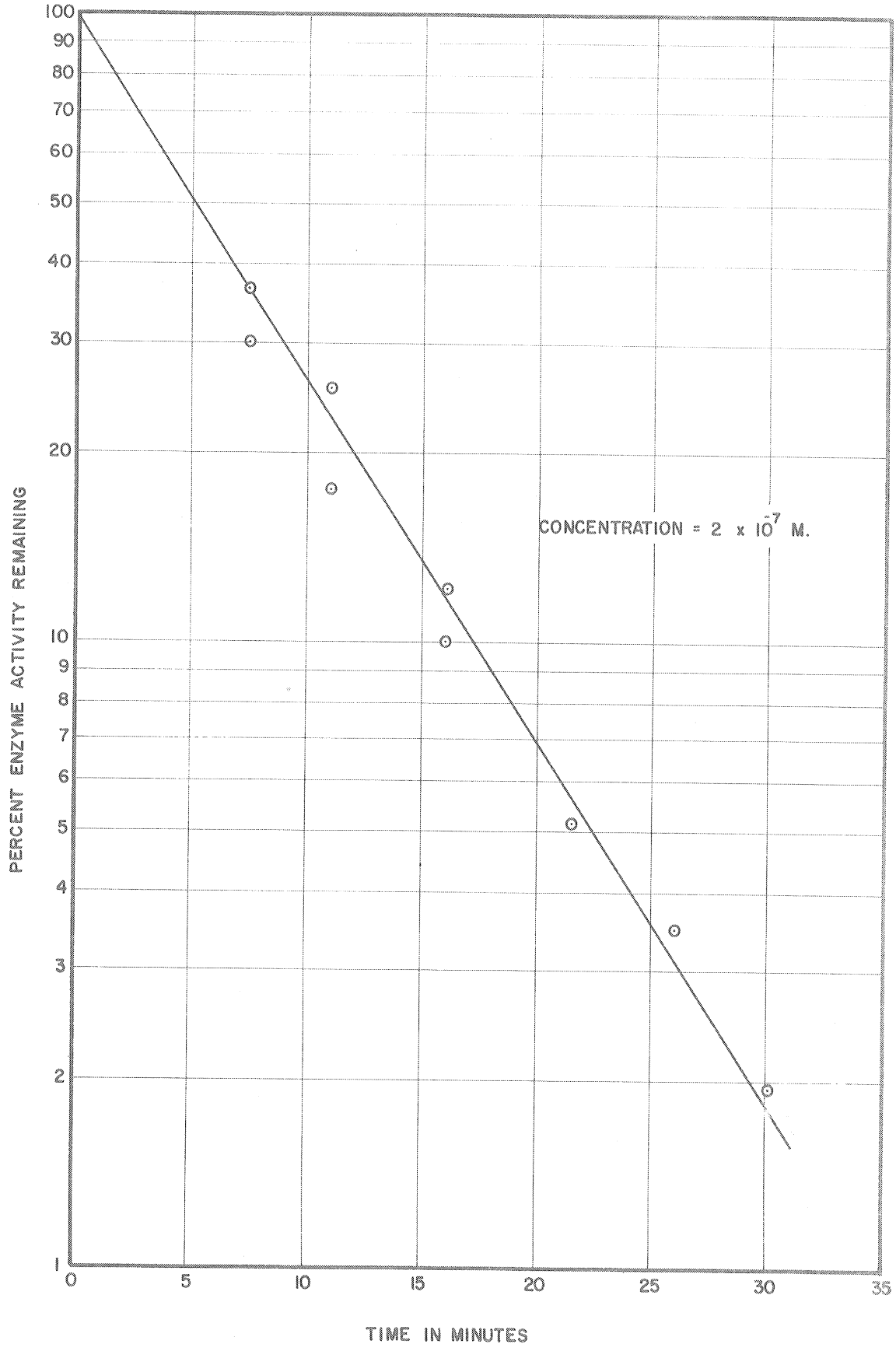

Fig. 12. Catalase solution loss of enzymatic activity at 24°C and in sunlight.

III. THE n-BUTYL BROMIDE SYSTEM

Samples of purified liquid n-butyl bromide containing DPPH (diphenylpicylhydrazl) at a concentration of 1.2×10^{-7} M were irradiated with fluorescent radiation of various energies in the range of from 5 to 25 kev, the bromine K absorption edge being at 13.48 kev. Damage to this system was determined in terms of the amount of free radicals produced as indicated by "loss" of DPPH scavenger. The amount of free DPPH remaining in a sample after irradiation was determined spectrophotometrically at 5200Å using a Beckman DU spectrophotometer. The Beckman was specially equipped to utilize micro-cuvettes to measure the optical density of extremely small volumes (0.150 ml) of sample solution.

Preliminary studies indicated that this system is reasonably insensitive to dose rate and DPPH concentration. A twentyfold increase in dose rate resulted in less than a twofold decrease in damage per unit energy absorbed. A tenfold increase in DPPH concentration resulted in less than a twofold increase in damage per unit energy absorbed. Apparently, then, the DPPH is scavenging successfully most of the free radicals formed. Dose rates of 1 to 8×10^9 photons absorbed per second, as determined using the SPG-1 proportional counter, were used in all irradiations.

Preliminary experimental results do not indicate any large anomalous damage response at the bromine K absorption edge. These preliminary results may be depicted, qualitatively, by the straight line plot of Fig. 13.

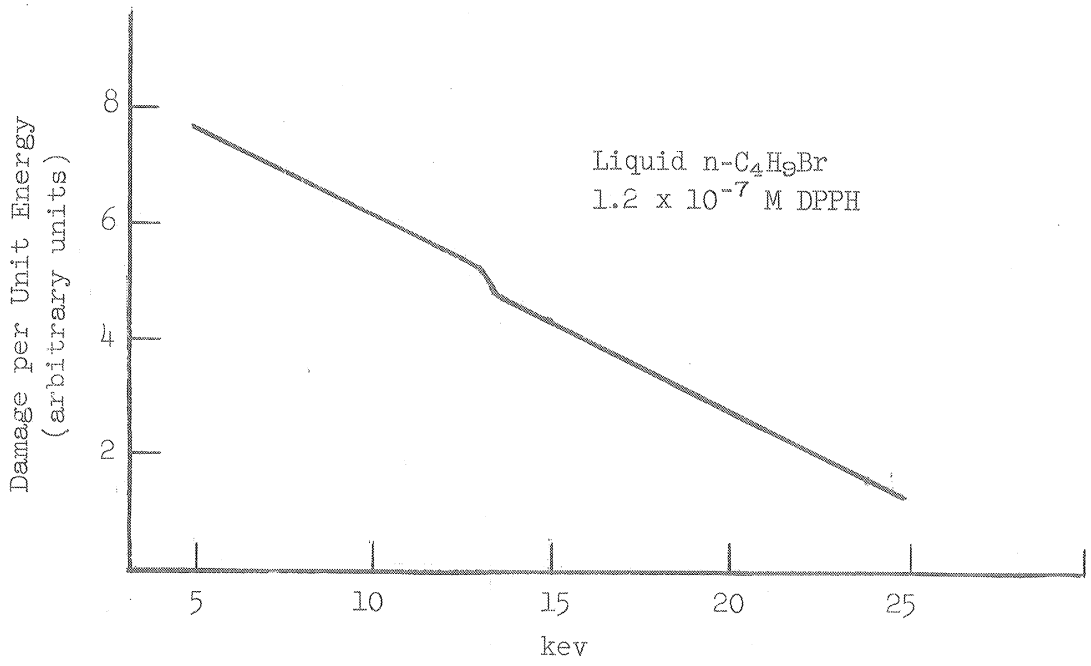

Fig. 13. Damage per unit energy (in arbitrary units) as a function of fluorescent X-radiation energy.

The reason for the negative slope in Fig. 13 is not known. It is possible that it is a reflection of dosimetry dependence. The (possible) slight decrease in damage efficiency at energies greater than the bromine K absorption edge may be due to loss of bromine K_α and K_β fluorescent radiation generated within the sample.

It should be noted that the absorption coefficient of n-butyl bromide depends largely on the absorption of the bromine atom. Below 13.48 kev, approximately 96% of the photons are absorbed by the bromine atoms, whereas above 13.48 kev more than 99% are absorbed by the bromine atoms. Thus a change at the bromine K absorption edge in damage per photon absorbed or per unit energy absorbed could also result if in some manner absorption in the bromine K shell, with its subsequent K fluorescence or Auger electron emission, results in the production of an amount of bromine atoms different from that resulting from L shell absorption.

Future experiments will be directed toward a more precise determination of the magnitude of the discontinuity in response at the bromine K absorption edge. These data will provide a basis on which to determine whether the loss of fluorescent radiation will account for the magnitude of the discontinuity.

IV. AN ORGANO-MERCURY SYSTEM

Samples of solid α-acetoxymercuri-β-methoxy-hydrocinnamic ethyl ester were irradiated with filtered and mixed (K_α, K_β) fluorescent radiation in the energy region of from 9.2 to 17.5 kev. The mercury L_{III} adsorption edge is at 12.282 kev. Damage was defined in terms of the amount of free mercury produced as a result of an irradiation. It was obtained by dissolving the compound in absolute alcohol and centrifuging the mercury. The mercury was then dissolved in concentrated H_2SO_4-HNO_3 and the amount of mercury produced (ca. 0.5 mg) was determined spectrophtometrically using dithizone complexing agent.

The compound R-Hg contains 40 weight percent mercury. In the energy region studied, the mercury atoms absorbed a minimum of 97% of the photons. Within experimental error, the damage per unit energy absorbed as a function of energy appears to be linear with no discontinuities at the mercury L_{III} absorption edge.

APPENDIX

X-RAY ENERGY RESOLUTION

In the plastic film and catalase experiments utilizing diffracted X-radiation, it was necessary to use fairly large slit widths to obtain a dose rate sufficiently high so that an irradiation could be performed in a reasonably short time. Even with the slit widths used, many irradiations were of 50 to 100 hours duration. Because of the large sample and therefore large slit widths used, the energy spectrum imparted to these samples included approximately the range of 0.95 E to 1.05 E, where E is the energy calculated from the X-ray Bragg angle setting.

For any given angle, and thus average energy, about 90% of the total energy arriving at the sample was found to be contained in a $2\theta = 5.0°$ Bragg angle range centered on the sample, as indicated by the data given in Fig. 14.

Percent of Total Energy	3.5%	23%	37%	23%	3.5%	
2θ Bragg Angle	$-\frac{5}{2}°$	$-\frac{3}{2}°$	$-\frac{1}{2}°$	2θ	$+\frac{1}{2}°$	$+\frac{3}{2}°$	$+\frac{5}{2}°$
Energy spectrum at certain angle settings			7.11			
	6.90	6.97	7.06	7.17	7.26	7.36
			13.48			
	12.61	12.95	13.29	13.63	14.01	14.39
			33.17			
	28.4	30.1	32.1	34.3	36.8	39.7

Note: K absorption edge, kev: iron = 7.11, bromine = 13.48, iodine = 33.17

Fig. 14. Percent of energy absorbed by a sample in $2\theta = 1°$ Bragg angle regions centered about the Bragg angle 2θ.

To obtain more nearly monochromatic radiation, a target fluorescent radiation technique was utilized. In these irradiations the X-rays emitted by a target radiator consisted of primarily 85% K_α and 15% K_β fluorescent emission. Dose rates were generally 10^3 to 10^4 greater than that obtained by the diffraction method.

The K_β radiation could be reduced preferentially by interposing a filter, generally of atomic number one less than that of the fluorescent radiator. The

K_α radiation is reduced in intensity by a factor of approximately three while the K_β is reduced in intensity by a factor of about 50 to 100. As a result, the filtered raidation is composed of about 99 to 99.5% K_α radiation. The dose rate is reduced only by a factor of about three.

Such filtered fluorescent irradiations will be utilized in analyzing further the damage response of the catalase, n-butyl bromide and organo-mercury systems.

In the energy region of from 5 to 35 kev, 35 K_α emission energies exist. It must be recognized, therefore, that the use of this fluorescent technique limits the investigations to only specified energies. For many studies, this number of discreet energy irradiations could, however, provide sufficient data with which to investigate the damage response of a system. More important, the existence of K_α and K_β resonance radiation effects, as suggested by the catalase system data, would be apparent only when irradiation was by means of target fluorescent radiation.