I. THE EFFECTS OF IONIZING RADIATION ON PLANTS: BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS

By SOLON A. GORDON

Division of Biological and Medical Research, Argonne National Laboratory

THIS symposium is addressed to a group heterogeneous in respect to biological discipline. To my knowledge it is also the first symposium in the AIBS on the effects of ionizing radiation on plants. It seemed appropriate, therefore, to make this first paper a generalized discussion aimed primarily at the plant biologist and not the radiation biologist. First, I will speak briefly on what ionizing radiation is; second, on what is thought to happen when such radiation is absorbed by relatively simple systems; and finally, on how several biochemical and physiological responses of the irradiated plant might be associated.

Attracting the interest of the plant worker to these phenomena is more than an abstract consideration. For example, I recall the recent Radiation Research Society meetings at which there was a symposium on radiation effects in biological systems. It was readily apparent that little work had been done on biochemical responses of the plant to ionizing radiation, and not very much more on physiological effects. It might be pointed out, of course, that the study of plant responses to ionizing radiation is a relatively new field, and that biological disciplines generally begin at a gross descriptive level. Visible changes are nevertheless terminal manifestations of micro-events; the sequence of phenomena that occurs when the organism is exposed to high energy radiation may be represented as in Fig. 1. Initially there is a change at the submolecular level of organization. If this change is not reversed within a relatively short time, altered physico-chemical patterns ensue either directly, or via modification in genic structure or relationship. This, in turn, gives rise to physiological changes that find expression in a gamut from minor alterations in form to death—the inability to reproduce at any organizational level. Obviously, kingdom demarcations between the plant and animal tend to disappear in moving from right to left in the sequence. As was implied a moment ago, the major fraction of the work on plant responses to ionizing radiation has been with morphological changes at the cellular, tissue or organ level.

I have used interchangeably the terms "high energy" and "ionizing" radiation. What distinguishes this type of radiation from other types?

Radiation may be considered as the movement through space of energy, in either corpuscular or electromagnetic form. Corpuscular radiation consists of streams of atomic or sub-atomic particles that can transfer their kinetic energy to any matter with which they collide. The particles may be negatively charged, such as the electrons in beta rays, positively charged, as the helium nuclei of alpha rays, or electrically neutral, as the neutrons. The energy of the particle is largely determined by its velocity. Its interaction with matter depends principally upon the atomic structure of the matter. Electromagnetic radiation, on the other hand, may be thought of as a self-propagating stream of particles, called photons, that possess zero rest mass and no charge. It travels with a constant velocity, the velocity of light. In motion, however, it behaves as if it were a series of waves, with both electrical and magnetic components; its energy depends on the frequency of vibration of these waves. Radio waves, visible light, x- and γ-rays are fundamentally similar classes of electromagnetic radiation that are separated on the basis of their respectively increasing vibration frequencies.

According to the principle established by Grotthus, only radiation which is absorbed can be chemically active. When radiation is absorbed, the energy transfer seems to occur in discrete packets. The photon or quantum is the amount of energy capable of being transferred on absorption of one of these energy packets.

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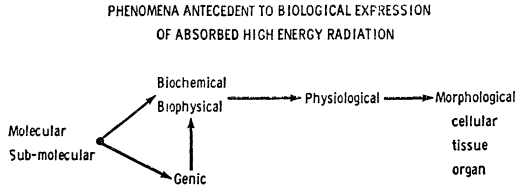


FIG. 1. PHENOMENA ANTECEDENT TO BIOLOGICAL EXPRESSION OF ABSORBED HIGH ENERGY RADIATION

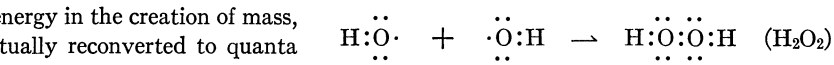
What happens to a molecule "excited" by the absorption of a photon? The energy can be dissipated by motion or thermal vibration. Or the energy can be liberated by fluorescence—the emission of another photon lower in frequency. If the total energy is high enough, it may be dissipated, in part, by ejection of an electron from the absorbing atom or molecule. In other words, an ionization or separation of electron charges results. I mentioned a moment ago that as we go from radio waves to light to x-rays in the electromagnetic spectrum the vibration frequency rises. But as the frequency rises, the quantum energy rises proportionately according to the relation $E = h\nu$, where h is a constant and ν is the frequency. Ionizing radiation is simply that portion of the spectrum where the photon frequency is high enough so that photon absorption results in a charge separation in the absorbing entity, generally by electron ejection.

A certain minimum amount of energy is required to overcome the force binding an electron to an atom or one atom to another. The lower radiation frequencies, radio waves and visible light, are not able to knock out electrons from substances of biological interest. As we move to frequencies over 10^{17} cycles per second, we move into a succession of electron-emitting phenomena in the x-ray range. First the photon gives up virtually all of its energy to an atomic electron, which is then ejected. As the frequency rises, more and more photons rebound from collision with electrons as scattered radiation of lower energy; the energy lost is transferred to the electron thrown out by collision recoil. Very high frequency photons above 10^{21} cycles per second (energies above, roughly, 10^6 electron volts) can lose their energy in the creation of mass, the mass being eventually reconverted to quanta of x-radiation. Corpuscular radiation of sufficient energy also produces ionization. It may do so directly by displacement of shell electrons, or by knocking out a proton from an atom with which it collides. Or, it may produce ionization indirectly after capture by the nucleus of an atom; the nucleus in turn becomes radioactive and disintegrates to liberate β- or γ-rays.

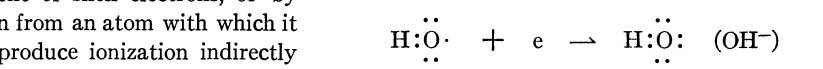
Two aspects of ionizing radiation might be stressed here. First, the energy transferred to an atom is often much greater than that required to eject an electron. Part of the excess energy appears in the expelled electron, now energetic enough to ionize other atoms with which it collides and to produce secondary electrons. After thousands of collisions, the electron has lost velocity or energy and is captured. If the electron is captured by an atom, a chemically reactive ion is produced. A molecule capturing an electron is usually unstable, decomposing to give reactive fragments called free radicals. Most of the ionizations initiated by x-rays are produced by the powerful secondary ejected electrons. This explains how a single x-ray photon produces many ionizations.

Second, an energetic particle can transfer some of its energy in flight to nearby atoms or molecules without causing ionization. This is called excitation or activation, since the atoms or molecules are now in a more energetic state. Thus, the total energy absorbed per ionization, or per ion pair formed, is about 30–35 ev, though only about 15 ev are required to ionize water, for example. The surplus of 15–20 ev is dissipated in molecular excitation. This is a considerable amount of energy when we consider that about 5 ev are required to break most organic bonds. If this surplus energy is spatially concentrated (i.e., not spread over too many atoms), it may, in turn, cause radical formation. Or it may activate chemical reactions, whose activation energies range between 1–10 ev.

The absorption of ionizing radiation, then, initially causes excitation and the production of free radicals and ions. These ions are different in character from the ions produced by the dissociation of salts in that they contain an uneven number of electrons. The unpaired or odd electron makes a free radical extremely reactive. Radicals are short-lived because of this reactivity (10^{-9} to 10^{-6} seconds in condensed systems). They will react with each other to form a primary chemical bond where two electrons are paired, *viz.*,



Or they will tend to become stabilized by gaining or losing an electron, *viz.*,



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Complex organic radicals tend to have longer lives, since resonance reduces the reactivity of the odd electron by distributing it over the whole molecule.

Photons of ionizing radiation are powerful enough to be completely democratic in regard to the molecular species with which they interact. Since protoplasm is structurally a colloidal suspension that is roughly three-fourths water, it is usually assumed that a major part of the energy transferred to a biological system occurs via its water. It is also considered that the biological effects of ionizing radiation are caused, in part, by the activated water. Fig. 2 shows one version of what happens when water stops quanta of ionizing frequencies. It serves to illustrate quite adequately the process of ionization and radical formation.

Along the lines we have discussed, Fig. 2 indicates an electron ejected from a water molecule with which a high-energy photon has collided, resulting in the formation of a hydroxyl radical (a). After many collisions, the electron is finally captured to yield a hydrogen atom or radical (b). These two uncharged radicals (H· and OH·) are chemically very reactive. They are powerful oxidizing and reducing agents and can readily break carbon-carbon or carbon-nitrogen bonds. In addition, hydrogen atoms can also bring about oxidation by dehydrogenation.

Hydrogen and hydroxyl radicals are believed to be the primary products of irradiated water. The presence of dissolved oxygen, however, can lead to the formation of other highly oxidative entities: hydroperoxyl radicals and hydrogen peroxide. The hydroperoxyl radical may arise from the reaction of a hydrogen atom with O_2 (c), or via the well-known affinity of oxygen for electrons (d). Hydrogen peroxide, in turn, may be formed by the stabilization of two hydroperoxyl radicals (e), or through electron capture by the hydroperoxyl group to yield the anion of H_2O_2 (f).

From Fig. 2 one would anticipate that the biological effect of an ionization would be potentiated in the presence of dissolved oxygen. The simplest explanation of such an oxygen potentiation of biological effect, which has been observed (but cf. Alper, 1956), would be that a greater number of reactive oxidative species are formed per ionization. While this may frequently occur in relatively simple *in vitro* systems, it is also probable that metabolic patterns are altered *in vivo* with changes in oxygen partial pressure and that this may influence the degree of radiation response.

The number of ionizations or ion pairs formed in an irradiated system can be either directly measured or indirectly deduced. Hence the number of solute molecules changed or inactivated per ionization can be approximated for the system as an index of the radiation potency. This index is known as the ionic yield (M/N). The most common dose unit used by the biologist in measuring ionizing radiation is the *roentgen* (r). For our purpose the r may be considered as the amount of energy dissipated in 1 gram of tissue or water in the production of 1.8×10^{12} ion pairs. This is equivalent to the absorption of approximately 93 ergs/g. The *roentgen equivalent physical* (rep) is a unit that facilitates comparison of various types of radiation. One rep denotes the release of the same amount of energy in a medium as would 1 r of x-rays.

I have used water as an illustration for the formation of ion pairs and radicals. Fundamentally similar electron phenomena take place when a solute molecule collides with a high-energy photon or particle. This, incidentally, touches on a controversial issue in the field of radiation effects. Does the energy responsible for initiating a solute change come *indirectly* from activated water or free radicals in the medium? Or does the solute itself act as a *direct* target for an ionizing particle? To pose the problem somewhat differently in terms you will hear developed later, breakage of chromo-

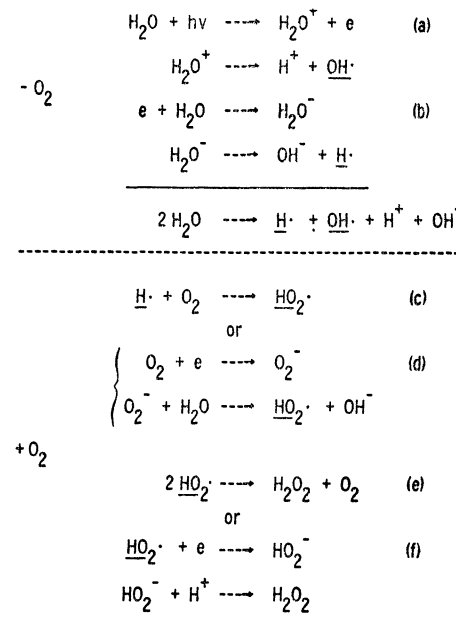

FIG. 2. THE ACTIVATION OF WATER BY IONIZING RADIATION IN THE PRESENCE AND ABSENCE OF OXYGEN

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somes is a significant effect of low doses of radiation. The breakage might occur in the track where an ionizing particle crosses the chromosome. Or the break may be the site from which an electron is transferred to a hydroxyl radical that had been formed nearby in the medium. It should be considered, however, that the locus of damage may be the termination of a sequence of electron transfers traversing the macromolecule, a sequence initiated at a site other than the one where bond cleavage occurs.

As Dale (1954) has pointed out, the closer the origin of an active radical to the molecule or structure on which it acts, the less important becomes the distinction between direct and indirect action. Without developing the matter further here, the problem is one that bears on the ultimate mechanism of radiation damage. By and large, the indirect action theory is more flexible in regard to the distance between primary ionization and the site of damage. It is also more applicable for explaining in vitro radiochemical events in aqueous media.

With emphasis on the indirect action hypothesis, what happens to molecular species of biological interest when irradiated in an initially definable aqueous system? Suppose a solution of solute A is irradiated by x-rays at a constant rate to yield product B. B reacts with the radicals in the medium at a low rate compared to the reaction rate of A, and the rate of disappearance of A is not limited by its concentration. Then the number of molecules of A that are changed will be directly proportional to the radiation dose. The ionic yield will remain constant till the concentration of A becomes limiting. Such zero order inactivation curves are shown in the oxidation of ferrous to ferric ion (Fricke and Morse, 1929), the decomposition of formic acid (Fricke, Hart, and Smith, 1938), and the oxidation of cytochrome c (Guzman Barron and Bonzell, 1950) by x-radiation.

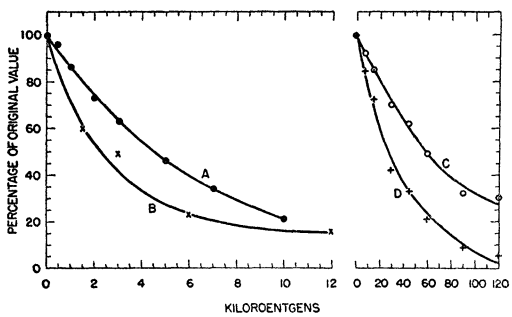

FIG. 3. EXPONENTIAL RELATION BETWEEN RESPONSE AND X-RAY DOSE IN SYSTEMS IN VITRO

Curve A, the decrease in concentration of the auxin indoleacetic acid (after Gordon and Weber, 1955). Curve B, the change in activity of carboxypeptidase against hydrolized edestin (after Dale, 1940). Curves C and D, the change in relative viscosities, corrected for the solvent, of nucleohistone and Na nucleate, respectively (after Sparrow and Rosenfeld, 1946).

TABLE 1

The concentration of various addends required for 50% protection of a 10^{-6} M solution of FAD against radiation damage (from Dale, 1942)

50% protection by:	
10^{-5} mol./ml.:	$10^{-7.5}$ mol./ml.:
Glycine	Glucose
Na oxalate	Sucrose
$NaNO_3$	KCNS
	Na formate
10^{-6} mol./ml.:	10^{-8} mol./ml.:
Leucylglycine	Fructose
Alanine	$NaNO_2$
$K_3Fe(CN)_6$	Na nucleate from yeast
$K_4Fe(CN)_6$	Na nucleate from thymus
10^{-7} mol./ml.:	
Na hippurate	

However, most organic molecular species yield irradiation products that are still capable of reacting with free radicals. The reaction products will "protect" the original solute. As the ratio of solute to product concentration decreases, the extent of protection increases and the solute concentration will decrease exponentially. Such exponential inactivation curves are observed on the irradiation of enzyme, auxin, and nucleoprotein solutions, as is illustrated in Fig. 3.

Obviously, co-solutes in an irradiated system may also compete for radicals and thus serve to decrease the rate of effect on the molecular species observed. Curves C and D in Fig. 3, for example, demonstrate the effect of protein in reducing the rate of viscosity change in DNA. Table 1, from the work of Dale (1942), further illustrates the phenomenon of protective effect. The table shows the concentration of various substances that will reduce by 50% the x-ray inactivation of a solution of alloxazine adenine dinucleotide, the prosthetic group of D-amino acid oxidase. Supplementing Fig. 3B, it indicates that nucleotides, as well as nitrite and fructose, are relatively effective protectants of the flavin dinucleotide. These studies, as well as many others in the literature, indicate quite clearly that the inactivation rate of a sub-

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stance irradiated *in vitro* is modifiable by the relative amounts and reactivities of accompanying substances.

With primary interest in the *in vivo* inactivation of metabolic determinants, I will from here on emphasize work of our own laboratory on the effect of ionizing radiation on the phytohormone auxin (Gordon and Weber, 1955; Gordon, 1956a). Most biological compounds will be inactivated with ionic yields between, roughly, 10^{-2} and 10^{0} when irradiated as pure solutions. The auxin indoleacetic acid is not unique in this respect, being inactivated with ionic yields in the order of unity under a variety of conditions. It is protected by dilute plant extracts, plant proteins being rather effective in this respect. For example, auxin was irradiated with x-rays in the presence of Fraction I protein of spinach, the mass protein of the leaf cytoplasm. With the protein at $1/100$ the concentration of the auxin, the ionic yield for auxin inactivation was found to be $1/100$ that of auxin irradiated alone. On the basis of estimated collision frequencies for the auxin and protein molecules with water radicals, this reduction in ionic yield caused by the protein indicates that 10^{4} radicals were deactivated by the protein for every radical that was effective in auxin destruction.

But in plant tissues such as the leaf or coleoptile auxin occurs at concentrations in the order 10^{-10} to 10^{-11} g. moles/gram. It is surrounded by a heterogeneous mixture of potentially protective solutes. Proteins alone, if considered as discrete molecular units, are in 10^{3} to 10^{4} mole excess. It thus appears highly improbable that low doses of ionizing radiation will directly inactivate auxin or any other multimolecular cellular solute to any appreciable degree. Several instances of the numerous experimental works tending to support this generalization may be cited. Guzman Barron (1954) and coworkers in particular have shown that the thiol group of both simple sulfhydryl compounds and sulfhydryl enzymes in pure solution is readily inactivated by x-radiation. The ionic yields were between 4 and 0.1. Yet we could find no decrease in the sulfhydryl titer (using the nitroprusside test) of *Avena* embryos immediately subsequent to an irradiation of 4×10^{4} r of x-rays. The same dose, though eventually lethal, did not inhibit succinoxidase activity in young *Avena* germinants. Similarly, in animals, a number of sulfhydryl enzymes do not decrease in activity following exposure to lethal doses of x-rays. An unchanged gross sulfhydryl concentration is not necessarily inconsistent with the inactivation of specific thiol groups essential to cellular function. There appears to be no evidence, however, that this happens *in vivo* as a direct radiation effect. In regard to non-thiol enzymes, Table 2 dramatically illustrates the relative insensitivity of two oxidative systems in the potato tuber. It took 50 kr of x-radiation to show an effect on the cytochrome oxidase, whereas this enzyme was unaffected by 3×10^{6} r of γ-radiation. It took the latter dose of gamma radiation to decrease tyrosinase activity by 50 percent.

In contrast, x-ray dosages of less than 100 r reduce, within minutes, the concentration of auxin in plants. The phenomenon was first observed by Skoog (1935). Since then numerous responses of plants to ionizing radiation have been attributed to destruction of the growth hormone. The reduction in auxin level following x-irradiation of young green plants is indicated in Fig. 4. Yet, as we indicated in discussing the concentration of auxin in plant tissues, appreciable auxin photolysis in vivo by the lower range of doses shown in Fig. 4 is theoretically implausible. Though this order of dose is sufficient to induce cellular damage, the actual energy absorbed is small. It is only enough to cause direct chemical change of several hundred molecules per μ^3 of tissue. This is virtually the concentration of the free growth hormone alone.

We are thus led almost inescapably to the following two possibilities: (1) the energy absorbed by the plant tissues is dissipated preferentially in auxin destruction; or (2) the energy absorbed inactivates molecules or molecular relationships that are responsible for the turnover of many auxin molecules. If so, there must be a mechanism that

TABLE 2

Cytochrome oxidase and tyrosinase activity of potato tubers following x- and γ-irradiation
(from Sussman, 1953)

Dosage	Type of Radiation	Cytochrome Oxidase Activity	Tyrosinase Activity
(r)		*mm³ O_2/30 minutes*	*mm³ O_2/30 minutes*
Control	—	119, 108	225
40,000	γ-rays	114, 117	220, 200
400,000	γ-rays	100	200
3,200,000	γ-rays	122	90, 110
50,000	x-rays	94	212

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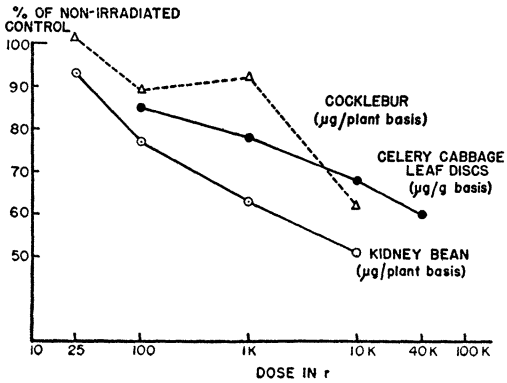

FIG. 4. CHANGES IN FREE AUXIN LEVEL IMMEDIATELY FOLLOWING X-IRRADIATION OF WHOLE PLANT SHOOTS OR SHOOT SEGMENTS

in effect "potentiates" or "multiplies" the energy originally absorbed. The decrease in auxin concentration would thus be an indirect result of a more primary radiation event. We were able to assess the probabilities of these alternatives by an experiment designed to evaluate the magnitude of direct destruction of the auxin in tissues.

Subapical tissues of the *Avena* coleoptile grow only by cellular elongation during the second and third day of germination. They require auxin for this growth, but cannot synthesize it. Growth in length of these tissues bears a proportional relation both to the auxin concentration supplied and to the amount of auxin which is destroyed during the growth process. An equivalence between growth and auxin destroyed can therefore be made. This equivalence was determined experimentally, and indicates that 5.2×10^{-13} moles of auxin disappear per mm. of elongation.

Suppose, then, coleoptile tissues are elongating under the influence of auxin. They are then irradiated. If auxin is destroyed by irradiation, the growth of the tissues will decrease proportionally to the amount of auxin inactivated. The coleoptile thus gives us a sensitive device for detecting auxin destruction *in vivo*. With estimates of the amount of auxin destroyed, and the amount of energy absorbed by the tissues, the radiation potency or efficiency can be calculated. Essential to the validity of this approach is the assumption that the potential ability of the tissues to grow, and to respond to auxin, is unimpaired by the radiation dose employed.

Subapical coleoptile sections were therefore immersed briefly in a weak solution of indoleacetic acid to maintain their elongation. They were then x-irradiated in a moist atmosphere and placed in water. The growth in the subsequent 12 hours was compared with non-irradiated controls. We were unable to find any reduction in growth resulting from irradiation until dosages of over 10^4 r were given. Apparently the extension of coleoptile sections, i.e., cellular expansion per se, is relatively resistant to ionizing radiation. This conclusion is consistent with other studies using plant organs more heterogeneous in growth pattern than the coleoptile (cf. Quastler and Baer, 1950; Gray and Scholes, 1951). Furthermore, since growth is proportional to the concentration of auxin in the tissues, any auxin destruction in vivo by low or moderate radiation doses would have resulted in decreased growth. This did not occur. From this we may deduce that auxin in the tissues was not sensitive to x-radiation doses below 10^4 r.

Between dosages of 10^4 and 10^6 r, growth inhibition progressively increases. When the sections were placed in auxin solution rather than water following irradiation, over 10^5 r were required to inhibit the subsequent growth. Otherwise stated, growth inhibitions following irradiation between 10^4 and 10^5 r could be reversed completely by addition of auxin. These dosages thus have no apparent effect on the growth potential of the tissues or on their reactivity to auxin. Therefore, let us assume that the growth inhibition following irradiations in the dose range of 10^4 and 10^5 r results from auxin destruction. Within this dose range, decrease in growth in mm. may be equated to moles of auxin inactivated, using the equivalence indicated above. The amount of auxin destroyed at each irradiation may then be calculated. This was done, and the ionic yield was found to decrease exponentially as a function of dose (r) according to the following approximation:

$$M/N = 6.6 \times 10^{-18} e^{-.01\,(r-1.5 \times 10^4)}$$

where

$$r \geq 1.5 \times 10^4$$

This equation indicates that where the growth inhibition may be interpreted as a result of auxin destruction, it initially takes 10^{17} ionizations to destroy one auxin molecule. Otherwise stated, the statistical probability of any auxin molecule being damaged *in vivo* by x-radiation is initially less than one chance in 100 quadrillion. As irradiation continues this probability *decreases*. It is evident that the efficiency of auxin destruction in these tissues

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is of relatively low order, and it is very probable that auxin in the plant is neither radiosensitive nor preferentially inactivated.

We are led, then, to the second possibility whereby the effect of low dose irradiation on auxin destruction is accomplished by a multiplier mechanism. An obvious candidate for the role is an enzyme whose damage will affect the turnover of many auxin molecules. You will recall from Fig. 4 that low doses of radiation caused the auxin concentration to drop in shoots of the kidney bean and cocklebur plants, and in leaf discs of the celery cabbage. These organs have active systems for auxin metabolism. The growth hormone is being constantly synthesized in the young leaves and meristems. At the same time it is being depleted by growth and by both enzymatic and non-enzymatic systems for auxin inactivation. On the other hand, subapical tissues of the coleoptile, where radiation does not decrease the auxin concentration, are unable to synthesize auxin and possess lower auxin depletion rates. These considerations suggested the following hypothesis to explain the effect of radiation on auxin economy. Though we know nothing of the kinetics of auxin metabolism in the plant, let us assume that the free growth hormone is a dynamic pool maintained as a steady-state system by concomitant biosynthetic and depletion reactions. If biosynthesis was preferentially and immediately radiosensitive, the rate of auxin formation would be decreased by, and during, irradiation. The level of the free auxin pool would drop, the magnitude and rapidity of the decrease being a function of the turnover rate of auxin. Conversely, the same result would ensue if the rate of auxin depletion was preferentially accelerated by irradiation. Therefore, the effect of x-rays on the biosynthesis and depletion of auxin was determined.

Young kidney bean plants, their roots shielded, were irradiated with x-rays. At various times after irradiation the free auxin level in the whole shoot was measured. The effects of various radiation doses are shown in Fig. 5. Doses between 25 r and 1 kr not only lowered the free auxin concentrations immediately following irradiation, but also depressed the auxin levels during the week following. With such doses a return to control level is attained in about two weeks. Recovery does not take place in this period, or subsequently, with the two highest dosages. Similar results to these were obtained when cocklebur plants were used.

We believe that such depressions of the free auxin level following x-irradiation of the plant are caused by a rapid curtailment of auxin biosynthesis rather than an accelerated auxin catabolism. This interpretation is based on a number of experimental studies that I will summarize.

1. We have not observed any manifestation of enhanced hormonal function and hence presumably increased rates of auxin utilization following irradiation. Where the radiation dose was large enough to have any effect, only inhibition was found in the elongation of *Avena* coleoptiles, in internode extension of *Phaseolus* stems, and in rooting of leafed *Populus* cuttings following irradiation, in each instance, of the total organ.

2. Auxin was added to breis made from irradiated and non-irradiated coleoptiles and *Xanthium* shoots. The breis were incubated both in the light and dark. Thus the effect of x-radiation on both photoinactivation as well as enzymatic and non-enzymatic auxin destruction could be estimated. In no experiment could we find any significant effect of irradiation on the amount of auxin recovered. In short, we could find no acceleration of any direct auxin depletion mechanism of the plant by x-radiation.

3. Growth of axillary buds is inhibited by the terminal bud of the shoot. The inhibition is caused by the auxin produced in and transported from

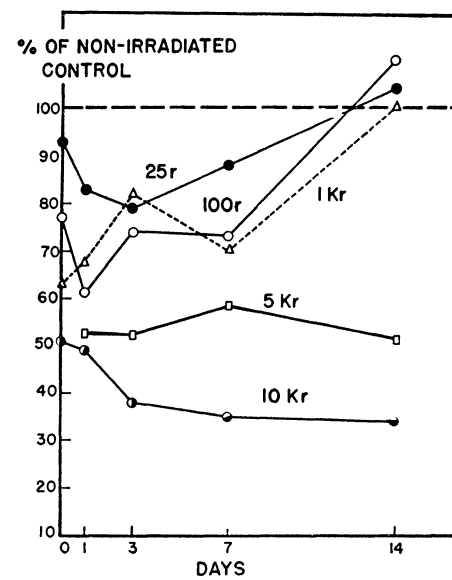

FIG. 5. RELATIVE FREE AUXIN LEVELS IN THE KIDNEY BEAN PLANT FOLLOWING X-IRRADIATION

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the terminal bud. If the terminal bud is cut off (or severely injured) the axillary buds develop. If a decapitated bud is replaced by an exogenous source of auxin, the growth of the axillary buds remains suppressed. An appreciable inhibition of auxin synthesis in the apical bud should result, therefore, in the growth of axillary buds.

The terminal bud of the cocklebur plant was irradiated with 200 r of x-rays, the remainder of the plant being shielded with lead. Subjacent axillary buds began enlarging. They grew continuously for the subsequent month, virtually no growth taking place in the axillary buds of non-irradiated plants. If auxin was supplied to the irradiated terminal bud by external application for only two days following irradiation, axillary bud growth was simply postponed for two days. They then duplicated the growth response of the buds on irradiated but not auxin-treated plants. If, however, auxin was supplied for 2 weeks following irradiation, the axillary buds which had been kept suppressed remained dormant, though they had not lost their ability to grow. It is clear, then, that auxin was able to reverse the effect of the x-rays on the terminal bud as measured by the response of organs normally kept dormant by auxin formation in that bud. The temporal pattern of inhibition of terminal bud effect on the axillaries at two days and recovery in 2 weeks is consistent with the pattern of decrease and recovery in free auxin levels exhibited in Fig. 5. These results offer a less equivocal substantiation of the radiation sensitivity of auxin formation.

4. Consistent with this interpretation are the auxin reversals of the growth inhibition following irradiation of crown-gall tumor cells. Auxin is apparently required for the cellular duplication that comprises most of the tumor growth, as well as for the initial transformation of normal cells. The crown-gall contains an active system for auxin biosynthesis and can convert tryptophan to auxin with relative efficiency. Klein and Vogel (1956) inoculated tomato plants at the second node with crown-gall bacteria and permitted carcinogenic transformation to take place. The plants were then irradiated with neutrons, x- and γ-rays. Following irradiation, the apical node was removed and either lanolin or lanolin-containing indoleacetic acid was applied above the inoculation site. Table 3 shows the inhibition of subsequent tumor growth by both the corpuscular and electromagnetic radiation, and the ability of auxin to reverse the radiation effect of the dosages below 5 kr.

TABLE 3

Inhibition of the duplication of crown-gall tumor cells in vivo by ionizing radiation and reversal of inhibition with indoleacetic acid (from Klein and Vogel, 1956)

Radiation	Dose r or rep/min	Total Dose	Av. Tumor Diameter as % of Control	
			Lanolin	Lanolin IAA
Fast neutrons	3.4	340 rep	95	103
	3.4	550 rep	28	100
	4.6	950 rep	7	90
X-rays	10.6	500 r	99	100
	10.2	1000 r	76	98
	12.2	1500 r	8	80
	47.5	5000 r	7	7
Gamma rays	12.2	1500 r	106	106
	12.5	2100 r	58	101
	12.2	2850 r	7	90

5. The most compelling confirmation of the lability of auxin formation in the plant comes from direct biochemical examination of the plant itself. There is considerable ground for the assumptions that the native auxin of hormonal function in growth is indoleacetic acid, and that indoleacetic acid originates by the degradation of tryptophan (Gordon, 1956b). Though the biosynthetic pathway is uncertain, there is also presumptive evidence that the terminal stage of the sequence is the oxidation of indoleacetaldehyde to the acid. The effects of irradiation on these systems were examined.

Mung bean seedlings were irradiated with various doses of x-rays. Cell-free homogenates were made of each dose group within several minutes after irradiation. Each was assayed to determine the rate at which it could convert tryptophan to indoleacetic acid and indoleacetaldehyde. The results are represented in Fig. 6. They indicate that an x-ray dose as low as 10 r results in an 11% inhibition of the enzyme system, with the extent of inhibition dropping exponentially with increasing dose. There was no decrease in the amount of indoleacetaldehyde concomitantly formed. On the contrary, the precursor of the auxin appeared to increase in inverse proportion to the acid as the dose was raised. The apparent pileup of the aldehyde suggested that the radiation block occurred in the enzyme system that oxidizes the aldehyde to the acid. This was tested by determining the effect of x-rays on the ability of the homogenates to convert the aldehyde to the auxin (Fig. 7). The rate of inhibition shows the same order of sensi-

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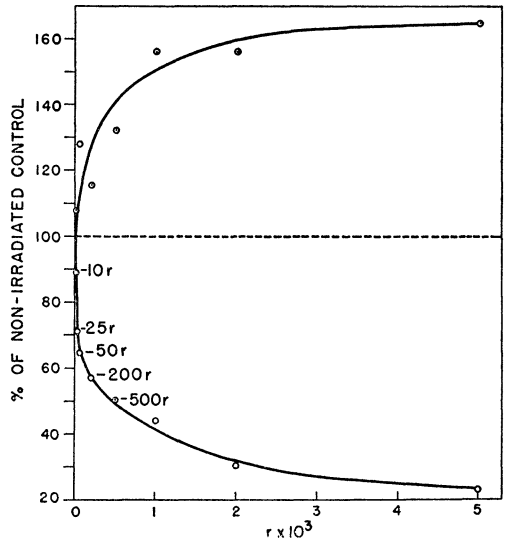

FIG. 6. CONVERSION OF TRYPTOPHAN TO AUXIN BY CELL-FREE HOMOGENATES OF X-IRRADIATED MUNG BEAN SEEDLINGS

The lower curve gives the relative amount of indoleacetic acid formed, the upper curve indoleacetaldehyde.

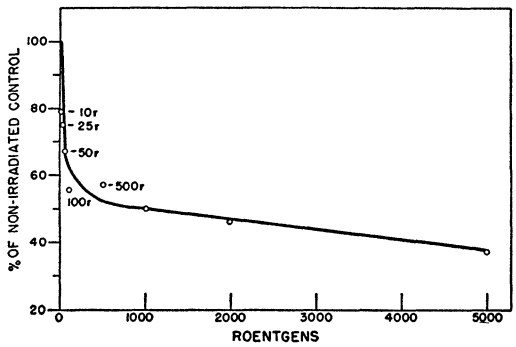

FIG. 7. CONVERSION OF INDOLEACETALDEHYDE TO INDOLEACETIC ACID BY CELL-FREE HOMOGENATES OF X-IRRADIATED MUNG BEAN SEEDLINGS

tivity and rapid diminution as does the total process of tryptophan conversion to auxin. It should be noted that 20 percent inhibition of the terminal enzyme in auxin formation resulted when the plant was irradiated with the dose of 10 r. Fig. 7 is explicit in its indication that the radiation damage occurs in the terminal stage of auxin biosynthesis.

In the preceding experiments the enzymatic damage was evaluated immediately subsequent to irradiation. What happens to the activity in the days following? The response of the auxin-forming system in the 2-week period after irradiation is given in Fig. 8. In these experiments tryptophan was infiltrated into the leaf and bud tissues of mung bean seedlings at each of the indicated times. The rate of auxin formation was then measured. Here again we have the same pattern of initial sensitivity and exponential decrease in extent of inhibition with increasing dose as shown both by the morphological studies and by the direct determinations of auxin levels. There is likewise the same pattern of recovery in biosynthetic ability: complete or virtually complete recovery in 1 to 2 weeks with doses below 1 kr, partial recovery with 2 kr and none at 5 kr. We thus have summarized three lines of experimental approach—physiological, morphological, and biochemical—that substantiate the relatively high sensitivity of auxin biosynthesis to ionizing radiation.

Might I emphasize that, in the biochemical experiments just described, enzyme activities were determined following irradiation of the plant. We are therefore dealing with an *in vivo* sensitivity. Secondly, enzymatic inhibition is detectable in preparations made within several minutes after irradiation. It may be inferred that cellular or nuclear replication is not required for expression of this radiation damage.

To clarify the latter inference, the intracellular distribution of the enzyme system was studied (Gordon, 1956c). It is found in the cells of young leaves and meristems as a non-particulate, soluble component of the cytoplasm. It is part of the complex "Fraction II" cytoplasmic proteins that fall between 0 and 4 Svedberg units in sedimentation velocity. This fraction constitutes practically all of the enzyme activities of the cytoplasm. With this knowledge, and with an approximation of the relation between radiation dose and the degree of inhibition, it is possible to estimate the number of

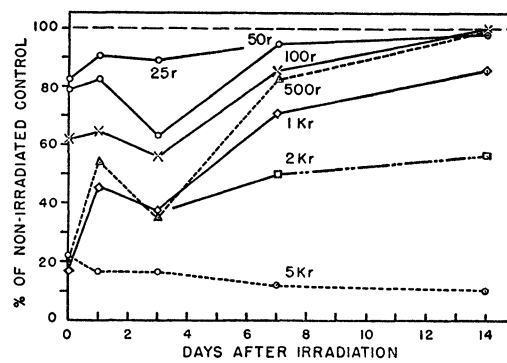

FIG. 8. CONVERSION OF INFILTRATED TRYPTOPHAN TO AUXIN BY MUNG BEAN SEEDLINGS AT VARIOUS TIMES AFTER IRRADIATION

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enzyme molecules destroyed per ionization. When this is done, one obtains the amazing value of an ionic yield in the region of unity, plus or minus several orders of magnitude. This is equivalent to saying that the energy absorbed is to a large degree diverted to the inactivation of the auxin-forming enzyme, disregarding the molecular excess of other cellular species. In effect, these enzyme molecules appear as relatively enormous radiation targets. What we have done, then, is to arrive at the paradox previously introduced: how one extranuclear molecular species can be inactivated in a heterogeneous milieu of protective substances.

We might at this point repeat the whole of the preceding discussion at the next lower ontogenetic level. This would bring us again to an explanation founded on the dynamic aspects of cellular metabolism—specifically, that the auxin enzyme undergoes rapid turnover, and that the locus of radiosensitivity is an entity involved in either the formation or the depletion of the enzyme. Because of our complete lack of information on the nature and kinetics of these systems, all we have done again is to pinpoint the problem of apparent energy channelling to particular loci (cf. Pelc and Howard, 1952a).

If the primary target of the radiation damage was a conjugated molecular complex the size of the chromosome or gene, the problem would be somewhat simpler in relating the amount of energy absorbed to a specific biochemical impairment. The cytoplasmic analogue of the conjugated macromolecules of the nucleus would be organelles such as the mitochondrion. The study just described on intracellular localization of the enzyme demonstrated, however, that it is an extranuclear soluble component of the cytoplasm. Hence we may also infer that the site of primary damage is likewise in the cytoplasm. Gray (1954) has pointed out in this context that a significant property of a large structure, destroyable by a single particle, is continuity. If we consider the cytoplasm *in situ* not as discrete autonomous molecular units but rather as a bonded, continuous micellar net, we have our enormous radiation target. It is a target that permits not only intramolecular but also intermolecular electron transfer. Hence it is synonomous with a volume wherein energy flow may occur irrespective of whether its flow is initiated by a photon, secondary particle, or free radical. It may be suggested that electron ejection from any site in the net will start an electron migration to that site from labile, i.e., readily oxidizable or broken, bonds. The auxin-forming enzyme or its progenitor may be at such a locus. Or, alternatively, the disablement of a mechanism for electron transfer, a system essential to the function of the enzyme, may be the explanation for the enzymatic damage. We can only be vague as to how the enzyme is inactivated in this association. There is no reason to assume, *a priori*, that the indoleacetaldehyde-auxin enzyme is the only enzyme impaired by low or moderate doses of ionizing radiation. This point about the specificity of damage will serve very well to introduce the terminal portion of my discussion.

In dealing with radiation damage, we must differentiate between the immediate, primary effects and the changes that arise from disruption of key cellular or metabolic processes. It is frequently quite difficult to distinguish between the two. Two criteria might be the magnitude of the radiation dose involved, and the time lag between irradiation and response. In regard to the criterion of dose magnitude, we have no experimental basis for assuming a qualitative difference in the initial "targets" or damageable entities, though certainly qualitative differences in secondary responses are dose-dependent. Pragmatically, however, there are threshold levels of molecular disorganization which must be attained before a specific type of cellular damage is perceived. With this qualification in mind, how unique is the *in vivo* sensitivity of the auxin-synthesizing enzyme?

As far as I am aware, no biochemical sequence of the many so far examined, including protein and RNA synthesis (Pelc and Howard, 1952b), has shown an immediate sensitivity to low radiation doses other than DNA biogenesis. The sensitivity of DNA formation has been demonstrated by the radioautographic studies of Howard and Pelc (1953) on root cells of *Vicia faba*, and is backed by the more extensive isotopic work on animals (Bacq and Alexander, 1955), particularly the works of Hevesy and of Holmes. Though the inhibition of DNA synthesis can be picked up in the animal within an hour, the time of the first perceptible plant response has not been precisely established. It is probable that the inhibition also occurs very quickly after irradiation. Synthesis of DNA takes place during interphase in meristematic tissues. Cells that have been irradiated at this mitotic stage do not elaborate DNA and have their mitotic activity delayed. The degree of inhibition is roughly dose-dependent, the effect of 35 r being detectable.

It will be recalled that the inhibition of auxin

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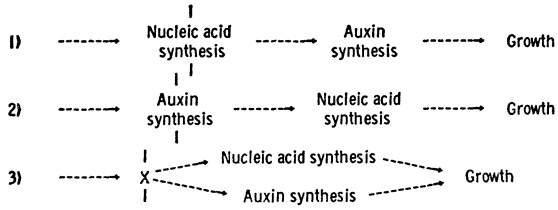

FIG. 9. POSSIBLE LOCI OF BIOCHEMICAL DAMAGE IN THE INHIBITION OF GROWTH BY IONIZING RADIATION

formation or tryptophan conversion tends to disappear within several days to two weeks, depending on the radiation dose, with no recovery above the dose range of 1 kr–2 kr (see Figs. 5 and 8). In oral discussion, L. H. Gray pointed out that this pattern of inhibition and recovery parallels almost identically the inhibition and recovery of mitotic activity in meristems. From the work of Howard and Pelc, it may be inferred that the inhibition of DNA synthesis causes the mitotic inhibition. Since both auxin and DNA are required for cellular replication, radiation damage to the growth of plant tissue may be related to auxin and DNA biogenesis in the relationships indicated by Fig. 9. These relationships postulate the not necessarily exclusive possibilities: 1) that DNA is required for and is synthesized sequentially previous to auxin formation, the radiation block occurring in the formation of the nucleic acid; 2) that the primary radiation block is in auxin synthesis, the auxin being required for the formation of DNA; 3) that the effect of the radiation is on an undefined entity in a reaction previous to and essential for both DNA and auxin synthesis. We are disregarding the possibility that the inhibition of both auxin and DNA synthesis is caused by dissimilar and unrelated radiation blocks. This omission is based on the similar dose responses and recoveries of the two systems.

The probability is low that the first of these interpretations is correct. This generalization is based on the following considerations. (1) Went's famous dictum "Ohne Wuchstoff kein Wachstum" has yet to be disproved. Without auxin, either endogenously synthesized or exogenously supplied, plant tissues will neither grow nor anabolize DNA (Skoog, 1954). (2) It is difficult to visualize how a reduction of DNA concentration in the nucleus can be manifest within minutes as a major loss in activity of a cytoplasmic enzyme. DNA synthesis is restricted to the nucleus, the synthesis of auxin to the cytoplasm. (3) The tips of coleoptiles are embryonic in ontogeny, although they are completely non-meristematic for ca. 2 to 3 days. Auxin synthesis in these organs is restricted to the tip. Yet the tips remain sites of auxin synthesis in this period although further elaboration of DNA has ceased.

These considerations as a whole suggest the second of the possibilities in Figure 9. The findings of Holmes et al. (1955) in Fig. 10, showing the DNA content of *Vicia* root tips as a function of distance from the tip, are pertinent in this connection. It is evident that the DNA content per cell increases with distance from the root apex, being about 30 percent greater in the fully elongated cell than in the cells of the terminal meristematic segment. In other words, DNA synthesis goes on in cells that do not synthesize auxin. At first glance this might seem to contradict the requirement of auxin synthesis for DNA elaboration. That it does not may be deduced from the fact that auxin is transported basipolarly from the production site at the tip to and through the cells comprising the region of elongation. Hormonal transport should satisfy any requirement for DNA synthesis.

Possibility number three would suggest an almost simultaneous effect in both the nucleus and

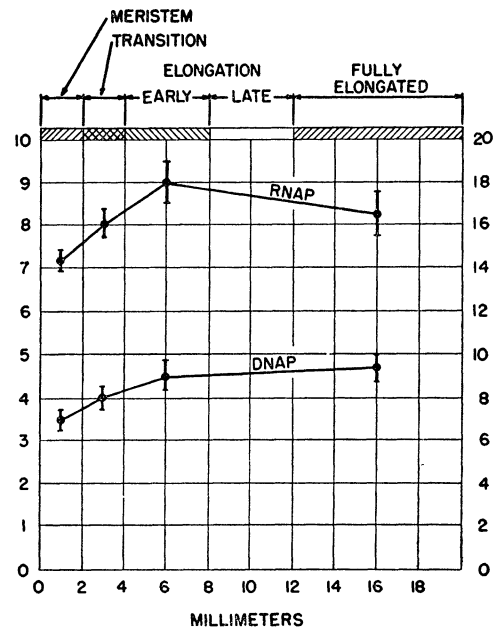

FIG. 10. THE RELATIVE DNA (LEFT ORDINATE) AND RNA (RIGHT ORDINATE) CONTENT PER CELL AT DIFFERENT DISTANCES FROM THE TIP OF *VICIA FABA* ROOTS.
(After Holmes, Mee, Hornsey, and Gray, 1955).

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cytoplasm of a common molecular lesion. It is difficult to evaluate this simultaneity rigorously without exact studies of the time required between irradiation and inhibition of the two systems. In this connection, the work of Quastler, of Gray and Scholes, and our own, by completely separate approaches, all indicate that elongating cells are highly resistant to radiation damage. Since such cells do not synthesize auxin but evidently do synthesize DNA, it would be a fascinating problem to investigate whether the ability of elongating and differentiating tissues to synthesize DNA was impaired by ionizing radiation. It would certainly clarify the relationships outlined in Fig. 9.

In conclusion, I hope I have brought into focus one of the fundamental enigmas in the field of radiation effects: how so little energy, absorbed with virtually no specificity, can trigger the sequence of phenomena that constitute the radiation syndrome. The worker with plants is fortunate in having on hand two radiosensitive biochemical reactions—reactions that are both chemically characterizable and immediately sensitive to low radiation doses. He is also fortunate that these reactions play so essential a metabolic role that they facilitate our interpretation of the morphologic response of the plant, and possibly also the animal, to ionizing radiation.

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