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Berkeley, California

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CRYSTALLINE LUMINESCENCE OF CELL-NUCLEAR CHEMICALS UNDER IONIZING RADIATION

Richard Lawrence Lehman
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CRYSTALLINE LUMINESCENCE OF CELL-NUCLEAR CHEMICALS UNDER IONIZING RADIATION

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ABSTRACT

An experimental study has been made of the delayed phosphorescence and thermoluminescence properties of 34 separate cell-nuclear chemicals exposed as crystalline powders to cobalt-60 rays at 77° K. In addition, the luminescence of the nucleic acid bases and related compounds was studied under irradiation with intense heavy-ion and electron beams. Under electron bombardment, the effects of ambient gases at various pressures have been examined at 77 and 295° K. The biological significance of these measurements is discussed.

The thermoluminescence-glow peak location, temperature-widths, and yields have been reported. The location in no case exceeded 180° K, and in most cases stood in the region 100 to 150° K. It was found that a given chemical may have several peaks, and similar chemicals may have glow peaks that differ in width, location, and intensity. Adenine and cytosine samples released 0.2 and 0.1%, respectively, of the absorbed gamma energy as thermoluminescence. In each case, the yields of molecular compounds that contained conjugated π-molecular orbitals approached these; other compounds such as lecithin had yields of the order of 10^{-7}. The activation enthalpies for thermoluminescence glows have been examined in detail, and a new method for their determination based on phosphorescence decay at 77° K has been used.

For those substances exposed to the particle beams, the emission spectra and yields at 250 to 600 nm under various gas pressures have been reported. Radiofluorescence energy yields up to 13% were measured for adenine at 77° K when the ambient oxygen gas pressure was

300 μ Hg. At 286°K the yield was 0.2%. Yields for the other nucleic acid bases were roughly 1/10 of these. The emission spectra were found to be bell-shaped bands in the region 350 to 540 nm with widths of about 100 nm. When adenine was exposed at 77°K in the presence of > 100 μ Hg of gas, the emission spectrum peaked at 490 nm. Under vacuum at 77°K, or at 286°K under all conditions, the spectrum peak shifted to 520 nm. Similar shifts were observed for the other compounds. A conceptual model, the exciton hypothesis, has been advanced to interpret the temperature and gas pressure effects on radioluminescence.

List of Symbols

β	Warming rate ($^\circ$K/sec) in thermoluminescence measurements; in Eqs. (4)-(6) it is a recombination coefficient.
ΔF	Free energy change in thermoluninescence reaction.
J	Rate of excitation of molecules by incident radiation.
K	Equals $\nu_t x$.
k	Boltzmann constant
L(t)	Intensity of luminescence at time t.
n(t)	Number of excited molecules at time t.
p	Equals $1/\tau$, the probability for radiative transition.
ΔS	Entropy change for thermoluminescence reaction.
s	Frequency factor--related to the natural vibration frequency of the system, to the entropy change in the thermoluminescence reaction, and to the transition coefficient.
T^*	Temperature at thermoluminescence glow peak.
T	Temperature as a variable.
t	Time as a variable.
τ	Meantime for luminescence decay.
ΔU	Thermal activation enthalpy for thermoluminescence reaction.
ν_t	Crystal vibration frequency.
x	The transmission coefficient.

CRYSTALLINE LUMINESCENCE OF CELL-NUCLEAR CHEMICALS UNDER IONIZING RADIATION

I. INTRODUCTION

This experimental study was motivated by the question "What can the luminescence of crystalline cell-nuclear chemicals reveal about ionizing radiation damage in living cells?" Since this is a new field, the study has been extensive and exploratory rather than intensive and particular. And although just a beginning has been made, it appears that crystalline luminescence technique may stand with electron spin resonance as a window through which an important aspect of radiation damage may be studied. Here, polycrystalline powders of most of the chemicals of cell nuclei have been irradiated with γ rays, heavy ions, and electrons, and their luminescence has been studied.

A. Relation of Crystalline Luminescence to Radiation Biology

The electron energy transitions associated with crystalline luminescence amount to a few eV. This is the same order of magnitude as for the normal excited states involved in molecular energy transfer, and in the primary chemical bonds. It is argued that ionizing radiation leads ultimately to excited electronic states of a few eV energy, and that the fate of these energy packets determines the nature of the biological damage. It is therefore of considerable importance to study the radiative transitions of excited electrons in condensed systems.

1. Electronic Organization of the Condensed Regions of Living Cells

In recent years, cell chemists have been obliged to examine the electronic nature of molecules in order to attack the major problems of cell-energy transfer and storage (Szent-Gyorgyi,[1-3] Arnon[4]). Concern with molecular electronics has been the gravity center of a dozen very recent conferences[5-11] and reviews[12-15] of radiation biology and chemistry. Electrical, optical, and theoretical studies of the electronic

structure of molecules have led to new insights into the action of high-energy phosphates (B. and A. Pullman),[16] mitochondria (Green),[17] proteins (Stryer),[18] respiratory pigments (McClure),[19] and drugs and carcinogens (A. and B. Pullman),[20] and into photosynthesis (Tollin),[21] energy transfer (Förster),[22] and bioluminescence (Seliger and McElroy).[23] There is thus considerable support for the view that the basis of normal cell-energy dynamics is the electron, and that the structural complexity of biomolecules and their aggregates provides simply a particularly suitable atomic scaffolding for transfer of electron excitation energy. The ultimate mechanisms of cell-energy transfer must thus be sought in the electronic levels of molecules and aggregates of molecules.

The electron micrographs by Sjöstrand[24] and Steffanson[25] reveal that the condensed cell structures--the chromosomes, the mitochondria, the chloroplasts, and others--are highly organized and densely ordered. Within such structures the molecular density is very great, approaching that of crystals, and almost certainly there exist local regions in which the excited electronic states are common to the entire region, as in molecular crystals.

2. Ionizing Radiation and Electrons in the Cell

For this discussion, the ionizing radiation is restricted to electron and electromagnetic beams of energy between, say, 20 eV and a few MeV, although the analysis applies significantly to all qualities and energies of high-energy radiation.

Virtually all the immediate biological damage from ionizing radiation is brought about by electrons. The initial event frees a high-energy electron which, after slowing-down processes (which may include brehmsstrahlung, secondary ionization, and local electronic excitations), may be scavenged by water or other molecules. The immediate result of the initial event is thus the creation of one (or more) excited electron-deficient molecules, and one (or more) electron-scavenging molecules in an excited state. In general these excited

molecules are formed within 0.01 psec of the initial event and are separated in space by a few molecular diameters at least.

The indirect-action theory of ionizing radiation emphasizes the situation in which both the excited molecules are, with high probability, water. The pioneer work of Dale established the importance of the indirect action of the H· and · OH radicals thus formed on enzymes in dilute aqueous solution.[26] The direct-action theory focuses on the case in which the initial (or secondary) ionizing event occurs in an important biomolecule. Here the subsequent electronic reactions and transformations of the "hit" molecule are emphasized.

In either case the problem of molecular de-excitation remains. The excitation energy may be stabilized within the molecule in the form of a chemical bond, it may be dissipated in the form of molecular or atomic vibrations, or it may be released as luminescence. Which of these de-excitation paths a given molecule takes depends upon its electronic nature and its molecular environment.

One indicator of the extent of conversion of absorbed radiation into chemical bond energy is the radiolytic yield of hydrogen gas. This yield for organic material, in or out of solution, is almost always between 1 and 10 molecules per 100 eV absorbed (Lindblom).[27] If one takes an average of 5 eV spent per molecule thus formed, then 5 to 50% of the absorbed energy is utilized in this way. The remainder of the excitation energy is yielded as heat and luminescence.

The radiation dose that kills mammalian cells is about 0.004 joule/g. For comparison, it requires about 4 joules/g to raise the temperature of water 1°K. Thus, the biological damage from local irradiation heating must be negligible compared with the effect of irradiation on chemcal bonds. Radiative de-excitation also appears to be an innocuous form of energy dissipation. Such luminescence in general has insufficient quantum energy to create secondary ionization. It is possible that much of this is locally absorbed and converted to vibration energy, part of which may be absorbed by chemical bonds, and part dissipated as heat.

3. The Direct Action of Radiation on Condensed Systems

The theory of indirect action in radiation biology is supported by the phenomena of the oxygen effect and of chemical protection. However, Dubinin et al. have found that an oxygen effect occurs during irradiation of dry polymers, and that the presence of bromine-ion- and iodine-ion-radical scavengers does not protect the cell against chromosome aberrations.[28] The work of Sapezhinskii and Émanuél'[29] on proteins and nucleic acids and of Howard-Flanders[30] on bacteria is also most readily interpreted by direct action. Chemicals, oxygen, humidity, temperature, etc. are capable of modifying this direct action of radiation on condensed systems. In short, whereas the indirect effect may be of greater importance in the relatively disperse cellular elements, the direct effect is more important in the condensed elements.

B. Electronic Energy Levels of Organic Molecular Crystals

1. Definition of a Molecular Crystal

In a molecular crystal, the forces of attraction between molecules are significantly smaller than the forces that bind the atoms in a molecule. Such crystals have low melting and boiling points, and evaporation occurs by whole molecules; these crystals are diamagnetic and are electrically nonconductors (Davydov).[31] Virtually all organic crystals are indeed molecular, and in particular the energy of the interatomic bond in an organic molecule is about 4 eV, whereas the energy in the intermolecular hydrogen and van der Waals bonds is 0.1 to 0.01 of this (Steffanson).[25]

By contrast, the forces in ionic and atomic crystals are relatively great, so that molecules lose their individuality. Here the potential energy bands for the valence electrons are common throughout the crystal.

2. The Molecular Orbital Terminology of Organic Chemistry

Further discussion of the electronic states in condensed organic systems requires the use of the following special terms.

a. Molecular orbital. This is represented by a wave equation which describes the energy, angular momentum, and magnetic orientation of a bound electron. It may be pictured as a "cloud" about the molecule that binds it. Similarly, an atomic orbital describes an electron bound to an atom. An orbital may be singly occupied, or occupied by two electrons having opposite ($\uparrow\downarrow$) spins. For example, the orbital notation for the ground state of the carbon atom is $1s^2\ 2s^2\ 2p_x\ 2p_y$. The three 2p orbitals ($m = -1, 0, +1$) have identical energies and are thus three-fold degenerate. In this case none of the orbitals contains two electrons in the ground state if any of the degenerate orbitals are unoccupied (Hund's rule).

b. σ bonds and π bonds. Single chemical bonds arise from the attractive forces exerted by electrons having paired ($\uparrow\downarrow$) spins in overlapping orbitals. The SPDF notation ($\ell = 0, 1, 2, 3$) of optical spectroscopy is used in the orbital symbols spdf. Thus the s orbitals have spherical symmetry and the p orbitals have axial symmetry. (See Fig. 1) The strongest chemical bonds are formed by that orbital configuration which allows maximum overlap. For this reason the four C-H bonds in methane are "hybrid," each being composed of a linear combination of one s and three p orbitals (sp^3); σ bonds are formed by spherical overlap of s orbitals or endwise overlap of sp, pp, or higher orbitals; π bonds are formed by sidewise overlap of sp, pp, and higher orbitals.

c. The π-bonding molecular orbitals. The biologically important bases of the nucleic acids (Fig. 2) are resonating molecules which possesses a number of mobile (π) electrons associated with the double bonds and the lone pairs of the hetero atoms nitrogen and oxygen. These bases also have electron pairs, localized at the unreduced nitrogen atoms, which are called n orbitals. The π electrons form a unique electrical cloud distributed over the entire molecular periphery and

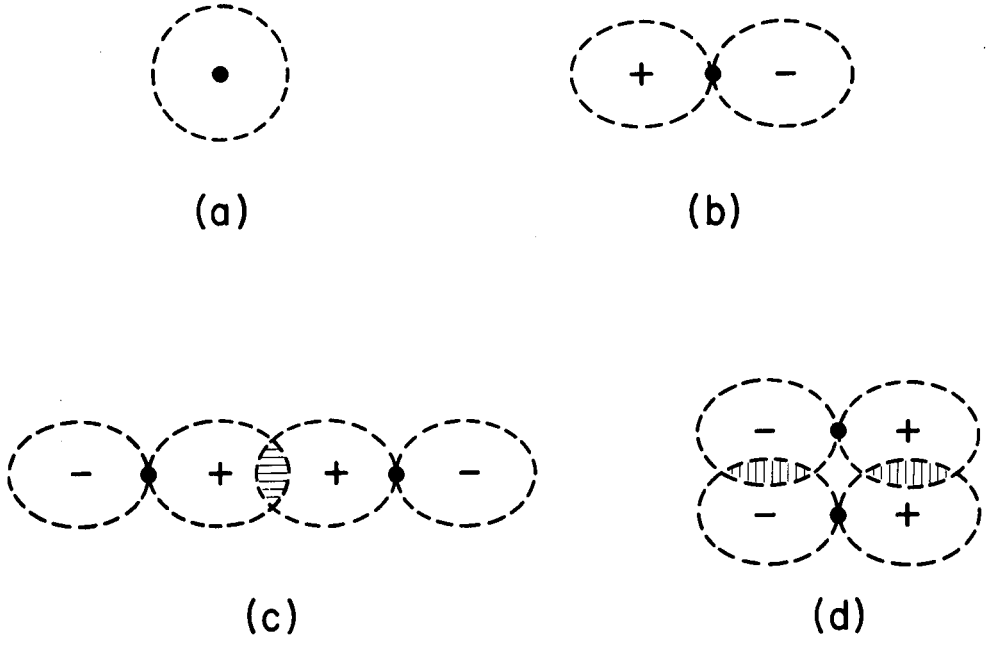

Fig. 1. (a). An s orbital.
(b). One of three possible p orbitals (the other two are at 90° to this alo g the other two space axes as determined by the magnetic quantum number).
(c). A σ bond formed from p orbitals.
(d). A π bond formed from p orbitals.
After Streitwieser (reference 32), p. 14.

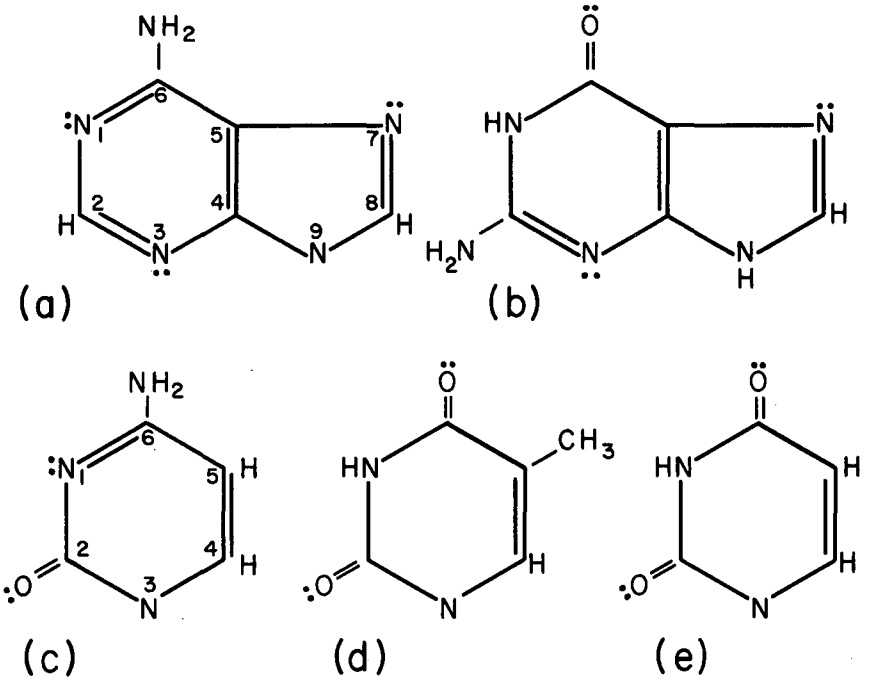

Fig. 2. Resonating ring structure of the nucleic acid bases.
a. adenine (6-aminopurine), 10 π electrons per molecule
b. guanine (2-amino, 6-ketopurine), 14 π
c. cytosine, 10 π
d. thymine, 12 π
e. uracil, 10 π
The dots give the electrons of the nonbonding n orbitals localized on the hetero atoms.

give rise to a molecular resonance energy of stabilization amounting to 2 to 4 eV per molecule (B. and A. Pullman).[34] The nature of these peripheral fused π bonds may be seen in Fig. 3 in a bond diagram of benzene.

d. Singlet and triplet states. Consider the spin possibilities of an excited state in which two molecular orbitals are singly occupied. The two electrons involved may have the same ($\uparrow\uparrow$) or opposite ($\uparrow\downarrow$) spins. The former case is that of a triplet state, the latter that of a singlet state. In the triplet state the parallel spins of the electrons prevent them from approaching closely. With opposite spins, close approach is denied only by electrostatic repulsion, which results in an increase in energy. For this reason a triplet state corresponds to a lower energy than a singlet state formed from the same orbitals.

3. Electronic States in Condensed Organic Systems

When an organic molecule lies in a pure crystal, its excited levels are shifted as indicated in Fig. 4. Each excited singlet state of a given molecule in the crystal is split into a band of energies. The ground states in molecular crystals are not split, although they are in general lower in energy (to the extent of the van der Waals interaction) than in isolated molecules. Triplet levels are relatively unaffected by condensation, according to a wave analysis by Tollin.[21]

The locally bound singlet excited regions are called exciton bands,[31] and the movement of excitation energy between molecules is named "exciton migration." This migration occurs without net movement of charge, and has been called "inductive resonance" by Vavilov and "resonance transfer" by Förster.[22] Molecular-conduction electrons are free within the crystal but require an additional amount of energy, the crystal work function, in order to escape into space.

Two types of energy transfer occur in organic molecular crystals: exciton migration of electromagnetic energy, and charge-carrier migration, the movement of conduction electrons and free positive holes. During their lifetimes excitons may give up a portion of their energy by phonon emission; that is, by participating in the lattice vibrations.

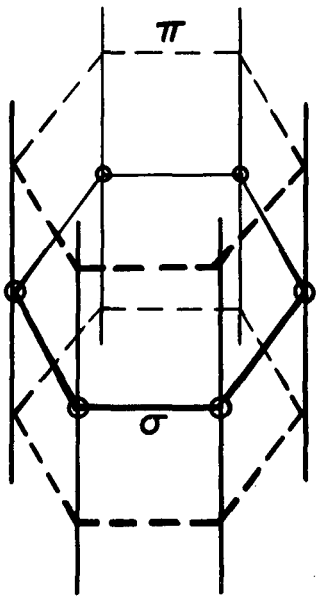

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Fig. 3. σ-π formulation of double bonds in benzene, showing σ bond and π bond.
After Roberts (reference 33, p. 20).

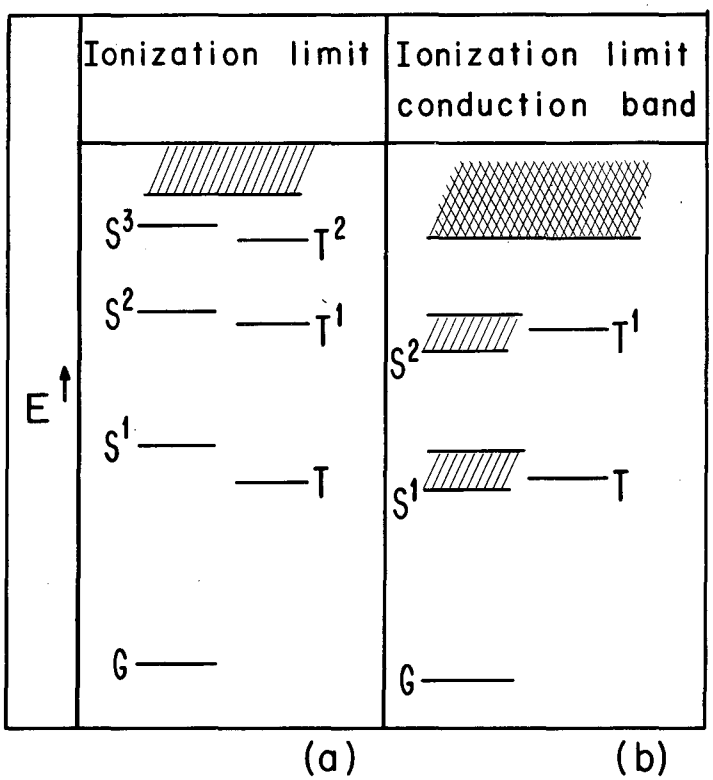

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Fig. 4. Schematic electron energy levels in
(a) isolated molecules, and
(b) molecular cytstals.
G = ground state; s and T_3 = excited singlet and triplet states.
After Tollin (reference 21).

C. Luminescence

This brief introduction is based in part on recent monographs by Curie[35] and Garlick.[36]

1. Luminescence

Luminescence may be defined as emission of light from a substance during or following absorption of energy such as that of ultraviolet light or high-energy particles. It may be distinguished from thermal radiation by its failure to obey Kirchhoff's law: the energy density of luminescent emission always exceeds that expected from thermal emission at the particular temperature. Also, luminescence may be distinguished from the Raman, Raleigh, Compton, and other scattering processes by the time delay following interaction. Scattering-process delay times are less than 0.01 psec whereas, for luminescence they are always greater than 1000 psec.

The special terms bioluminescence and chemiluminescence may be used to describe light energy emitted during chemical reactions; photoluminescence and radioluminescence refer to light emission following stimulation by optical and high-energy rays and particles, respectively; and triboluminescence is used to describe light emitted from crystals under intense mechanical strain such as grinding

Although no precise separation between fluorescence and phosphorescence is possible, one may arbitrarily define fluorescence by setting a delay time of less than 10 μsec. Phosphorescence is then luminescence with a delay time greater than 10 μsec. In this definition thermoluminescence, the release of light upon heating, is a special type of phosphorescence–namely, a phosphorescence of long duration which may be intensified by heating. Electroluminescence describes "cold" light emitted from a substance under the action of a strong electric field.

2. Monomolecular Decay of Luminescence

Wherever the delay of emission is due to a mean lifetime τ of the excited electronic state that has a probability $p = 1/\tau$ for radiative

transition to the ground state, first-order kinetics apply. If n molecules are excited at any instant, J being excited per second by incident radiation, the kinetic equation

$$\dot{n} = J - pn \tag{1}$$

obtains. The luminous intensity L is given by pn. If excitation begins at t = 0, L rises according to

$$L = L_0 \left[1 - \exp(-pt)\right], \tag{2}$$

where $L_0 = L(\infty)$. The luminescent decay (J = 0) is then

$$L = L_0 \exp(-pt). \tag{3}$$

3. Bimolecular Decay

Since the time of Becquerel[37,38] it has been known that not all phosphors follow the simple exponential decline, and he proposed in 1867 a bimolecular model to explain the observed "hyperbolic" decays. In this, photoconductivity was observed to accompany luminescence. It was inferred therefore that luminescence required the formation of free charged carriers within the phosphor–and that their return to normal states was by random recombination with empty luminous centers. If charge-carrier recombination with vacant centers is the rate-determining reaction, the kinetics follow the form of a simple bimolecular chemical reaction. In this analysis, there are n carriers (and n empty centers) at any moment, and if J centers are emptied per second by excitation, then

$$\dot{n} = J - \beta n^2, \tag{4}$$

where β is a recombination coefficient for free carriers and empty centers.

In this case, $L = \beta n^2$, and

$$L = J \left[\tan t (\beta J)^{1/2}\right]^2 \tag{5}$$

for the rise and

$$L = L_0 \left[1 + t (\beta L_0)^{1/2}\right]^{-2} \tag{6}$$

for the decay of luminescence.

It has since been found--by Garlick and Wilkins[38] and by Randall and Wilkins[39]--in most photoconducting phosphors, that trapping and retrapping of the charge carriers produces a nonexponential decay which only roughly follows Eq. (6).

4. Metastable States

Jablonski[40] in 1935 proposed an energy-level scheme (Fig. 5) for an emitting center in an organic molecule. In this, the excited system F may "relax" to the metastable M state. Radiative transitions M$\leftrightarrow$G are forbidden and M$\rightarrow$ F requires thermal activation, the probability for which is

$$p = s \exp(-\Delta U/kT). \tag{7}$$

The decay kinetics are the same as for exponential decay,

$$L = L_0 \exp(-pt), \tag{8}$$

except that here p = p(t). In more recent thinking, the state M corresponds to a trapped electron, and the coefficient s to the "frequency factor."

Because Eq. (7) is important in the thermoluminescence work reported in Section III, it is instructive to present another derivation of it, first given by Williams and Eyring.[41] The free energy F = U-TS has the role of a thermodynamic potential at constant volume. The rate of a monomolecular chemical reaction (here the emptying of traps) that requires a thermal activation depends on the free-energy change ΔF by a Boltzman factor

$$p = K \exp(-\Delta F/kT) = K \exp(\Delta S/k) \exp(-\Delta U/kT), \tag{9}$$

where ΔU and ΔS are respectively the enthalpy and entropy of activation. The factor $\exp(\Delta S/k)$ is the probability (the system having reached the necessary activation energy ΔU) that that configuration will occur which will permit the trapped electron to escape. The factor K may be set equal to the product $K = \nu_t x$, where x is a "transmission coefficient" and ν_t is the crystal vibration frequency approx 10^{12} or 10^{13} sec^{-1}, and is sometimes called the "attempt-to-escape" frequency. Thus the "frequency factor,"

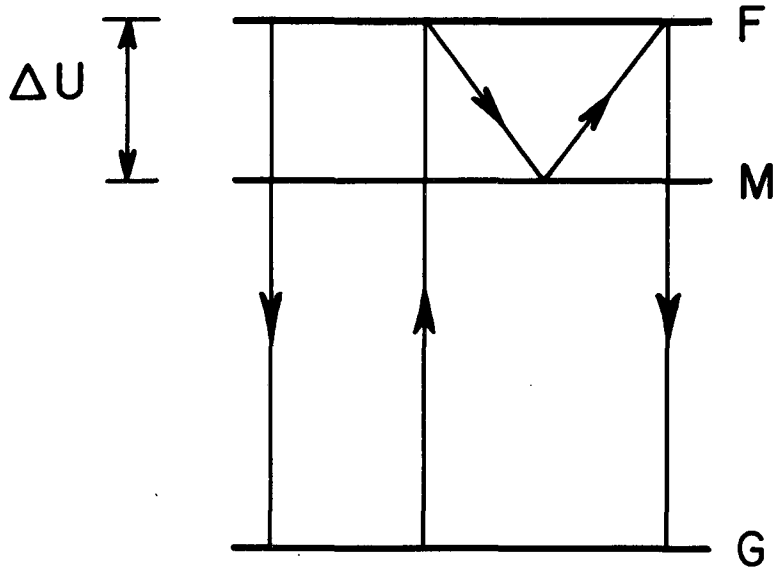

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Fig. 5. Energy-level scheme for an emitting center in an organic molecule.
After Jablonski (reference 40).

$$s = x\nu_t \exp(\Delta S/k), \tag{10}$$

is only indirectly related to the local thermal vibration frequency, and for $s << \nu_t$, it means $\Delta S < 0$, or x is very small.

If phosphorescence from a metastable state is temperature-controlled, then, combining Eq. (3) and (8), one has

$$L = L_0 \exp\left\{-s \exp(t\Delta U/kT)\right\} . \tag{11}$$

Assuming that the thermal activation of the metastable electron is the rate-limiting process (monomolecular kinetics), one may derive the equation $L(T)$ for the thermoluminescence glow curve as follows. Here

$$dn/n = s \exp(-\Delta U/kT)dt, \tag{12}$$

where n is the number of metastable centers at time t. For uniform warming at β° K/sec,

$$dT = \beta dt \text{ and } \ln(n/n_0) = -\int_{T_i}^{T} \frac{s}{\beta} \exp(-\Delta U/kT')dT'; \tag{13}$$

hence

$$L(T) = n_0 \, s \exp(-\Delta U/kT) \exp\left\{-\int_{T_i}^{T} \frac{s}{\beta} \exp(-\Delta U/kT')dT'\right\} . \tag{14}$$

In this, $n_0 = n(t=0)$, and s is the frequency factor. Sims has utilized this equation for comparison with his experimental glow curves of KH_2PO_4 crystals, and has found that the agreement was good.[42]

The following are properties that may be deduced from the thermoluminescence equation (14):

(a) For a given n_0, s, and β the maximum emission occurs at T^*, which is proportional to ΔU.

(b) For fixed n_0 and ΔU, T^* shifts to higher temperature as β is increased or as s is decreased.

(c) The area of the glow curve is proportional to n_0, and therefore to the absorbed dose, but the shape and T^* for a given s, β, and ΔU are independent of n_0.

(d) At low temperatures

$$L \approx n_0 s \exp(-\Delta U/kT); \qquad (15)$$

therefore the initial part of the glow curve may be used to find ΔU, without knowing s, from the slope of the plot of ln L vs 1/T.

5. The Value of Thermoluminescence Studies

In principle, thermal activation first removes electrons from shallow traps (ΔU small), and then from deeper ones. For this reason the thermoluminescence glow curve should give the energy distribution of trap depths in the excited crystal. Because certain thermoluminescence peaks strongly depend on ambient gas pressure (see Sec. III), and other peaks disappear before measurement by a relatively rapid phosphorescent decay, glow curves do not always give this distribution. Yet in spite of this, thermoluminescence remains a powerful tool for the study of metastable states in crystals.

6. Configurational Coordinate Model for Emission Centers

In this model the potential energy of a center is plotted against a configurational coordinate which may be, for example, the distance between the center and neighboring atoms. Normally the ground state and first excited states are given, as in Fig. 6, and the vibration states are presented as horizontal lines, separated by $h\nu$, the phonon or (infrared) lattice vibration quantum.

Optical transitions are vertical, since they occur quickly before the system can relax to a new minimum potential energy configuration (the Franck-Condon assumption). Thus in general the absorption AA' occurs to a relatively high vibrational state--and in losing energy by means of local vibrational exchange the zero-point energy B of the excited state is attained. Luminescent emission B-B' may then occur. The final energy loss to A takes place by a series of phonon emissions (vibration transfers). At CC' the potential curves closely approach, and it is possible that the system may undergo the nonradiative transitions BCC'A by means of vibration exchanges. The probability for for the BC transition is given by

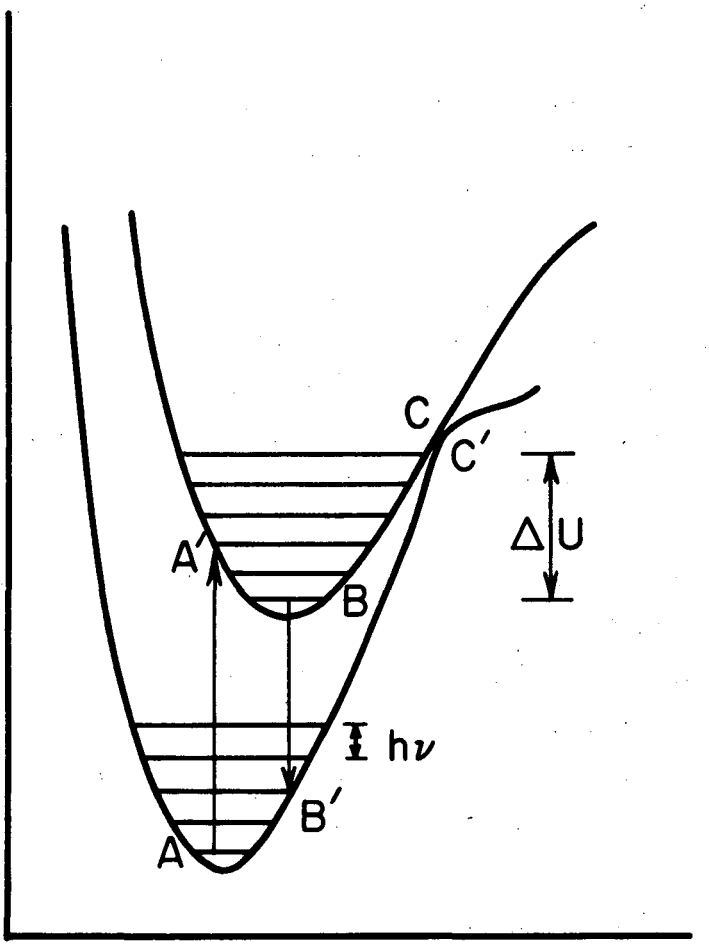

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Fig. 6. Configurational coordinate plot of an emission center.

$$P_{BC} = \nu \exp(\Delta U/kT), \qquad (16)$$

where ν is the frequency of vibration, (10^{12} or 10^{13} sec^{-1}).

This model is useful in understanding the frequency shift observed in fluorescence and phosphorescence between the absorbed and emitted light. When the transition BB' has a low probability (for example, if it is a triplet-singlet transition), the model also helps explain the competing nonradiative mode. And, as emphasized by Klick,[43] the broad Gaussian emission spectra of phosphors even at the lowest temperatures may be explained by the extent of zero-point energy of B, which is predicted by wave mechanics. Klick was able to predict fairly well the temperature dependence of the width of spectral emission for a number of inorganic phosphors, using a one-dimensional harmonic oscillator model. This suggests that to a good approximation the vibrations of these centers are a form of radial pulsation.

The major shortcoming of this model is that it does not describe luminescent mechanisms that involve excitation or charge transport within the crystal lattice. For these phenomena, the use of crystal band theory is more helpful. The configurational coordinate model therefore is particularly useful for luminescence that arises from the excited states of a fixed center, such as those of a thallium ion in NaI(Tl). In practice it is difficult to experimentally obtain an energy-configuration diagram for a luminescent center. Measurement of absorption and emission spectra, of decay constants, and of luminescent efficiency is required over a wide temperature range; this has been done for only a few inorganic phosphors.

D. Luminescence of Organic Molecular Crystals

The theoretical description of lattice imperfections, impurity centers, surface effects, intermolecular energy transfer, or influence of crystal vibrations on electronic spectra of organic crystals has barely begun (Hochstrasser).[44] One result of this incomplete knowledge is the controversy over the $n \rightarrow \pi^*$ transitions in the intense 240–280 mμ absorption of the purines, pyrimidines, and nucleic acids (Zimm and

Kallenbach,[45] Gellert,[46] and Kasha[47]). It is known that the lowest singlet excited states are unoccupied π^* antibonding levels,[45] and therefore each absorption band may contain several close-lying $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transitions. Their relative importance is the subject of controversy.

1. $n\pi^*$ Transitions

Shimada has observed a 0.01-sec phosphorescence of a 10^{-3} M frozen solution of pyrimidine at 90°K.[48] The broad emission spectrum (360 to 450 nm) following mercury lamp excitation was attributed to a $\pi^* \rightarrow n$ triplet-singlet transition. The observation of n, π^* transitions is somewhat surprising, because in general they should be weak, owing to the spatial separation between n and π orbitals, and the contribution of only the s components of the sp^2 orbitals on the N atoms to the transition moment.

2. Absorption Modes

In any case, it is the n and π orbital transitions in the nucleic acid bases that occur in the visible and near ultraviolet, whereas transitions of the σ orbitals lie in the far ultraviolet. The infrared absorption of the nucleic acids has been reviewed by Norris.[49] Peaks have been defined at 3, 6, 8, and 9 μ, which correspond to single-bond (N-H, C-H, O-H) stretching, double-bond stretching, P=O vibrations, and C-O-C and C-O stretching, respectively. There are other infrared absorptions to 30 μ and beyond that correspond to particular configurations of the nucleic acid molecule. The region 3 to 30 μ represents vibrations of 10^{13} to 10^{14} sec^{-1}. This may be compared to the crystal vibrations that occur at the lower frequencies of 10^{12} to 10^{13} sec^{-1}.

3. Emission Modes

Most condensed organic systems have unique emission spectra independent of the energy of excitation: fluorescence occurs from the lowest excited singlet states, and phosphorescence from the lowest excited triplet states. No emission is observed from the higher electronic states even though their lifetimes (10 to 100 psec) are exceedingly

brief. Therefore it must be assumed that an excited molecule transfers vibration energy to its neighbors within approx 1 to 0.1 psec, the period of crystal vibration. Since the molecular vibration frequencies are 0.1 to 0.01 psec, any excess vibrational energy remaining after crystal excitation is rapidly dissipated into lattice phonon states. The reverse process, the transfer of energy via lattice vibration→molecule vibration →electron excitation→luminescence is exceedingly inefficient, as may be inferred from attempts at optical detrapping.

4. Optical Detrapping

Stoddard has studied the effects of illumination on the metastable states of x-irradiated single crystals of sodium chloride.[50] Upon warming a crystal irradiated at dry-ice temperature he observed glow peaks at 198, 253, 318, 363, and 553°K. If instead of warming the crystal after such an irradiation, he cooled it to 77°K in the dark, and then played 400 to 1200-mμ monoenergetic light on it, subsequent warming revealed a strong bleaching of the upper peaks and a strong potentiation (i. e., enhancement) of the 198°K peak. In another study, a crystal irradiated at room temperature was annealed at 373°K, then cooled to 77°K and illuminated. In this case, the 553°K peak was bleached, and the lower peaks, especially the 198°K one, were potentiated. It was found that the 450-nm light was about five to ten times as efficient in these bleaching-transfers as shorter (400 nm) or longer (2600 nm) light waves.

For these peaks the thermal activation energy is 0.4 to 0.6 eV, and yet the 1-to 3-eV light quanta were incapable of permanently detrapping the electrons. Instead, they effectively raised electrons from deep traps into empty shallow ones. In this work, the illumination presumably acted directly on the trapped electrons; but in the following study, the illumination was 1.5 μ, a wavelength that acts on molecular vibrations. Pure single anthracene crystals were irradiated with 385-nm light at 77°K by Bryant et al.[51] Upon warming, three conductivity peaks were measured, at 203, 233, and 263°K. A 12-min infrared-lamp treatment did not alter these peaks, nor did an equal photon dose at 1.5 μ.

Section III also reports futile attempts to alter metastable electronic excitation by use of intense infrared beams.

5. Oxygen and Impurity Effects

Oxygen is a well-known agent for quenching fluorescent solutions, and Bowen and Williams have suggested that this effect may be explained by a triplet exchange collision between the normal (triplet) oxygen and the singlet-excited molecule.[52] For bioluminescence of the sea pansy, however, Cormier has found that molecular oxygen is essential.[53]

Oxygen also is important in solid-state luminescence. Thirty-seven years ago Hamano reported that cholesterol and certain other dry substances were able, following x-ray or ultraviolet radiation in the presence of oxygen, to expose photographic plates.[54] Grossweiner and Matheson have found that oxygen had no effect on the x-ray fluorescence intensity or buildup of pure ice, but that ice frozen in the presence of 0.2 atm of oxygen gave a thermoluminescence over a broad temperature range with no well-resolved peaks.[55] Small quantities of hydrogen peroxide, however, quenched both the fluorescence and thermoluminescence.

Vladimirov discovered that the spectrum and quantum yield of the several amino acids in separate solutions are different from the total spectrum and yield of the same amino acids incorporated in a protein molecule, and further that the fluorescence and afterglow in tryptophan-containing proteins are almost entirely due to this amino acid.[56] He also found that the tryptophan-deficient protein serum albumen gave a very high fluorescent yield (20%) in acid and alcohol solutions, but not otherwise. Under these conditions the hydrogen bonds are broken; for this reason he suggested that the hydrogen bonds between tyrosine residues and the protein normally quench fluorescence. If this phenomenon is important, then the local protein or nucleic acid environment (O_2, pH, etc.) may determine the ultimate radiation sensitivity of the cell: under certain circumstances the unwanted excitation energy is efficiently released as luminescence, and under others it is locally absorbed in the chemical bonds.

Oxygen and impurity effects are also well known in organic semiconduction and electron spin resonance-fields related to luminescence. For example, a 0.1% tetracene-doped single crystal of anthracene was found by Bryant et al. to give a completely different conductivity glow curve than a crystal of pure anthracene.[51] Vartanyan and Rozenshtein report that the dark conductivity of certain xanthene dyes was increased in the presence of oxygen at lower temperatures, but not at temperatures of 50 to 100°C.[57] Moreover this effect was reversible.

In electron spin resonance work, the gaseous and water environments during and after irradiation have marked effects on radical yield, quality, and lifetime. For instance, nitric oxide treatment of tyrosine and a few other amino acids and sugars after irradiation in vacuum causes ESR signals to vanish (Smith).[15]

6. Luminescence Yields

The absolute fluorescence quantum efficiences of many organic substances in solution have been reported by Melhuish.[58] For all the substituted heterocyclic compounds listed, the efficiencies ranged from 0.02 to 0.89. That of anthracene in infinite dilution of benzene was 0.26, a value that is also typical for the solid organic scintillators. Thermoluminescence yields have not been reported for organic chemicals, but Daniels et al. have reported that the fraction of absorbed gamma energy stored in lithium fluoride crystals as trapped electrons is less than 10^{-4}.[59]

7. Emission Spectra

Weinberg et al. have studied the low-temperature thermoluminescence emission spectra of γ-irradiated tyrosine, tryptophan, phenylalanine, and trypsin.[60] All these substances were found to have roughly bell-shaped spectra in the region 450 to 550-nm with widths of 100 to 150 nm. Phenylalanine presented a second peak, at 300 nm, 25 nm wide. In all cases a gradual spectral shift to the longer wavelengths was observed during warming. Sharma has put 2 to 5-kV electrons on

benzene, toluene, and p-xylene at 89°K.[61] The fluorescence spectra consisted of systems of rather broad bands in the visible and in the ultraviolet; the visible bands were more intense. The phosphorescence spectrum of p-xylene at 89°K showed the visible bands, but the ultraviolet bands were absent.

The 77°K phosphorescent spectra of uv-stimulated solid organic films of xanthene dyes are also roughly bell-shaped, with peaks in the region 500 to 550 nm, and 150 nm wide. In some cases one or two shoulders are present (Vartanyan and Rozenshtein).[57] Peaks of this same shape in the region 470 to 490-nm and 100-nm wide also were reported for dilute frozen (77°K) solutions of ovalbumin, RNA, and DNA (Sapezhinskii and Émanuél').[29] The same spectra were obtained under stimulation by either uv or cobalt-60 rays; and the phosphorescent decay was exponential in each case, with lifetimes of the order of 4 sec.

Solutions of purines, pyrimidines, and their nucleoside and nucleotide derivatives have been subjected to fluorescence spectrometry by Udenfriend and Zaltzman.[62] With the exception of free thymine, the purines and the derivatives were without fluorescence. Adenine and its derivatives fluoresce weakly and only in acid solution; guanine fluoresces most intensely of the nucleic acid bases and may be measured in acid hydrolysates of nucleic acids by its 340-nm emission.

Zadorozhnyĭ and Naboikin have observed a strong Stokes' shift in fluorescence of organic H-bonded systems.[63] They feel that the rupture of these bonds during electronic transitions in emission or absorption may give rise to this shift toward the longer wavelengths.

The fluorescence intensity of pure ice under x rays was observed by Grossweiner and Matheson to fall by a factor of 7 upon warming from 77 to 200°K.[55] The fluorescence spectrum was a band, at 385 nm, 80 nm wide.

8. Introduction to Thermoluminescence

The experimental findings of the several studies of thermoluminescence of organic chemicals irradiated at low temperature may be summarized as follows:

(a) Sharma–using thin films of benzene, toluene, and p-xylene irradiated at 89° K with 2 to 5-keV electrons–found:[61]

(a. 1) Thermoluminescence may be accompanied by a temperature-independent phosphorescence of exponential decay time of a few seconds.

(a. 2) The difference between the shorter-wavelength edges of the fluorescence and phosphorescence emission gives an estimate of trap depths of approx 1 eV, in disagreement with the 0.3 to 0.4 eV estimated by the method of glow-peak temperature.

(a. 3) Glow peaks are rougly bell-shaped, and a given substance may have several such peaks.

(a.4) Glow peaks between similar chemicals vary widely in their temperature-width, their location, and their intensity.

(a.5) Sharp flashes were recorded when the temperature was raised to the vaporization point.

(b) F. J. Bryant et al. –using single crystals of anthracene irradiated by ultraviolet at 77° K and examined by the conductivity-glow-curve method–found:[51]

(b.1) The general conductivity glow-curve pattern was similar from crystal to crystal, and was largely independent of surface roughness or internal cracks.

(b.2) See (a.3) above.

(b.3) The glow curve is drastically changed and reduced in intensity by the presence of 10^{-3} mole/mole of tetracene.

(b.4) Increasing the warming rate from 0.08 to 0.37 deg/sec shifts the glow peak from 250 to 265° K.

(b.5) An x-ray dose of 10^{5} rads at 77° K destroys the major glow peak.

(c) Carter–using γ-irradiated polycrystalline amino acids and proteins at 77° K–found:[64]

(c.1) Glow curves from proteins are not the sums of the glows of the constituent amino acids.

(c.2) See (a.3) and (a.4) above.

(c.3) The chemical composition exerts the greatest influence on glow curves, although impurities may have strong influence on the glow of weakly emitting amino acids.

(c.4) The crystalline system plays a minor or negligible role in determining glow curves.

(c.5) The glow peaks of the ring-type amino acids are roughly 1000 times as intense as those of the nonresonating type. All the intense glows are in the region 100 to 140° K.

(d) Augenstine et al. –using γ-irradiated polycrystalline amino acids, proteins, and related substances at 77° K–found:[65]

(d.1) The thermoluminescence trap depths of proteins and polypeptides, based on glow-curve analysis, are 0.02 to 0.17 eV, and their glow peaks are in the range 106 to 118° K. Glow intensities are in general 10^{-3} times that of the 173° K NaCl peak, but range from zero in cytochrome c to 10^{-2} times the NaCl peak in egg albumen.

(d.2) The trap depths of the amino acids are generally in the 0.2-eV region, and the glows of the ring-type acids are roughly 10^{-1} to 10^{-2} times the 173° K Na Cl peak and intensity.

(d.3) The glows of D- and L-phenylalanine are in fair agreement, but that of the D-L racemic mixture is completely changed and greatly reduced in intensity.

(d.4) The glows of L-glutamic acid and poly-L-glutamic acid are roughly of the same (low) intensity, but the latter lacks the peaks at 141 and 165° K observed in the former.

(d.5) For the protein enzyme trypsin, doses from 240 rads to 7.2 Mrads may be plotted linearly with area under the 110° K glow peak.

(e) Weinberg et al. –using γ-irradiated polycrystalline amino acids and proteins at 77° K in 1/2 atm of helium–found:[60]

(e.1) The glow-peak intensity measurements agree to ± 50% (for given experimental conditions) for samples from the same chemical batch.

(e.2) A temperature-insensitive glow at 77° K contributed 5% of the total glow peak of trypsin; the remainder requires a thermal activation energy of about 0.10 eV.

(f) Some general phenomena have been observed in studies of thermoluminescence:

(f.1) In inorganic crystals, continued irradiation to high doses (0.1 Mrad) creates new high-temperature peaks (Daniels).[59] Saturation and even a back reaction may occur at higher doses (Sims).[42]

(f.2) Sims also noted sharp flashes superimposed on the glow of potassium dihydrogen phosphate in the 123 to 143° K region. (See also Sec. III, Table I.)

(f.3) The increase of 173° K glow-peak area is roughly linear with increasing surface area of crystalline sodium chloride samples irradiated at 77° K in 400 μ Hg of oxygen, helium, and argon (Nelson).[66]

E. Summary

The foregoing has given a glimpse of what crystalline luminescence can reveal about ionizing radiation damage in living cells. In brief, cell aggregates are densely organized structures, composed of molecules that have the peculiar ability to transfer, store, absorb, and emit energy in packets of a few electron volts. The action of ionizing radiation is direct on such systems and ultimately creates local excited electronic states of energy of a few electron volts. Whether the cell aggregates stabilize this energy internally as chemical events, emit it as optical radiation, or dissipate it as local vibrations may determine the ultimate radiation sensitivity of living cells. The method of de-excitation depends to a marked extent on the immediate gaseous or "impurity" environment, and on the electronic nature and configuration of the molecules.

Organic crystalline luminescence provides a unique tool for the study of these effects under controlled conditions. For instance, even excited states that are exceedingly transient at body temperature may be held in their primary form at the temperature of liquid nitrogen, and thus studied more conveniently than in the living cell.

On the technical side, organic crystalline luminescence is largely an empirical science whose interpretation in terms of basic principles has barely begun. Optical transitions involve π and n molecular orbitals. Such transitions are capable of modification by local interaction--for example, with hydrogen bonds and with molecular vibrations. In condensed systems optical transitions generally occur only from the first excited singlet and triplet states; any excess excitation energy is rapidly transferred to molecular vibrations. It may be for this reason that optical detrapping is difficult.

Quantum fluorescence efficiencies of the heterocyclics vary between 0.02 and 0.89, and thermoluminescence yields are small, less than 10^{-4}. The purines, except thymine, do not fluoresce in solution; adenine fluoresces only weakly; and quanine most strongly in acid solution. All the nucleic acids and bases absorb strongly at 260 nm. Emission spectra consist of one or more bell-shaped bands, of width 100 to 150 nm, for a wide variety of biochemicals.

Thermoluminescence glow peaks are roughly bell-shaped and occur mainly in the region 100 to 170° K. A given chemical may have several peaks, and similar chemicals may have glow peaks differing in width, location, and intensity. The glow peaks of the ring-structure amino acids are 1000 times as intense as those of the other amino acids. The crystalline system and impurities are relatively unimportant in these glows. The thermoluminescence curve of proteins is not the composite sum of the amino acid glows.

In the experimental study reported herein, most of the chemicals present in the cell nucleus have been subjected to luminescence analysis in pure crystalline powder form. The preliminary work consisted of a study of the delayed phosphorescence and thermoluminescence properties of a wide variety of chemicals following irradiation at 77° K by cobalt-60 rays. The glow-peak location, temperature widths, and yields are reported.

The second phase of this work consisted of examining the luminescence of the nucleic acid bases and related compounds under irradiation with intense electron and heavy-ion beams. Under electron bombardment, the effect of ambient gases at various pressures on their luminescence has been studied. The energy yields and emission spectra for fluorescence, thermoluminescence, and delayed phosphorescence are reported.

II. EXPERIMENTAL METHOD

A. Equipment and Methods for the Gamma Irradiations

1. Irradiation Facility

The Lawrence Radiation Laboratory cobalt-60 gamma irradiation facility was used. It consists of two cobalt-60 sources, which at the time of these studies contained totals of about 700 curies and 25 curies, respectively. These sources have been described in detail elsewhere.[67,68]

2. Chemicals

The special cell-nuclear biochemicals were supplied in the purest commercial grades by Sigma Chemical Company of St. Louis, Missouri (Mr. Louis Berger, of this company, has written that the nucleic acid bases were crystallized from hot aqueous solutions without use of organic solvents.) The more common chemicals were of analytic reagent grade. The dry chemicals were in polycrystalline powdered form, and were stored in a freezer.

3. Sample Holder

The sample holder for the irradiations may be seen in Fig. 7. It consisted of a base assembly and sample cup, a brass V-tube barrel, a heavy Teflon coupling mount, and end windows. Details of the base assembly are given in Fig. 8. All the metal parts were blackened by the "Ebonal C" process of alkaline oxidation. The smaller-diameter arm of the V-tube barrel is so oriented that infrared light may be put on the sample through the small germanium end window. The larger-diameter arm serves as the light tube from the sample through the quartz end window to the photomultiplier apparatus. The V-tube arms are segmented so that Teflon gaskets may thermally insulate the end-windows. The Teflon gaskets provided air- and liquidtight seals.

4. Photomultiplier

Several RCA 1P28 quartz-window photomultiplier tubes were used. The light-sensitive photocathode material of these tubes is Cs_3Sb, which has an S-5 spectral response that peaks at 340 mμ and has a useful wavelength range from 220 to 600 mμ. The tubes used were carefully

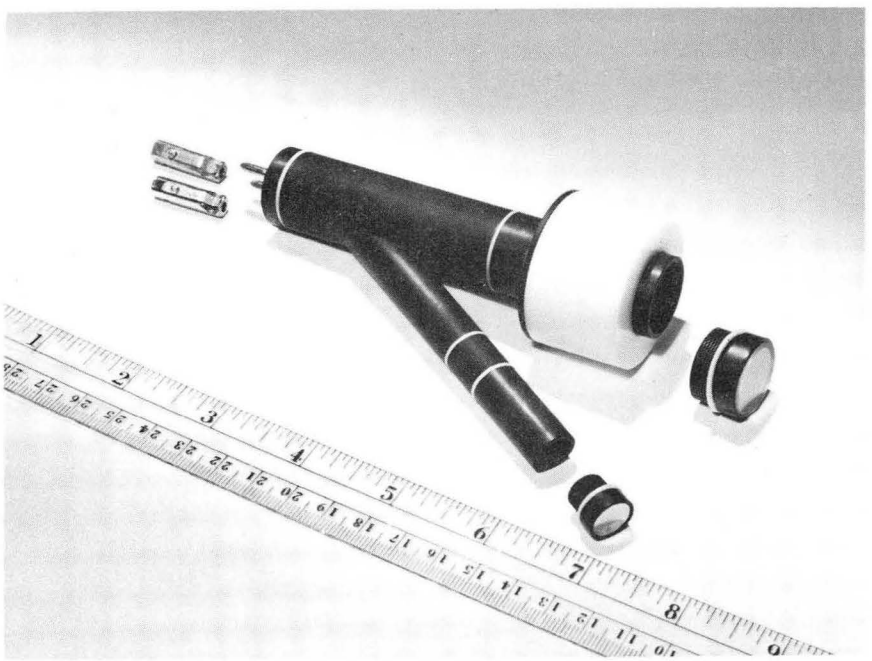

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Fig. 7. Sample holder for gamma irradiations.

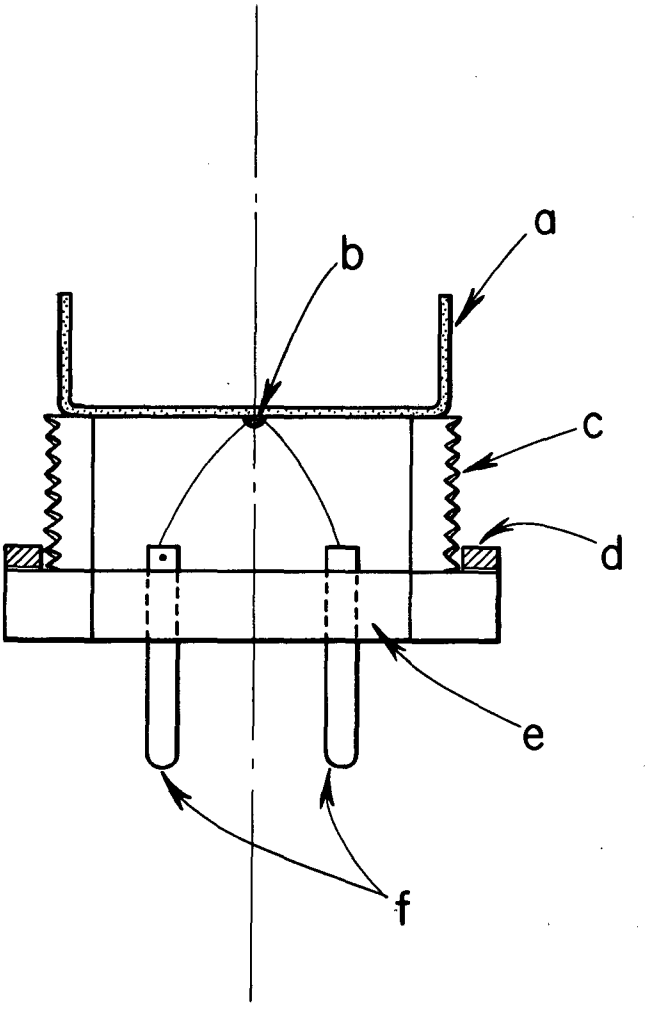

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Fig. 8. Details of base assembly.
a. Copper cup, blackened by "Ebonal C" process.
b. Thermocouple leads—and junction.
c. Stainless steel.
d. Teflon gasket.
e. Pressed wood plastic.
f. Copper-constantan thermocouple jacks.

selected to have a dark-current output of 0.3 to 0.4 nA when operated at 980 V. The photomultiplier tube was housed in the apparatus shown in Fig. 9. The apparatus consisted of a Teflon coupling mount, a camera shutter, and the aluminum tube-head mount. The metal parts were black-anodized and the outer Teflon surface was covered with black tape.

5. Amplifier and the Recording Equipment

The photomultiplier tube was supplied with 980 V via a standard voltage-divider box from the regulated 2-kV voltage supply. The light signal was amplified by the E-H Company model 215-RL electrometer-voltmeter fitted with a 10^8-ohm precision resistor. The amplified light signal was displayed in the Leeds-Northrup X_1 X_2 model G type-S potentiometer strip-chart recorder. The temperature of the sample cup (Fig. 8) was simultaneously recorded on the strip chart by use of the copper-constantan thermocouple leads and coupling jacks.

6. Experimental Procedure

A 10- to 200-mg sample of the chemical to be tested was weighed on a precision balance and put into the sample cup. The cup assembly was screwed into the body of the holder until the Teflon gasket was tight, so that the sample was sealed in air at 292° K at 40 to 60% relative humidity. The bottom two-thirds of the sample-holder assembly was then immersed in liquid nitrogen contained in a one-pint vacuum Dewar. Three minutes was required for the sample to reach liquid-nitrogen temperature.

Next, the Dewar holding the vacuum flask was remotely carried to the irradiation position inside the cobalt-60 source. Approximately 2 min elapsed during the process of getting the sample into and out of the large source housing. When the irradiation was completed, the photomultiplier assembly was coupled to the sample holder, and the latter was quickly taken from the liquid-nitrogen-immersion Dewar and seated in the heater. The heater consisted of wire coils housed in a ceramic base. From it heat was delivered to the lower two-thirds of the sample holder. The heater base contained the sockets for the thermocouple jacks. By varying the ac voltage to the heater coils a constant

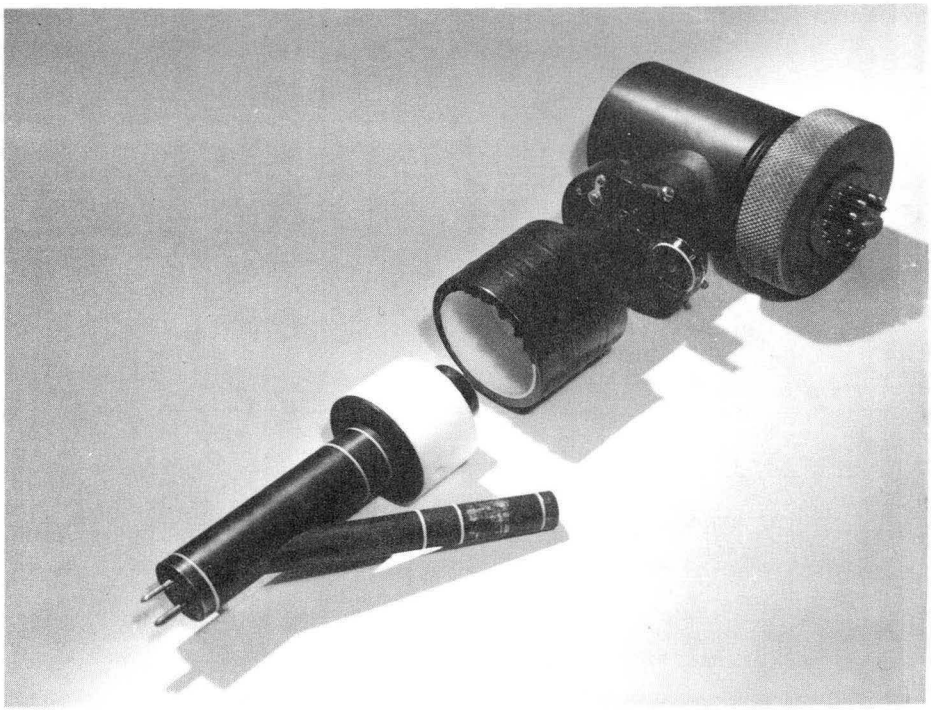

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Fig. 9. Photomultiplier housing.

rate of sample warm-up was obtained. The rate could be varied between 8 and 70°C per minute.

7. Dosimetry.

The radiation fields within the cobalt-60 sources were measured with polished cobalt glass pieces 15×6× 1.4 mm. The reading equipment was the Bausch and Lomb spectrophotometer type 33-29-40 together with the Sorenson and Company ac voltage regulator model 1000-3. The measured radiation field at the sample location was 93 roetgens/sec. This was found to be in agreement with interpolated values obtained by the Fricke ferrous sulfate method and by special colored-liquid dosimeters[69] supplied by the Edgerton, Germeshausen & Grier Company. In each case it was assumed that an exposure dose of 1 roetgen delivered an absorbed dose of 1 rad, or 100 ergs/g.

B. Equipment and Methods for the Particle Irradiations

1. Heavy-Ion Accelerator

The Lawrence Radiation Laboratory heavy-ion accelerator can produce variable-intensity ion beams of atoms of mass between helium and argon at fixed energy of about 10 MeV per nucleon. Its use in biophysics irradiation has been described by Brustad.[70]

For this work oxygen (+6) and carbon (+4) ion beams were used at beam intensity levels of 1.2 to 4 nA, measured by a 0.1-mil insulated aluminum foil mounted behind a 3/8-in.-diam collimating hole, immediately in front of the target sample holder. The beams were delivered in 2-msec pulses at the rate of 12 to 15 pulses/sec.

2. Electron Linear Accelerator

The Lawrence Radiation Laboratory Linear Accelerator[71] produced intense beams of 5-MeV electrons. The beams were delivered in 6-μsec pulses at rates between 7.5 and 30 pulses/sec.

3. Chemicals

The chemicals were the same as those used in the γ irradiations. For use in the target sample holder it was necessary to press the

powdered polycrystalline chemicals into 3/4-in. -diam 1-g pellets by use of a hydraulic ram and the pellet-press assembly in Fig. 10. The pellets were stored at 0°C over a desiccant ($CaSO_4$). A new pellet was prepared and put into use when its predecessor had accumulated 10^7 rads.

4. Target-Sample Holder

The target sample holder was designed so that particle beams would play on the pellet directly. The holder may be described with the aid of Fig. 11. The particle beam entered brass port B and struck the pellet, which was mounted by means of double-sided masking tape on the copper target mount D. The target luminescence passed through quartz window C and coupling mount A to the spectrometer-analyzer and phototube. The copper-constantan thermocouple leads E passed through the brass base plate to the target mount. A stream of liquid nitrogen flowed from F to D to G to cool the target mount during the low-temperature measurements. Dry nitrogen gas, flowing under roughly 10 to 30 psi along the same path, warmed the target mount during thermoluminescence runs.

All the metal parts in the optical end of the holder were blackened by the "Ebonal C" process of alkaline oxidation. The holder had vacuum-tight rubber O-ring seals at all coupling surfaces. Hidden in the brass port B and located 1 in. from the target mount was an aluminum collimator-plug with a 1/2-in. -diam central hole drilled along the beam axis. This plug allowed the beam to play exclusively on the mounted pellet.

5. Conditions During Irradiation

The experimental setup as it was used at the electron linear accelerator is shown in Fig. 12. Here the target sample holder and support assembly were separated from the end of the accelerator beam tube by 3 in. The beam-port tube of the holder slipped past an O-ring vacuum seal into the support assembly as shown. The support assembly was fitted with a National Research Corporation vacuum ion gauge mount above, and with a vacuum mount below.

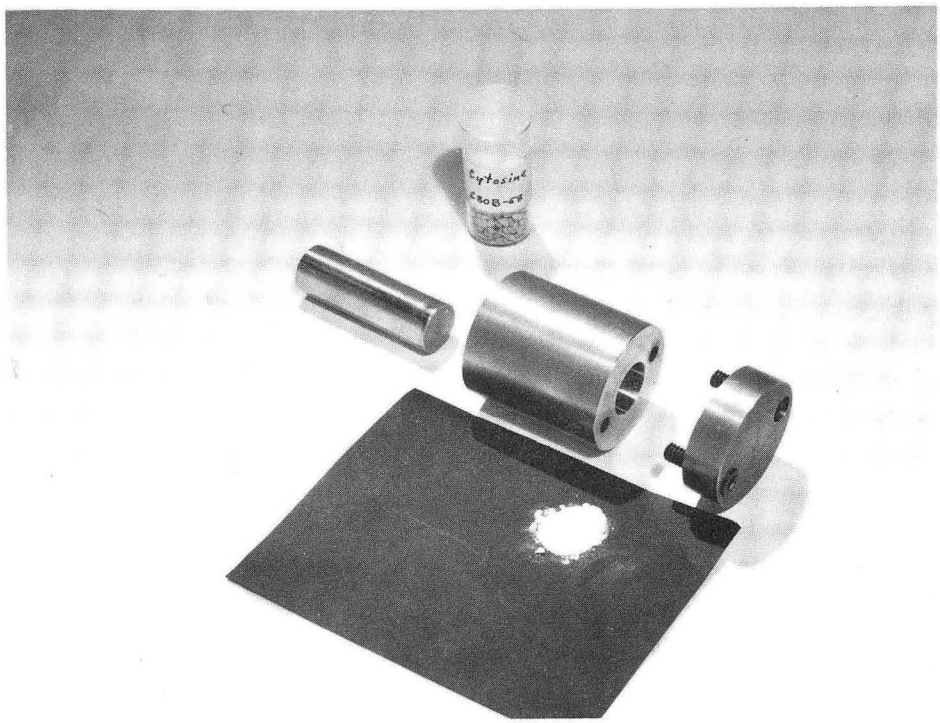

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Fig. 10. Pellet press assembly.

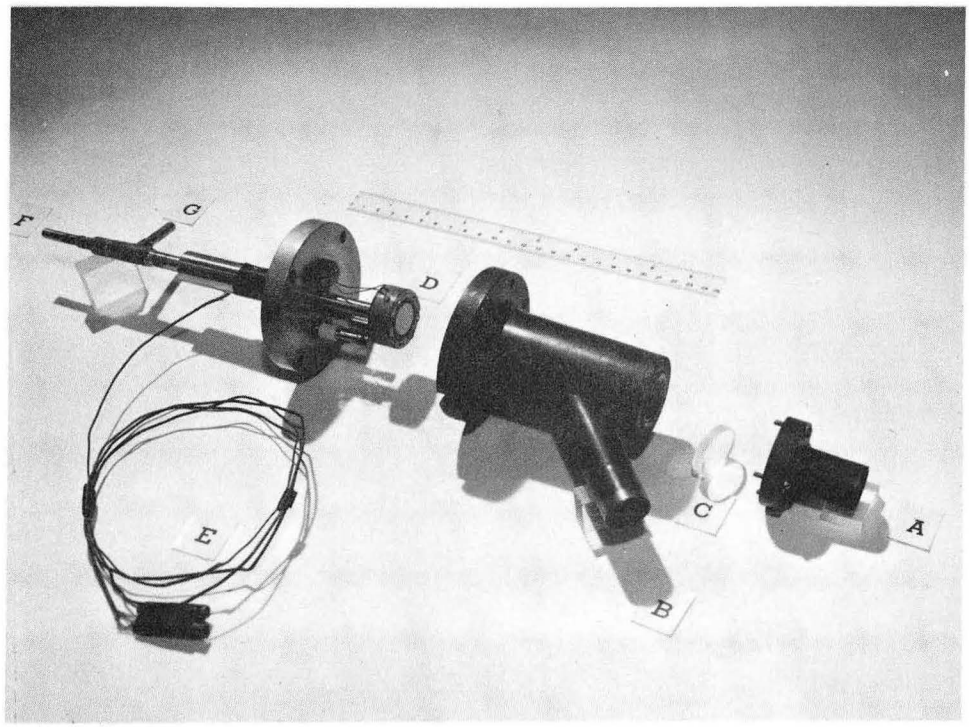

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Fig. 11. Target sample holder.

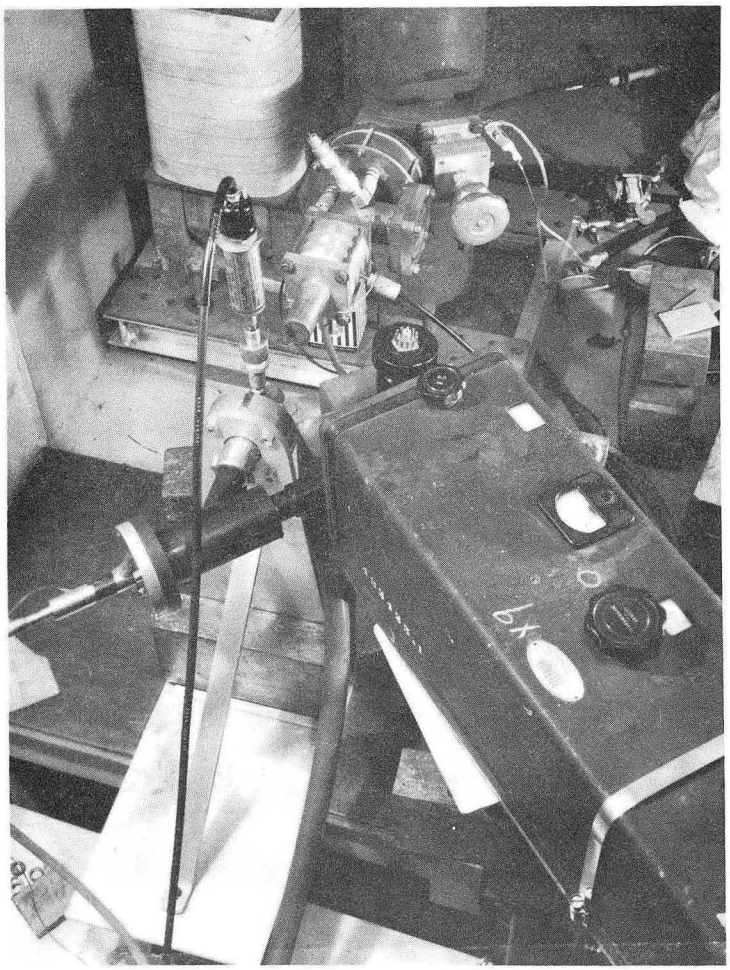

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Fig. 12. Setup for electron irradiations.

A needle valve in the vacuum line allowed variable quantities of dry nitrogen, oxygen, or helium from commercial cylinders to enter the target sample holder via the support assembly. The high-vacuum system consisted of a mechanical roughing pump backed by a mercury diffusion pump.

For the work at the heavy-ion accelerator, the beam-port arm of the target holder entered a support assembly mounted on the end of the accelerator-tank beam tube. There the pellets were irradiated in the vacuum of the accelerator tank itself.

6. Dosimetry

Pellet-sized cobalt-glass dosimeter plates were exposed at the target site to the electron beams. The reading technique and equipment are given in Subsec. II-A.7. It was found that the dose rate was 3800 rad/sec when the accelerator was operated at 0.05 V and 30 pulses/sec. When a piece of microslide glass was mounted at the target site and irradiated, a circular area just slightly less than the 3/4-in. pellet was darkened.

No dosimetry was attempted at the heavy-ion accelerator. It was assumed that the readings of the foil beam monitor (Part A) gave a true measure of the beam current on the pellets. The heavy ions were of course stopped in the first 0.40 mm of the pellet and the local rad dose was unknown but very high. However, a certain amount of beam energy was put into the pellet surface and a certain amount of light energy was emitted. The luminescence yields in this case were simply the latter divided by the former.

7. Calibration of the Photomultiplier-Amplifier-Recorder System

A Sylvania Company 0.02-W electroluminescent disk was used as a distributed constant low-intensity light source to simulate the experimental glow. The disk and the blackened-brass light tube are shown in Fig. 13. The brass tube has the diameter of the light orifice inthe sample holders, and the exact length of the pellet-to-spectrometer coupling distance. Thus inserting the brass tube into the spectrometer coupling and holding the face of the disk tight against the open end

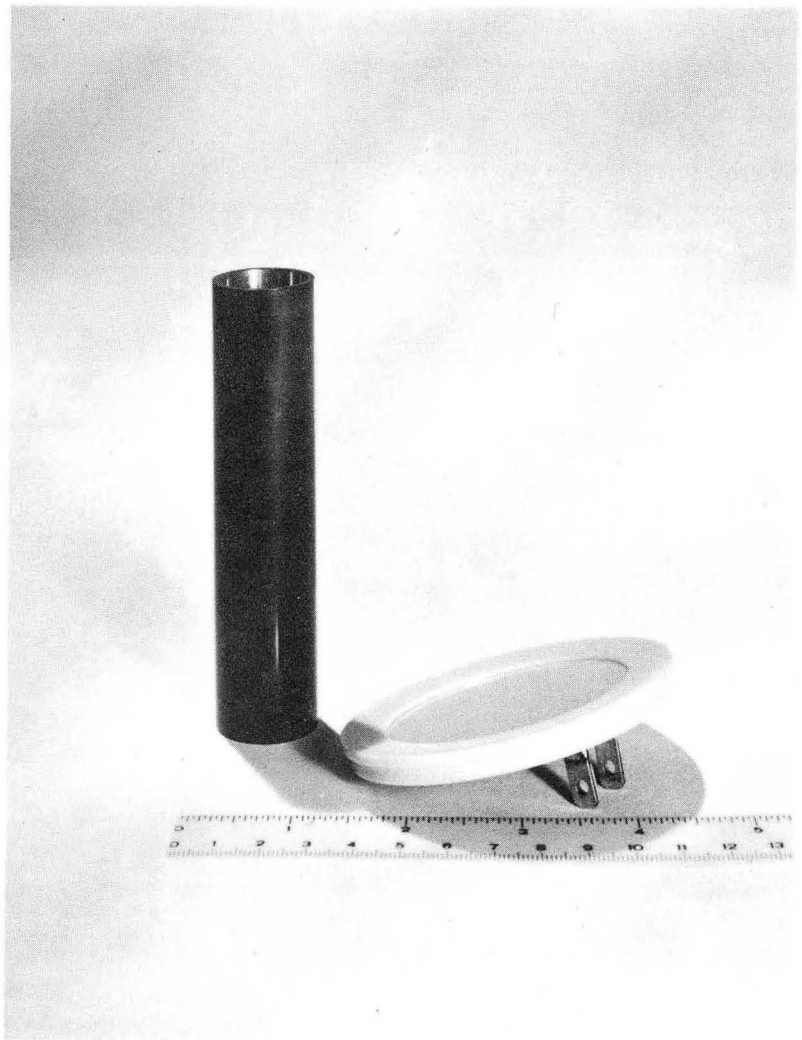

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Fig. 13. Electroluminescent disk and accessory tube.

achieved the same optical geometry as was used in the experiment. Thus, when the brass tube was inserted into the spectrometer coupling and the face of the disk was held tight against the open end, the experimental optical geometry obtained.

The electroluminescent disk, with 30.0 volts 60-cycle ac applied to it, was found to glow with total radiant intensity of 0.82 μ watts/steradian, and to have a bell-shaped emission spectrum at 500 nm, 80 nm wide at half intensity. The luminous and radiant intensity of the disk were measured at the National Bureau of Standards by use of a calibrated vacuum photoelectric tube fitted with a removable luminosity filter. The measured intensity is believed to be accurate to ±20%.

The electroluminescent disk intensity was strongly dependent on voltage: it changed at a rate of 2.5% per 0.10-volt shift from 30.0 V, and the total decrease in intensity in going from 120 V to 10V ac was by a factor of one million. The emission spectrum did not shift over this range of intensities. These properties coincide with those of an electroluminescent ZnS phosphor described by Butler and Waymouth.[72]

8. Spectrometer

The spectrometer consisted of the monochromator analyzer of a Beckman model DU. A simple flat-mirror coupling input was used, and an RCA 1P28 phototube was the output light detector. The phototube housing was identical to that used in the gamma-irradiation studies, but without the light shutter and Teflon collar. The housing was permanently attached to the face plate of the DU. The optical band-pass width of the DU at 2.0 mm (wide open) entrance slit varies with wavelength, as given in Fig. 14. In the same figure may be seen the IP28 phototube relative spectral response, and the "total" relative phototube-DU response.

The High-voltage supply, electrometer-voltmeter, and strip-chart recorder were the same as in the gamma-irradiation studies.

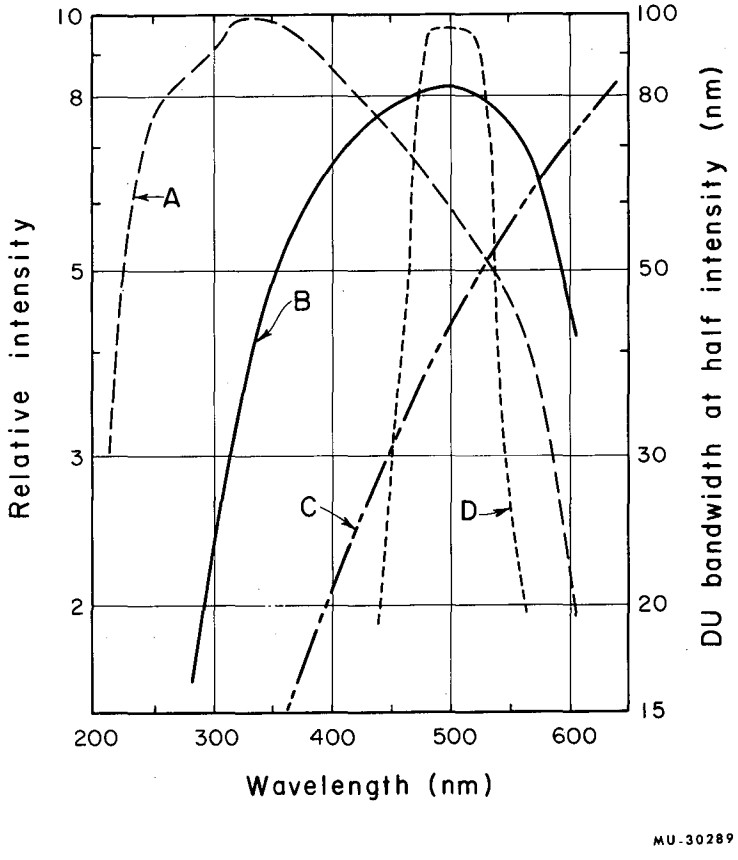

Fig. 14. Spectral response of detectors.
A. RCA 1P28, S-5 response.
B. "Total" DU and S-5 response.
C. DU response.
D. Emission spectrum of electroluminescent disk light standard.

III. EXPERIMENTAL RESULTS

The luminescence properties of thirty-four organic chemicals irradiated at 77° K by cobalt-60 rays, and those of the nucleic acid bases and related compounds irradiated by heavy-ion and electron beams, are reported in this section.

A. Luminescence Following Low-Temperature Gamma Irradiation

1. Thermoluminescence

The glow-peak temperature, duration, and yield of radiant energy for three dozen chemicals in powdered or quick-frozen-liquid form are presented in Table I. Because all the glow peaks are roughly bell-shaped, the three given measured parameters (temperature-location, duration at half maximum, and yield) are sufficient to describe them. In each case the glow measurements were begun between 2.5 and 4 min after the end of the exposure.

On the basis of Table I, two things may be emphasized:

a. The glow-peak temperatures stand without exception in the region below 180° K.

b. Certain cell-nuclear chemicals under suitable conditions may release 0.1 to 0.2% of the locally absorbed gamma dose as thermoluminescence.

From Fig. 15 it can be seen that in each case of high glow yield, the molecular structure contains conjugated double bonds (π orbitals), or, in the case of inorganic crystals, electronic excited states common to the entire crystal. For comparison we have found that $PbCl_2$ crystals have a low-temperature glow-peak yield of about 0.02. The starred chemicals have been entered in Fig. 15 at their approximately correct places as estimated from relative intensity values reported by Augenstine et al.[65]

The yields were obtained by comparison of the glow intensities of the samples with the radiant intensity of a calibrated electroluminescent disk at the same optical location. It was found that the average glow-peak intensity of 1 μwatt could be duplicated when the calibrated disk was operated at 30 V (60 cycles).

Table I. Thermoluminescence of various gamma-irradiated chemicals[a]

Chemical	Heating rate (°K/m)	T^{*} (°K)	Duration (sec)	Yield $\times 10^{-6}$	Comments	Sigma Chemical company lot number
Adenine	70	120±10	31±5	2400	480±50 nm width	A-12B-255
5' ADP-Na salt	8	98	420	35[b]	LNp	121B-790
	70	120	45	37[b]		
ATP-di-Na salt	54	80	≈20	16	two peaks	121B-785
	54	125	36	200		
ATP-di-Na salt	70	95-114	40	24	dissolved in H_2O	
Aniline	54	105	66	240		
Anthracene	54	98-111	27	28		
Benzene	54	95	10	17	lesser peaks 80°, 118°, 145°	
Carbon tetrachloride	32	135	70	0.016		
Catalase	70	80-147	60	8	hint of peak at 185°K	
Cephalin	70	90-105	≈15	0.16	two peaks	C-40B-65
	70	148	60	0.28		
Cholesterol	70	124	20	28	two peaks	C-121B-95
	70	183	26	17		
Cytosine	70	115	15	72	three peaks	C-80B-58
	70	130	25	640		
	70	180	35	360		
Sodium chloride	70	200	35	600	two peaks	
	70	250	40	180		
DNA - Calf thymus	70	138	29	2.8	highly polymerized	Type I
DNA-tetra-Na Salt	70	153	30	1.5		Type II
DNA-Grade III	70	120	29	22.		Grade III
DNA-free acid	70	125	33	6.1	tail to 210°K	Type IV
	70	~110	~30	6.4	sat. sol'n	

(cont.)

Table I. Thermoluminescence of various gamma-irradiated chemicals (cont.)

Chemical	Heating rate (°K/m)	T* (°K)	Duration (sec)	Yield×10-6	Comments	Sigma Chemical company lot number
β-DPN	12	115	135	11.		111B-692-7
β-DPN	70	132	12	12		
β-DPNH	70	83	6	0.56		121B-625
Ether, anhyd.	70	93	11	4.4	lesser peaks 80°, 100°, 150°; two peaks	
	70	135	12	2.8		
Glycine	36	77-150	150	0.032		
Guanine	70	108±4	24±6	10.	pk. 510 nm; LNp	
Histone-calf thymus	70	113	50	14.	LNp	H-101B-96
Lecithin	70	77-160	70	0.28		2-120B-81
Linoleic acid	54	100	90	1.9	tail to 185°K	
Naphthalene	54	102	14	7.5	lesser peaks at 80°, 164°K, 202°K, two peaks	
	54	132	22	22.		
Phenanthrene	70	102-225	110	~1600.		
Phenol (single crystal)	54	100-230	150	?	about 100 sharp spikes	
Polyethylene	70	118±4	31±3	400	LNp; transparent sheet; two peaks.	
	70	155±5	24±3	105		
Protamine-SO_4	70	77-150	60	1.6	LNp (salmine)	
Pyrimidine	70	105	45	1.2	long tail	Cal Bio. 5481
Sucrose	54	120	30	1.2	LNp; lesser peaks at 150°, 220°	
Sucrose	70	175	12	0.0080	sat. sol'n	
Sucrose	70	105	30	1.4	hot syrup cooled in air	
Thymine	70	105	22	30	tail to 265°K	T81B-267
Toluene	70	110	60	160	two peaks	
	70	153	18	20		
Uracil	70	120	35	36	resembles thymine glow; two peaks	
	70	160	54	28		
Tap water	11	174	60	0.0040	required 10^5 rad exp.	

[a] Exposures at 93 rad/sec to Co^{60} γ rays. Exposure range 0.3 to 50 krad. Samples sealed at 1 atm air, 21°C, 40 to 60% RH.

[b] Partly engulfed in a temperature-independent phosphorescence of 4× this yield.

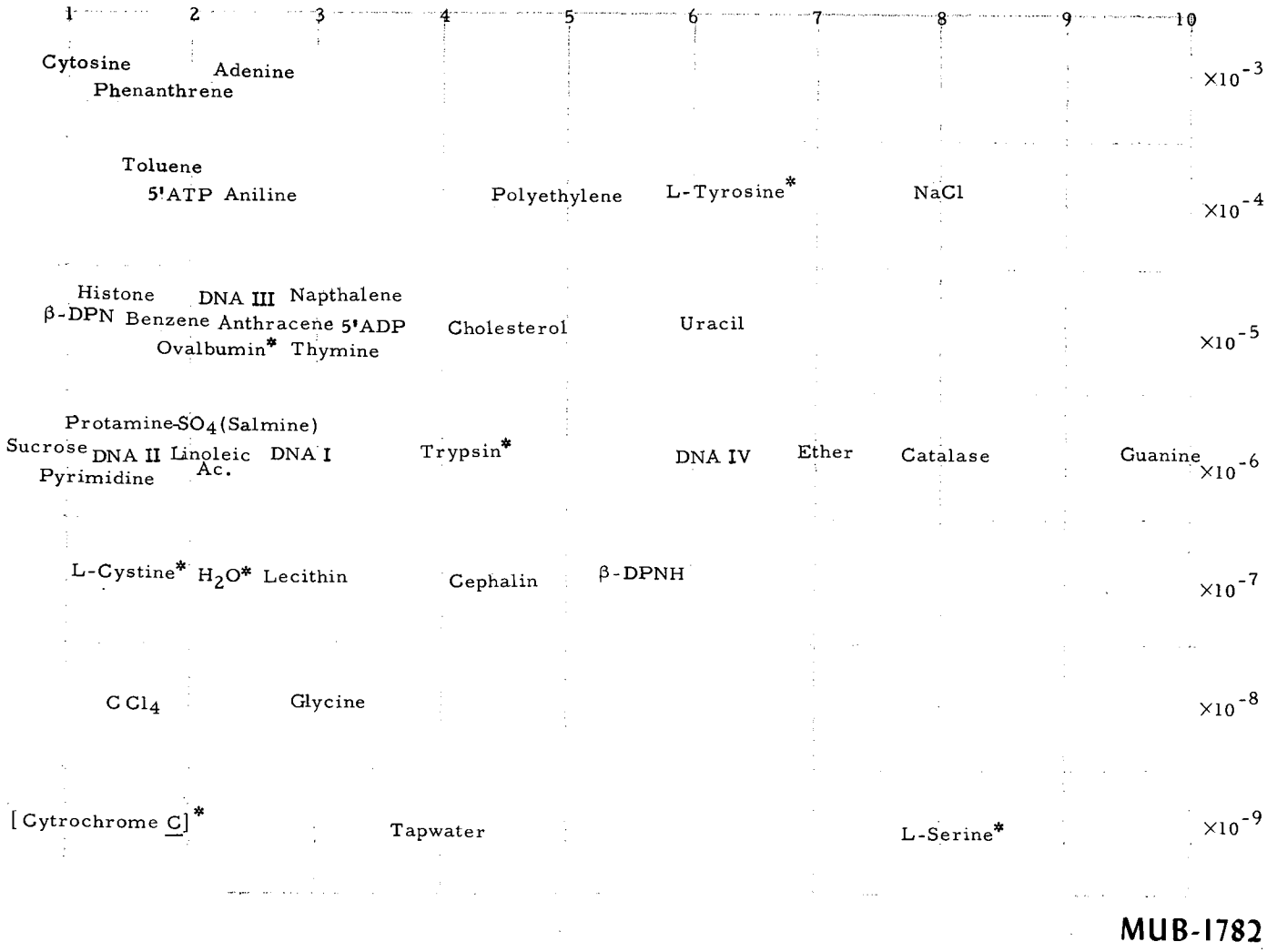

Fig. 15. The low-temperature glow-peak yields.
(Starred values from Augenstine et al., reference 65.)

2. Phosphorescence

Those chemicals that, 3 minutes after the end of the gamma-irradiation, glowed with at least 0.1 the glow-peak intensity have been noted "LNp" in Table I. In histone and protamine sulfate, this "phosphorescence" was observed to be simply the emptying of traps (via the low-temperature end of the Boltzmann distribution tail) which belong to broad low-temperature glow peaks of small thermal activation energy (< 0.15 eV). The same mechanism was observed in sucrose and sodium chloride, except that sharp peaks (>0.2 eV) in the region 77 to 85°K caused the phosphorescence.

Strong delayed phosphorescence was observed in 5'-adenosine diphosphate (ADP) crystals and in a piece of sheet polyethylene. When these substances were warmed at 8°K/min a phosphorescence of mean life about 6.6 min was found in the time region 4 to 22 min after irradiation. Typical glow peaks were superimposed on this phosphorescence. However, at a heating rate of 70°K/min, the mean life of the phosphorescences dropped to about 0.8 min (the glow peaks were still clearly superimposed). The 4- to 22-min phosphorescence yields (140 and 50×10^{-6} for ADP and polyethylene respectively) remained constant at both warming rates.

3. Attempts at Optical Bleaching

When an adenine sample in a liquid nitrogen bath was irradiated for 30 sec with 10 mW of 1.2- to 3.5-μ radiation from a 250-W heat lamp, a 50% reduction in the 120°K glow was observed during subsequent heating. However, when adenine was similarly exposed for 30 sec to about 1 W of 4.0- and 5.5-μ radiation from a 500-W silicon carbide glow bar, it was not affected. Neither did a 22-min delay in the dark nor a 30-sec exposure to room daylight have an effect.

A single measurement on a cytosine sample that had received about 100 mW of 1.20 to 2.4-μ radiation through a silicon window for 4 min revealed a 75% reduction in the 125°K peak and a 50% reduction in the 182°K peak. The thermoluminescence of alkali halide samples was much more profoundly affected than that for adenine and cytosine.

For instance, a 30-sec exposure of KCl crystals to an infrared lamp completely bleached the low-temperature glow.

B. Luminescence Following Irradiation by Heavy-Ion Beams

Oxygen ions at 10 MeV/ nucleon give up their energy locally as dense spikes of excitation along their paths. And because these ions come to rest in the region that they have excited, they are a particularly interesting tool for the study of the local oxygen effect. These measurements allow comparison of the effects of two different excitation modes on luminescence.

1. Fluorescence

The fluorescence yields and spectra of eight cell-nuclear chemicals under irradiation with heavy ions at 77° and 295°K may be seen in Table II. (The term "fluorescence" is used here to describe luminescence emitted during irradiation. It includes all the phosphorescence with mean life < 1 second and the initial few seconds of phosphorescence of longer duration.) It should be noted that the yields are minimum estimates, not corrected for that portion of the fluorescence which is self-absorbed, in pressed crystalline pellets. This correction could amount to a factor of 10 or more. Nonetheless all values are of the order of 2×10^{-5}, and there is no consistent pattern. Considerably different spectra and yields were observed when adenine was irradiated at 77°K with carbon and with oxygen beams.

In each case the emission spectrum consisted of a single sharp or broad peak located in the 350- to-525-nm region. With oxygen beams the peak widths at half maximum intensity varied from 90 to 225 nm, but most were in the neighborhood of 100 to 130 nm.

An unexpected transient fluorescence "buildup," which may be related to the on-off method of taking measurements, was observed in about one-half the runs. During this work the heavy-ion beam was turned on for 5 sec (12 pulses/sec), then off for 5 sec while the monochromator dial was changed to the next wavelength band. The "buildup" was a fluorescence during the first few 5-sec exposures that was 1.5 to 2 times the intensity obtained by subsequent exposure at the same wavelength setting.

Table II. Luminescence of certain chemicals during heavy-ion irradiation.[a]

Chemical	Temp. (°K)	Yield[b] ($\times 10^{+6}$)	Spectrum[c] (nm)
Adenine	77	17.	505-525-562[a]
Adenine	77	1.2	425-470-525
Adenine	295	55.	465-522-575
ATP	77	8.0	393 <430> 530[d] <445>
ATP	295	8.0	396-460-550[d]
Cytosine	77	18.	452-504-556
Cytosine	295	33.	465-505-555
DNA	295	3.4	465-525-585
Guanine	77	8.8	360 <410> 585 <520>
Guanine	295	5.0	360 <375> 585 <500>
Sucrose	295	3.2	405-463-545
Thymine	77	28.	315-350-470[d]
Thymine	295	23.	315-354-440[d]
Uracil	77	24.	440-511-576
Uracil	295	15.	435-500-572

[a] The first entry was irradiated with 120-MeV C^{+4} ions at 43 mW intensity--all others with 160-MeV O^{+6} ions at 33 to 95 mW. All samples irradiated at residual air pressure of 1 to 5 μ Hg.

[b] Ratio of mW of measured light energy emitted to mW of heavy-ion energy put into the surface of the 1-g chemical pellets. Not corrected against E-L disk standard spectrum, 465-500-535 nm.

[c] Wavelength at peak intensity--and that on each side of peak at half intensity.

[d] Spectrum tail to 680 nm.

2. Phosphorescence

An exceedingly small phosphorescence of mean life between 0.5 and 2 sec was observed in all samples irradiated at 77° K and in all but guanine, thymine, and adenosine triphosphate (ATP) at 295° K. The yields amounted to approximately 0.01 the fluorescence yields, and the phosphorescence emission spectra lay in the region 450 to 600 nm. The absence of this afterglow was quite evident on the declining edges of the 320-to-450-nm fluorescence "spikes" of thymine and guanine.

3. Thermoluminescence

Attempts were made to generate the 120° K adenine glow by use of heavy-ion energy inputs up to 2000 joules. Neither with oxygen nor with carbon beams was there any evidence of a low-temperature glow. Under the experimental conditions of 1 to 5 μ Hg residual vacuum the glow yield did not exceed 10^{-10}. Similarly, no glow was observed in sucrose irradiated by doses to 500 joules.

When the photomultiplier is coupled directly to the optical arm of the sample holder and the DU is eliminated, sensitivity is increased about 300 times. A guanine pellet was irradiated at 77° K with such a setup to an energy input of 70 joules of oxygen beam. An initial phosphorescence fell, by a factor of 100, to approx zero during the first 40 sec postirradiation. Upon heating, a small sharp glow peak appeared at 93° K and a broad peak at 124° K that had a tail to 200° K. The total yield was 0.32×10^{-9}, with the residual gas pressure of 1 to 5 μ Hg.

C. Luminescence Following Irradiation by 5-MeV Electrons

Measurements were begun at the electron linear accelerator in order to obtain the high irradiation dose rates required to study the luminescence spectra of organic crystals. The studies with heavy-ion beams and preliminary work with electrons had revealed that the ambient gas pressure during irradiation has a profound effect on radioluminescence. For this reason, during these runs the sample holder and mount were separated from the electron-beam tube (Fig. 12) and had an independent high-vacuum system that admitted controlled amounts of gas.

1. Fluorescence

The radiofluorescence yields and spectra of the five nucleic acid bases are reported in Table III. (Again, "fluorescence" refers to luminescence emitted during irradiation.) As with the heavy-ion beams, the yields are the ratios of the radiant (visible) energy to the energy taken from the electron beam by the pellets. An experimentally determined factor of 10 has been applied in all cases to compensate for the self-absorbed light deep in the pellet. There was no fluorescence "buildup" with the 5-sec-on, 15-sec-off routine.

The maximum fluorescence yields occurred at 100 to 300 μ Hg of oxygen pressure, and the yields were in general greater at the lower temperatures. Otherwise there were no general patterns. Under certain conditions adenine was observed to release up to 13% of the absorbed dose from 5-MeV electrons as visible radiation.

The emission spectra were found to be bell-shaped with peaks in the region 440 to 540 nm and widths from 65 to 160 nm. The spectrum of a sample at constant temperature was not strongly altered by a change in gas pressure. A change in the quality of the gas or in the temperature of the sample, however, did modify the emission.

2. Phosphorescence

a. Adenine. The data for measurements of the 77°K afterglow of adenine under widely different conditions of gas quality and pressure may be simply stated: at pressures greater than 30 μ Hg the adenine phosphorescence yield and spectrum are identical with the thermoluminescence yield and spectra for the given conditions. With good precision it was possible to estimate the magnitude of the 120°K glow peak from that of the 77°K afterglow. A number of careful spectral measurements also failed to reveal a difference from the glow peak spectrum, 440-490-550 nm (see Table IV).

Table III. Luminescence of certain chemicals during 5-MeV electron irradiation.

Chemical	Temp (°K)	Gas pressure	Yield ($\times 10^3$)	Spectrum (nm)
Adenine	77	< 1 μ Hg air	5.8	490-520-590
Adenine	77	5 μ Hg O_2	80.	435 (475, 500) 550
Adenine	77	300 μ Hg O_2	130.	438 (475, 500) 550
Adenine	220	25 μ Hg O_2	3.4	480-530-580
Adenine	245	25 μ Hg O_2	2.4	490-540-580
Adenine	283	<1 μ Hg air	2.1	495-535-580
Adenine	286	26 μ Hg air	2.1	490-535-593
Adenine	286	110 μ Hg O_2	2.1	485-525-615
Cytosine	77	<1 μ Hg air	0.62	450-500-555
Cytosine	77	200 μ Hg O_2	4.8	425-480-535
Cytosine	80	1 atm O_2	0.80	450-480-532
Cytosine	100-125	130 μ Hg O_2	1.40	447-480-537
Cytosine	290	3 μ Hg air	0.80	495-520-560
Cytosine	290	110 μ Hg N_2	0.13	470 (500, 520) 545
Cytosine	290	1 atm N_2	0.16	475-510-545
Guanine	77	10 μ Hg air	5.3	462-510-565
Guanine	77	110 μ Hg O_2	11.0	460-507-560
Guanine	280	30 μ Hg O_2	0.17	-- 525 --
Guanine	275	1 atm O_2	0.09	-- 500 --
Thymine	77	150 μ Hg O_2	52.	430-460-535
Thymine	294	16 μ Hg air	0.032	-- 480 --
Uracil	77	<1 μ Hg air	1.2	410-450-520
Uracil	77	200 μ Hg O_2	1.2	415-460-515
Uracil	77	200 μ Hg N_2	1.2	395-440-520
Uracil	290	<1 μ Hg air	0.008	400 (440, 520) 560

Table IV. Thermoluminescence of electron-irradiated chemicals[a]

Chemical	Heating rate (°K/m)	Gas pressure	Peak temperature T^*(°K)	Duration (sec)	Yield $\times 10^{+6}$	Spectrum (nm)[b]	Comments
Adenine	44	1 atm N_2	124	38	1200	none	No DU
	44	1 atm O_2	112	30	2400	440-490-550	
	55	1 atm He	120	33	1200	440-490-550	
	55	<100 μ, any gas	140	45	See Fig. 15	440-490-550	
ATP	44	30 μ air	--	0	0.24 max.	none	No DU
Cytosine	55	all conditions	177±7	95	See Fig. 16	445-490-545	
Guanine	55	110 μ O_2	--	0	16.	-- 500 --	
Thymine	55	220 μ O_2	121	91	32.	430-460-530	
	55	< 220 μ N_2	110±2	72	25.	430-460-530	
Uracil	55	all conditions	139±6	101±10	100.	425-465-525	

[a] Dose rates varied, but were near 3300 rad/sec

[b] Peak wavelength, and wavelength at half maximum on each side, at T^*.

The decay of the adenine afterglow was quite special also: the intensity followed

$$I \propto 1/t, \quad \text{for} \quad t > 1 \text{ sec.} \tag{17}$$

That is, the decay is logarithmic, with slope of -1. This decay has the interesting property that its instantaneous "mean life" always equals the time elapsed since the end of the irradiation.

As the pressure drops below 30 μ Hg, the phosphorescence persists in the absence of thermoluminsecence, but with decreasing yield. No measurements were taken at room temperature.

b. Cytosine. The 77°K phosphorescence of cytosine followed the same pattern, except that the yield never exceeded 0.1 of the thermoluminescence yield at pressures above 200 μ Hg. At lower pressures a weak afterglow persisted in the absence of a glow peak. Here also the decay obeyed Eq. (17), and the afterglow spectrum coincided with the glow-peak spectrum.

c. Uracil. At 77°K the uracil afterglow yield was about 1/20 of the 140°K glow-peak yield under all conditions. The emission spectrum was measured, 420-470-520 nm, and found to coincide with that of the glow peak. The decay also obeyed Eq. (17).

d. Thymine and guanine. The low-temperature phosphorescence of these substances was weak but measurable. In no case did the yield for thymine exceed 0.1 the 121°K glow peak yield.

3. Thermoluminescence. The glow-peak yields and emission spectra for the nucleic acid bases are presented in Table IV, and a typical glow curve may be seen in Fig. 16. Although the yields varied widely between the different samples, the emission spectra were remarkably similar and constant under different gases and pressures. However, in each case there was a definite and measurable shift to the longer wavelengths as the glow-peak temperature was exceeded, i. e., on the descending part of the glow peak. This shift amounted to about 20 nm.

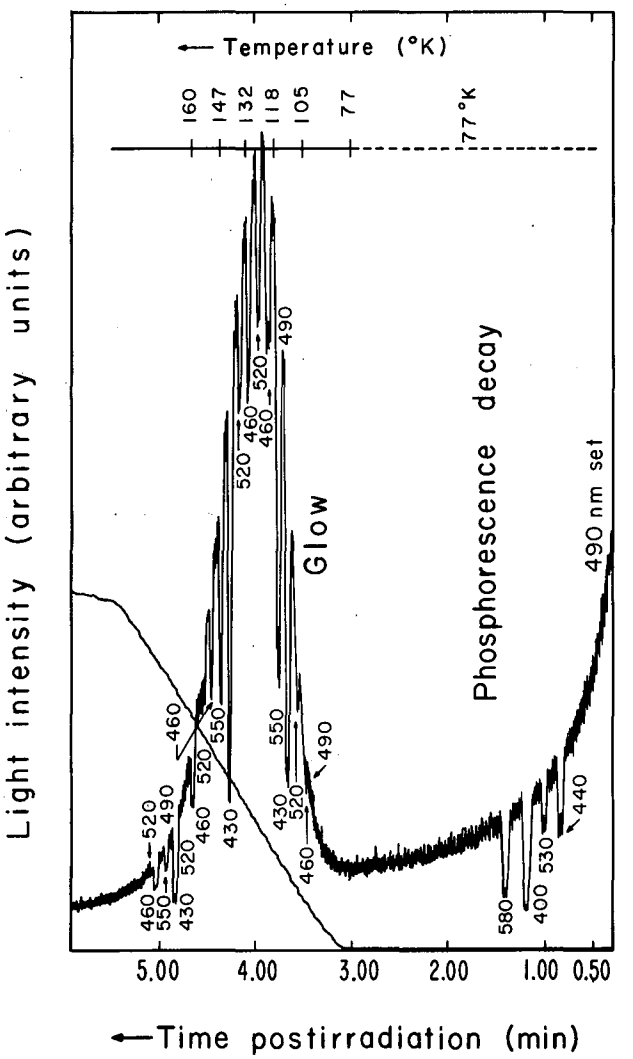

Fig. 16. Glow and phosphorescence decay at 430 μ Hg N_2 pressure. (Adenine pellet, 1 g; 7×10^4 rad 5-MeV electrons.)

The pressure dependence of the glow-peak yield for adenine may be seen in Fig. 17. In no case was a measurable thermoluminescence glow observed when the ambient pressure was less than 20 μ Hg. When dry nitrogen or oxygen gas or air was present, there was a remarkable drop in the glow-peak yield in the region 20 to 30 μ Hg. A similar drop occurred with helium at somewhat higher pressure. An optimum gas pressure for thermoluminescence was found at about 100 μ Hg: at this point the yields were 5 times those at 1 atm N_2 and 1.5 times those at 1 atm O_2. At 1 atm pressure, the yields in oxygen were consistently twice those in nitrogen.

A similar pressure dependence was observed for the glow-peak yields in cytosine, except that the sharp drop occurred in the region 100 to 200 μ Hg, and the dependence on oxygen and nitrogen differed. The cytosine pressure dependence as well as that of uracil and thymine may be seen in Fig. 18. The latter substances exhibited the same glow-peak yields at all pressures. In a single attempt to measure the guanine glow in 110 μ Hg of oxygen, none was observed.

The adenine, uracil, and thymine glow-peak yields at 1 atm pressure were found to roughly coincide with yields obtained from cobalt-60 irradiation. However, with electrons, the cytosine peak was only 1/20 that with γ rays. The giant 130°K cytosine peak was never observed following electron bombardment.

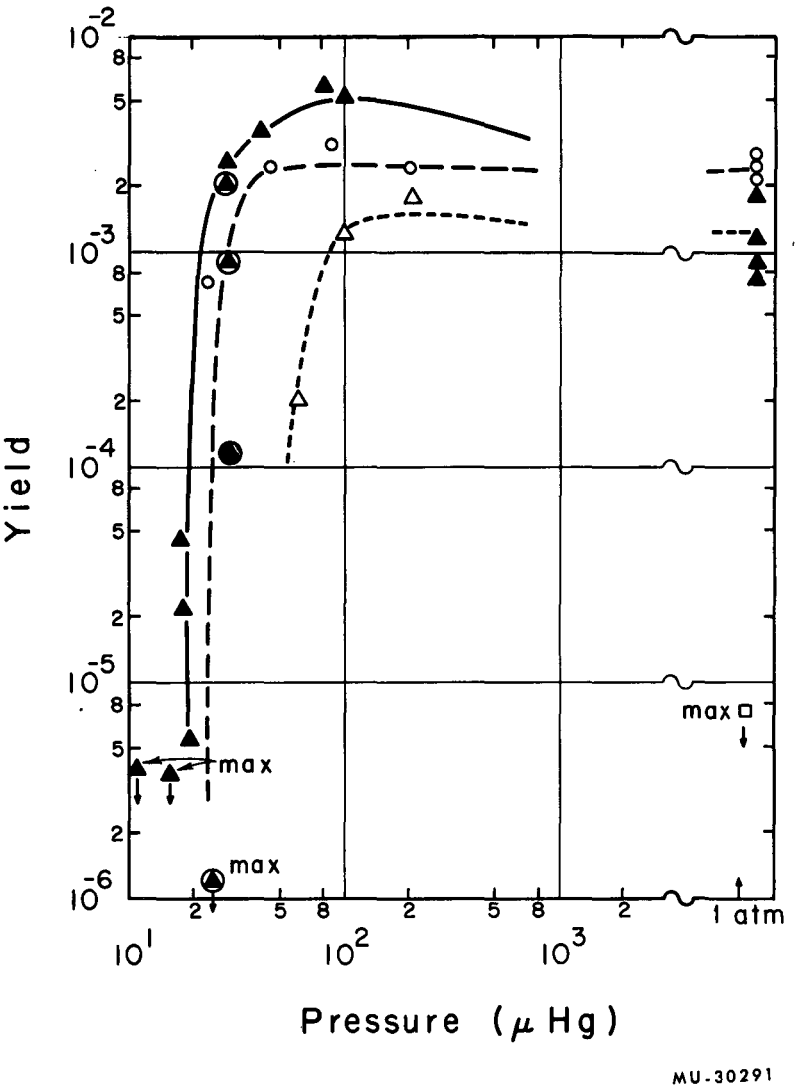

Fig. 17. Thermoluminescence yield of adenine at low gas pressure

o oxygen ▲ nitrogen
● air △ helium
□ blank run (no adenine)

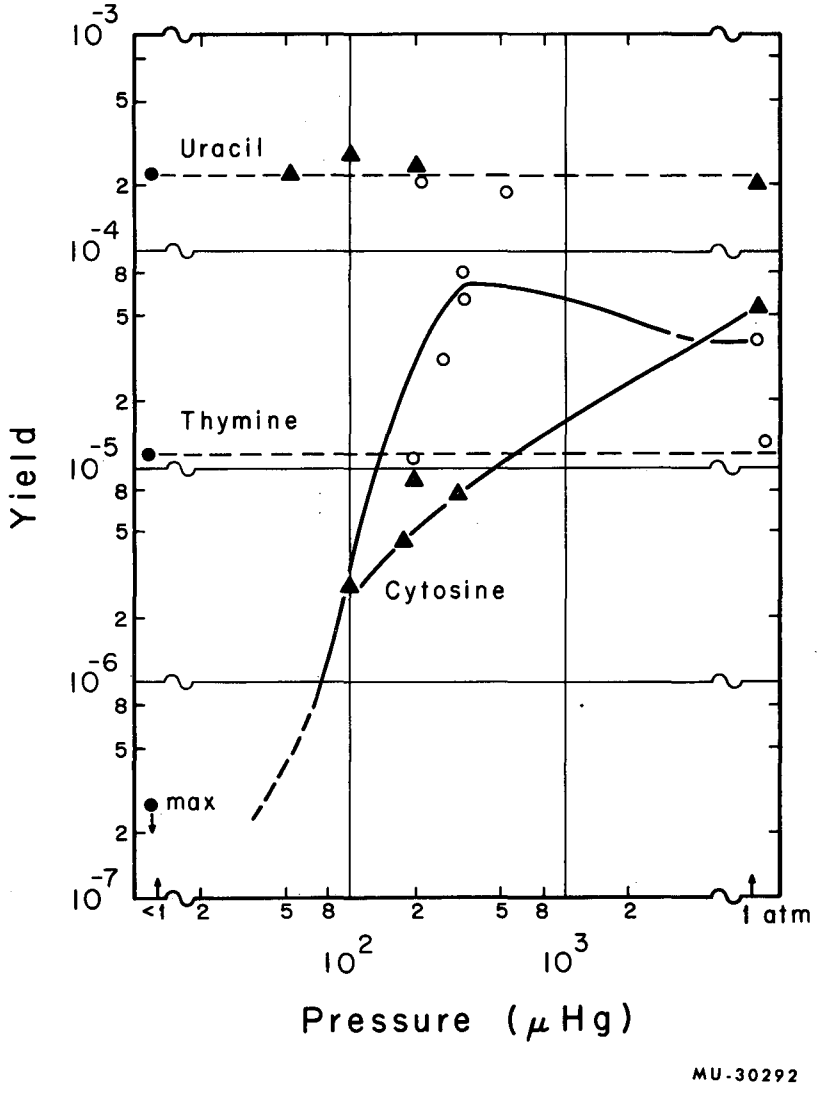

Fig. 18. Thermoluminescence of uracil, thymine, and cytosine at low gas pressures.
● air ▲ nitrogen o oxygen

IV. DISCUSSION

A. Interpretation of the Thermoluminescence Data

1. The Problem

The principal value of glow-curve measurements is in the study of trap depths. The problem that arises in interpreting glow curves is therefore: after obtaining by experiment the temperature T^* of the maximum glow, to derive from it the depth ΔU of the traps. The glow-curve equation (14) reveals that ΔU is proportional to T^*, but only for given values of the frequency factor s and the heating rate β. The frequency factor is at the heart of the problem, and the remainder of this section concerns its relation to T^* and ΔU.

By use of Eq. (14) the entire glow curve may be computed, point by point, by numerical integration. However, such calculations are very tiresome, so it is desirable to find ΔU directly from T^*. Now, T^* is determined by setting $\frac{dL}{dT} = 0$. This gives

$$\beta \frac{\Delta U}{kT^*} = s\, T^* \exp\left(-\frac{\Delta U}{kT^*}\right), \tag{18}$$

in which ΔU is defined as an implicit function of T^*. Equation (18) has been solved by the technique of successive approximation for certain values of s/β, and the solutions have been presented graphically in Fig. 19. The full nature of the problem is revealed by these graphs.

For example, when adenine crystals are irradiated at 77°K and then warmed at 1°K/sec, a glow-peak is observed at 120°K. It can be seen with the help of Fig. 19 that ΔU may lie between 0.07 and 0.32 eV, depending on the value of s selected. The same figure shows that there is a strong dependence of ΔU upon s for any T^*.

The determination of ΔU by use of Eq. (15) without knowledge of s is equally unsatisfactory. For instance, Sims,[42] using Eq. (14), found that values of $s = 10^{11}$ and $\Delta U = 0.485$ were required in order to duplicate the 195°K glow peak of KH_2PO_4 crystals. Yet for the same peak, an Arrhenius plot of Eq. (15) gives $\Delta U = 0.30$; this corresponds to $s = 2 \times 10^8$ according to Fig. 19.

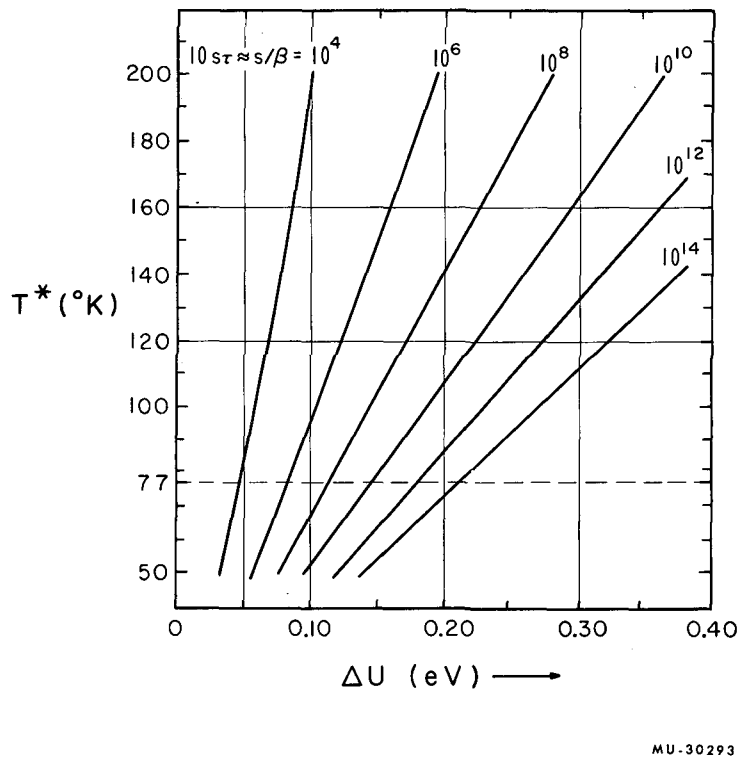

Fig. 19. Solutions of the glow-peak equation (18).

2. The Solution

The problem is that Eq. (18) contains four variables, and only two (β and T^*) are known from a single glow-curve measurement. A second independent observation of the relationship between s and ΔU is therefore required if the equation is to be solved. Measuring the phosphorescent decay at 77°K and using Eq. (7) provides such an independent measurement. Determination of ΔU for adenine is an example of this method.

When an adenine sample was allowed to remain 22 minutes in the dark at 77°K prior to the glow measurement, there was no appreciable attenuation of the glow peak yield. If there had been a slow phosphorescence during this time ($\approx$ 1300 sec) at the expense of the glow peak, then such a decay (of $\tau < 1000$ sec) would have been evident by a reduction in the yield. Therefore $\tau_{77°}$ for the 120°K adenine peak is greater than 10^3 sec. This observation sets a lower limit of 10^8 sec^{-1} for s, and of 0.17 eV for ΔU for the adenine glow.

The relationship between the phosphorescence decay times ($\tau = 10^2$, 10^3, 10^4, 10^5 sec) at 77°K and the dependence of various glow-peak temperatures upon s and ΔU is presented in Fig. 20. A careful examination of this plot is helpful in the interpretation of the thermoluminescence data:

(a) The glow-peak temperature of 100°K must be associated with a frequency exceeding 3×10^{10} sec^{-1} in order to have $\tau_{77} > 100$ sec. This no doubt is the principal reason that very few glow peaks were observed at $T^* < 100$°K in this work: the low-temperature peaks decayed away in the 3- to 4-minute delay between irradiation and heating.

(b) For the same reason, the trap depths associated with $T^* < 100$° must exceed 0.16 eV in this work.

(c) Histone and protamine sulfate, which were observed to have a 77°K phosphorescence 200 sec after irradiation and whose glow peaks are approx 115°K, may be placed with $s = 10^8$ sec^{-1} and $\Delta U = 0.16$ eV.

(d) For all the other glow peaks, the trap depths may be limited to ± 0.05 eV by using Figs. 19 and 20. For instance, take the 105°K

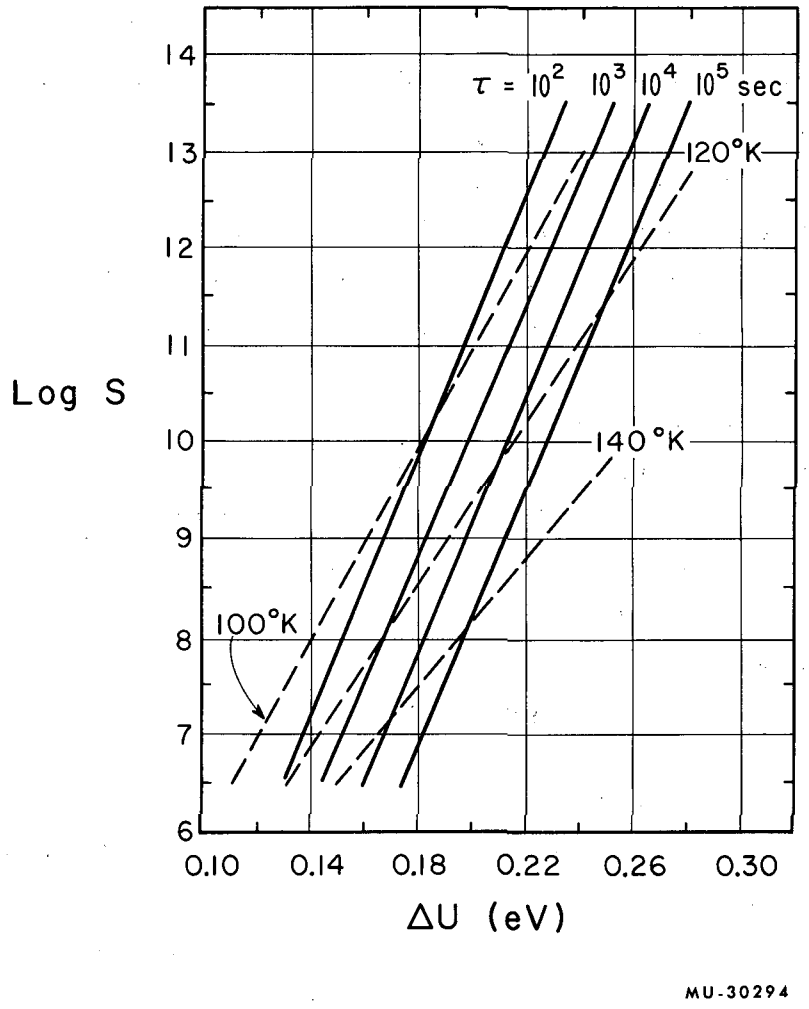

Fig. 20. Solutions of the phosphorescence equation (7).
see dashed curves for τ=0.1 sec.
see solid curves for T=77° K.

aniline peak. It is known that $\tau > 10^3$ sec, otherwise a measureable 77°K phosphorescence would have been recorded. Therefore, from Fig. 20, $s > 5\times10^{11}$ sec^{-1} and $\Delta U > 0.22$ eV. From Fig. 19, with $s = 10^{14}$ sec^{-1} (the maximum conceivable value), $\Delta U_{max} = 0.27$ eV. In this case, therefore, the limits on ΔU are 0.22 to 0.27 eV, and on s are 5×10^{11} and 10^{14} sec^{-1}.

(e) The paucity of glow peaks in organic powder samples of $T^* > 170°K$ remains to be explained. It is possible that in these substances trapping sites of depth > 0.30 eV are not present in appreciable numbers. If such relatively high-temperature peaks are found, it is likely that the $\tau_{77°}$ will be great—the order of years or more.

3. Relation Between τ and β

In Sec III. B. 2 the strong 77°K phosphorescence of ADP and polyethylene was mentioned. When these substances were heated at two different rates, the mean life of the phosphorescence changed. This is of course expected of temperature-dependent phosphorescence: during constant heating the exponential form remains, but the "mean life" shortens directly as the heating rate is increased.

It was in fact discovered that the parameter s/β in Fig. 19 very nearly equals 10 $s\tau$ for every T^* and ΔU as obtained from Fig. 20. Setting $10\ s\tau = s/\beta$ gives the relation $\beta\tau = 0.1$. That is to say, for $\beta = 1°K/sec$ we have $\tau = 0.1$ sec. In thermoluminescence this is of some interest in that when $\beta = 1$, then at T^* the mean life of the emptying traps is about 0.1 sec. And of course when $\beta = 0.1$, then $\tau = 1$ sec at T^*. This relationship gives a quantitative basis for the well-known phenomenon of intensification of glow-peak height by means of increased heating rate.

4. Comparison of Gamma- and Electron-Induced Glow Peaks

The adenine peak location, yield, and emission spectrum remained remarkably constant under excitation by these different modes. However, there was when cytosine was irradiated with electrons a surprising reduction in yield, and the giant glow peak was absent.

In no case did the glow-peak temperature exceed 180°K, and with the exception listed above, the glow curves for electrons resembled those for γ rays. This implies that the electrons behave similarly to cobalt-60 rays in their interaction with organic crystals. It is noteworthy that the strong electron beams were capable neither of grossly modifying the luminous centers nor of creating new ones.

B. Role of Gas Pressure

1. The Dependence of Glow Yields Upon Gas Pressure and Quality

The observations to be explained are:

(a) The thermoluminescence of adenine, cytosine, and guanine vanished at low gas pressures (< 20 μ Hg), while that of thymine and uracil remained unchanged to pressures < 0.1 μ Hg.

(b) The 120°K adenine peak shifted to 140°K at low pressures, (about 100 μ Hg), and to 112°K when the ambient gas was oxygen at 1 atm

(c) The thermoluminescence and 77°K phosphorescence emission spectra coincided, although there was a definite but small shift to longer wavelengths as T^* was exceeded.

(d) The glow-peak emission did not change when nitrogen gas was replaced by oxygen or helium.

2. Dependence of Fluorescence Yields Upon Gas Pressure and and Temperature

The observations to be explained are:

(a) With the exception of cytosine, the fluorescence yield at room temperature roughly equalled the low-temperature glow-peak yield. For cytosine, this holds true if the γ-ray glow yield is used.

(b) The 77°K optimum fluorescence yield (at 200 μ Hg O_2) was roughly 100 times the glow yield except for uracil, for which the factor was only 12.

3. Clues from the Emission Spectra

The measured emission spectrum for a given substance was found to have the same shape under the different experimental conditions. Remarkably, the wavelength peak was at either one of two positions.

(a) At room temperature, and at 77°K under vacuum, the peak locations coincided. The emission peak under heavy-ion irradiation also generally occupied this spectral position.

(b) At 77°K, under gas pressure exceeding 100 μ Hg, the emission spectrum was the same as that for the thermoluminescence (and the 77°K phosphorescence).

4. Exciton Hypothesis

The dependence of luminescence yields and emission spectra upon temperature and local gas pressure requires an explanation. To meet this requirement the following hypothesis for the role of gases in the radioluminescence of organic crystals is advanced.

(a) The ionizing radiation produces electronic excitations in the crystals with energy packets of a few electron volts. Some of these excitations are "localized" below the conduction bands and give up their energy to lattice vibrations. Another portion has sufficient energy to enter the conduction bands and travel to discrete molecular luminescence centers or to sites where nonradiative deactivation may occur.

(b) The ambient gas penetrates into the crystal lattice, thereby increasing the characteristic lattice vibration frequency, and changing the potential-energy states of the luminescence centers.

(c) At low temperature, the excitons formed below the conduction band have a greatly reduced probability for vibrational energy loss. They therefore are effectively trapped, although they do not necessarily remain at one location.

(d) The heat applied in thermoluminescence serves as the activation enthalpy for the diffusion (release) of the trapped excitons. This enthalpy is slightly decreased (0.04 eV) by the local presence of gas molecules.

(e) The major effect of the gas, however, is the perturbation of the potential-energy states of the luminescence centers. One of the many possible perturbations that is consistent with the observations is given in Fig. 21. In excited state 1, the probability for nonradiative transitions via CC' is great. This probability is reduced in favor of a radiative loss in state 2. Also, in accordance with measurement, the emission peak frequency DD' is slightly greater than that for BB'.

5. Implications of Exciton Hypothesis

It must be emphasized that this model is simply an interpretation and that there is no compelling evidence for it. Nontheless the hypothesis usefully predicts the following things:

(a) Increased conductivity at T^*.

(b) Indifference of the molecular emitter to the type of gas present.

(c) Reduced molecular deactivation yield (broken chemical bonds, etc.) at low temperature when gas is present.

(d) Thermoluminescence potentiated after irradiation in vacuo by a penetrating gas.

(e) Bimolecular reaction kinetics.

C. Comparison of Measurements

No energy yields for thermoluminescence of organic compounds have been reported; that for LiF was less than 10^{-4} (Daniels et al.).[59] Here it was also found that in general the low-temperature glows had yields less than 10^{-4}. However, the yields of crystalline powders of adenine and cytosine were 1 to 2×10^{-3}. There was confirmation of the earlier observations that organic conjugated ring molecules have yields 1000 times as great as other molecular types, that the glow peaks of similar compounds differ in width, location, and intensity, that the glow peaks occur generally below 170°K, and that the emission spectra are bell-shaped and about 100 nm broad.

During thermoluminescence of single crystals of the hygroscopic substances phenol and KF, a series of sharp < 1-sec spikes was

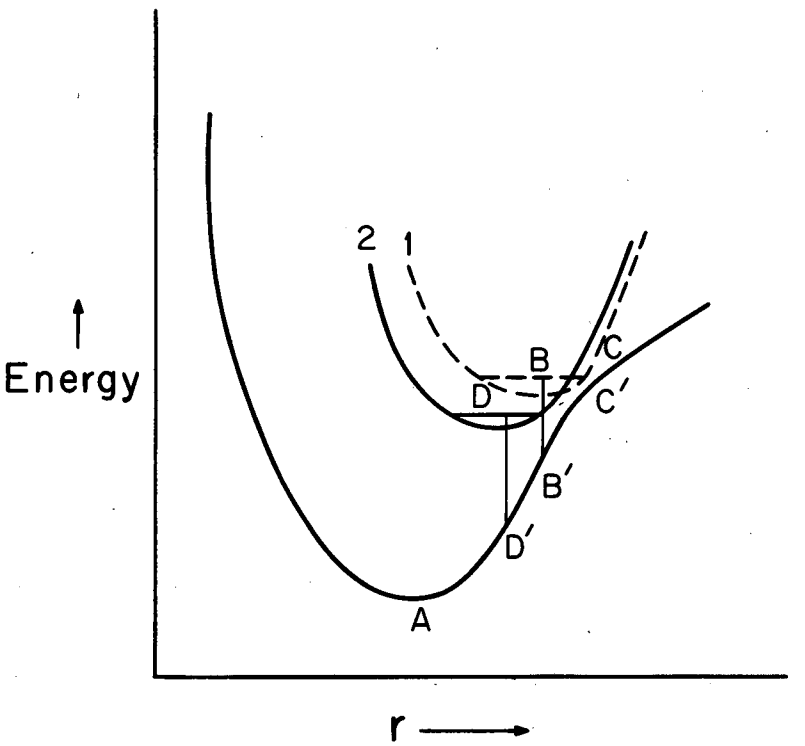

Fig. 21. Hypothetical coordinate shift.
1. Position of excited state in pure crystal.
2. Position when gas molecules have entered the crystal.

recorded instead of the normal glow at low temperatures. Sharma[61] and Sims[42] have reported similar phenomena. The latter author believes these spikes are a form of triboluminescence.

The fluorescence energy yields under electron bombardment at certain local gas pressures and temperatures exceeded 10% for adenine, but generally stood in the region 0.1 to 5.0%. These yields are comparable to those observed in organic solid and liquid scintillators.

The discovery that organic thermoluminescence may be strongly influenced by the local gas pressure has been independently confirmed by measurements using tyrosine and certain other substances (Carter).[73] The reproducibility extremes for the glow yield of a given chemical batch were found to be about ± 50%.

Optical de-excitation of irradiated material at 77°K was found (in agreement with the data of Bryant et al.[51]) to be inefficient at wavelengths exceeding 2 μ. Large exposures with an infrared lamp did not reduce the glow yield by more than the normal ± 50% fluctuations.

The exciton hypothesis may be compared to a model proposed by Voevodskii and Molin,[74] which predicts a reciprocal relation between the radiolytic yield of H atoms and luminescence yield. Briefly, their model attempts to explain the low yields for the organic compounds that possess resonance stability. They feel that the energy position of the lowest excited state is critical. If this exceeds the dissociation energy for the C-H bond, then the probability for bond rupture is high. If it does not, then radiative de-excitation becomes more probable. The exciton hypothesis has no provision for bond rupture once the exciton has reached a luminescence center. The only nonradiative decay mode from the lowest excited state envisaged in the configuration coordinate model is vibration exchange. However, it is a simple matter to add a third potential surface (to Fig. 21) representing a bond-rupture decay mode. In such a figure all three possible modes would then be pictured, and the probabilities for each would depend upon the relative positions of the potential surfaces as determined by local configuration and the liquid and gas environment. Such a tripotential figure, however, will be rather difficult to precisely construct for a given substance.

There is a recent observation that supports the basic feature of the exciton hypothesis. Brocklehurst and Pimentel[75] recorded glow peaks at 10, 14.5, and 19°K when solid nitrogen had been irradiated with electrons at 4.2°K. In this case it is difficult to conceive of the diffusion of anything except excitons upon thermal release.

D. Crystalline Luminescence and Radiation Biology

It was argued in Section I that the problem of excitation energy transfer and release in the living cell must be solved by considering the electronic nature of the biomolecules, that the action of ionizing radiation is direct on condensed cellular elements, and that absorbed energy is in the form of packets of a few electron volts. In this view the radiosensitivity depends upon the particular de-excitation mode: thermal vibration loss, luminescence, or conversion to chemical potential energy.

Only the last mode has heretofore received serious attention in radiobiology--and, in general, studies of chemical yields do not reveal much about molecular electronics. The measurements reported here demonstrate that cell nuclear chemicals may under certain conditions release as luminescence a large fraction of the energy absorbed from high-energy radiation beams.

There is of course a considerable jump between the crystalline state of purified biochemicals and the condensed state of various molecules in the living cell. It is possible, however, that exciton migration occurs within such structures and that the molecules are not electronically isolated. The latter is true without question in single proteins, in chloroplasts, in DNA molecules, and mitochondria, and the molecules in these structures are densely ordered. The cyrstalline state is approached in the living cell to this extent.

Several future biophysics investigations by use of luminescence technique suggest themselves:

(a) Yeast cells quick-frozen in solutions containing β-Mercapto-ethylamine (MEA) provide a simple system for radiation protection studies (Smaller and Avery).[76] These authors observed that when this system

was irradiated at 77°K and then warmed, at 125°K the water radicals vanished without interaction with the yeast and MEA radicals. It will be of interest to determine if this disappearance is associated with luminescence, and is unique for ice at this temperature; 125°K is near T^* for the strongest thermoluminescence in organic crystals.

(b) It may be of great importance to the electronics industry as well as to biophysics to examine pure single organic crystals in order to obtain dark-, photo-, and radioconductivity data for correlation with luminescence properties under various environmental conditions.

(c) Organic crystalline thermoluminescence may be developed and put to use in radiation dosimetry. By their nature, such substances are "tissue-equivalent."

(d) A series of investigations of the various conditions that enhance crystalline luminescence will be of interest. It may be possible to attack the problem of radiation protection by this technique. For instance, certain compounds that are otherwise innocuous may be added to the diet so that they may enhance the intracellular fluorescence mode of electronic de-excitation.

(e) Studies of luminescence of organic crystals in the region 4 to 77°K will prove helpful in determining the nature of the initial excitation energy.

(f) The importance of H bonds in luminescence has been emphasized by Vladimirov[56] and by Zadorozhnyĭ and Naboikin.[63] An effort to this end may be rewarding. It is possible that the major function of these (and induced dipole bonds) is an electronic coupling of components in an electrical system rather than a purely structural linkage.

(g) The examination of luminescence decay of organic crystals by use of fast-pulse electronics will be very interesting. Knowledge of the fluorescence decay kinetics will allow greater understanding of this mode of electronic de-excitation.

(h) Studies designed to determine the necessary conditions for change in the relative heat, luminescence, and chemical yields suggest themselves, as does also

(i) The testing by luminescence analysis of crystals grown under conditions whereby gases have been rigorously excluded.

V. SUMMARY AND CONCLUSIONS

Organic crystalline powders have been exposed to high-energy radiations and examined by luminescence analysis. It was observed that temperature and gas pressure have profound effect upon luminescence yields. The fluorescence energy yields under certain conditions may rise to 13%, the phosphorescence and low-temperature thermoluminescence yields may rise to 0.01 of this. The emission spectra under various conditions and excitation modes were found to be bell-shaped in the region 350-550 nm and 100 nm wide. The activation enthalpies for thermoluminescence glows have been examined in detail and a new method for their determination, based on luminescence decay at 77°K, has been used. A rather broad conceptual model for the interpretation of the observations--the exciton hypothesis--has been advanced.

It is argued that luminescence is one of three fundamental de-excitation modes in radiation biology, that there is justification for analogy between the electronic states of condensed systems in living cells and those in molecular crystals, and that luminescence analysis may stand with electron spin resonance as a new window through which unique aspects of radiation biology may be studied.

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