Fluorometric and Phosphorometric Analysis

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This review is the 15th of a series of biennial reviews on fluorometric and phosphorometric analysis covering the literature indexed by *Chemical Abstracts* during the period from December 1973 to December 1975 and is intended to contain materials published since the last review (*116A*). The authors wish to acknowledge the contributions of Professor Charles E. White (deceased) and Dr. Alfred Weissler who so ably prepared the previous biennial reviews.

Because of the large number of publications in the area of fluorescence some selection criteria were deemed necessary. Publications from obscure foreign journals are generally omitted, the area of solid-state inorganic luminescence includes but a few representative articles, and coverage of photosynthesis and related topics is but a brief selection. Atomic fluorescence and fluorescence x-ray emission analysis are excluded because they appear in other Analytical Reviews. Undoubtedly some selections reflect the authors viewpoints but hopefully serious omissions are kept to a minimum.

Several reviews covering general aspects of luminescence as an analytical technique have appeared (*6A, 14A, 63A, 86A, 88A*). Fluorescence methods were reviewed for polymer research (*85A*) and trace analysis (*50A*). Energy corrected spectra were used in the determination of trace elements, in measurements of quantum efficiencies, and in the elucidation of biochemical reactions (*112A*). Apparatus and experimental techniques used to measure the fluorescence and phosphorescence of nucleic acids are of interest (*46A*). Analytical applications of fluorescence and clinical nephelometry were investigated by Arnold (*4A*). The text by Pesez and Bartos titled "Colorimetric and Fluorimetric Determination of Organic Compounds" contains material of general applicability (*93A*). Riehl edited a book titled "Introduction to Luminescence" published in German which covers a number of photophysical phenomena such as electroluminescence and thermoluminescence (*98A*). Reviews of fluorescence (*59A*) and phosphorescence (*2A, 84A*) measurements have been published. A text titled "Fluorescence Spectroscopy" edited by Pesce contains a number of special interest chapters such as solution effects on absorption and emission spectra (*91A*). Of particular interest to workers in the biological areas is the book edited by Steiner entitled "Excited States of Proteins and Nucleic Acids" (*107A*). Chapters on instrumentation, principles, excited-state properties of nucleic acids, and fluorescent protein conjugates make this text a valuable addition to the literature.

The application of time-resolved spectroscopy appears to be gaining momentum in both fluorometric and phosphorometric analysis. This approach has opened up areas of analyses that would have been difficult if not impossible to investigate by classical approaches to luminescence. Some of the theoretical aspects of time-resolved emission spectra have been discussed (*30A*) and procedures to deconvolute fluorescence decay curves by a least-squares method have been presented (*115A*). Binary mixtures of metals were quantitatively analyzed by a time-resolved atomic fluorescence technique (*55A*). Application of time-resolved spectroscopy to organic crystals (*57A*) and thin films (*40A*) revealed differences in decay modes not observed in the total spectra. Separation of prompt and delayed fluorescence in carbonyl compounds is described (*17A*). The extension of time-resolved fluorescence techniques to the picosecond domain continues to provide answers to questions regarding relaxation processes as well as revealing more subtle problems (*44A, 70A, 97A*). Molecular biology has provided particularly fruitful applications in studies of proteins, nucleic acids, and membranes (*68A, 114A, 121A*). An interesting application of time-resolved phosphorimetry for the qualitative and quantitative analysis of drugs points to future work in this area (*41A, 42A*).

Fluorescence lifetime measurements continue to provide fundamental information regarding radiative and radiationless processes. A significant isotope position dependence of the radiationless decay rate was demonstrated in methyl substituted and deuterated benzyl radicals (*15A*). Fluorescence quenching upon high energy excitation with γ and x rays was investigated (*13A, 25A*). The influence of intramolecular and environmental effects on fluorescence properties was shown in aromatic compounds (*10A, 29A, 83A, 113A*). Laser excitation was used to measure fluorescence risetimes and lifetimes of dye molecules (*81A, 110A*). Localized symmetry and matrix effects in inorganic complexes were elucidated by luminescent spectra and lifetime data (*45A, 89A, 95A, 111A*). Quenching effects have been reported for several diatomic molecules and radicals (*16A, 34A, 96A*). Inner filter effects on quantum efficiency measurements were discussed (*78A, 79A*). Fluorescence standards reported by Chen should serve to minimize the interlaboratory variability of fluorescence lifetime measurements (*20A*).

Theoretical approaches to the calculation of radiative lifetimes remain only semiquantitative indicators of experimentally determined data (*19A, 99A*). Concentration depolarization effects (*11A*) and a numerical method for equilibrium polarization (*74A*) were investigated. Frequently, errors in fluorescence lifetime measurements may be corrected by the method of moments and the best fit between experimental data and a plausible functional form determined (*37A, 47A, 48A, 49A*). Computer simulation of fluorescence quenching at varying distances from a given emitting species displayed the influence of concentration on the fluorescence signal (*94A*). A new method was introduced for the determination of chemical kinetic and diffusion constants with the development of the theory and experimental realization of fluorescence correlation spectroscopy (*28A, 71A*). The application of this technique to small molecule–macromolecule interactions may prove extremely valuable.

Intramolecular and intermolecular perturbations are particularly important in luminescence spectroscopy. A discussion of quasiline spectra is of interest (*87A*). Magnetic resonance techniques in excited-state studies have been utilized in investigating relaxation processes (*3A, 23A, 35A, 39A*). Internal (*26A, 33A*) and external (*1A*) heavy atom effects were examined. The dependence of radiationless decay rates upon vibrational state and excitation wavelength was found in several environments (*51A, 64A, 109A, 118A*). Intersystem crossing effects were measured for several carbonyl compounds (*69A, 80A*). Heat pulses were used to examine the dynamics of electronic relaxation in crystals (*5A*). Delayed emission in single and mixed crystals was investigated and environmental masking of small intrinsic differences among molecular properties discussed (*52A, 77A, 119A*). Intermolecular processes involving solvation effects were interpreted using a statistical model (*92A*). The influence of pH and proton transfer in protein and organic molecule luminescence was demonstrated (*9A, 31A, 36A, 73A*). The dependence of radiative and radiationless rate constants on excitation energy (*117A*), elec-

tron impact (*43A*), and pressure (*104A*) was studied.

Studies of photosynthesis and related molecular systems are subjects of an extensive literature. Only a sampling of citations is present here. The influence of salts (*7A, 66A, 72A*), light (*32A, 65A*), and other environmental factors (*24A, 54A, 75A, 76A, 90A, 103A, 108A*) on the fluorescence of chloroplasts was the subject of many investigations. Orientation effects in chloroplasts and photosynthetic bacteria were studied by observing the influence of a magnetic field on the fluorescence spectra (*22A*).

Low temperature fluorescence was measured in *Chlorella* whole cells (*100A*). Concentration and viscosity effects were shown to be dependent on the nature of the central metal ion in chlorophyll (*53A, 56A*). In vivo measurements of chlorophyll are subject to limitations as demonstrated by quantum yield and fluorescence lifetime measurements (*56A, 67A, 82A*). Chlorophyll fluorescence quenching by in vitro compounds and reversible inhibition of photosynthetic electron transport has been studied in green algae (*8A, 12A*). Structural changes influencing chlorophyll fluorescence were shown to be induced by ions and molecular aggregation (*38A, 58A, 61A*). Characteristics of chlorophyll fluorescence provided subject material for several articles (*102A, 105A, 106A, 120A, 122A*), and phosphorescence of chlorophyll and related molecules (*21A, 60A, 101A*) was detected. One report (*62A*) indicated some disagreement with previously published data.

Fluorescence lifetimes of α- and β-carotenes (*18A*) and the effect of chelated metal ions on primary photophysical properties of metalloporphyrins (*27A*) were determined.

INSTRUMENTATION

Fluorescence instrumentation has been discussed generally (*16B, 40B, 41B*) and several new instrument designs and/or modifications have been described: filter fluorometers (*14B, 15B*); specialized optical (*24B, 78B*) and detection systems (*28B, 35B, 74B*), including photon counting (*2B, 12B*); polarized emission measurements (*58B, 77B*); a fluorometer for determination of near-infrared emission (*48B*); a submersible fluorimeter for in situ measurements in large bodies of water (*37B*); a flight-qualified multiwavelength laser fluorosensor for remote sensing (*46B*); and electronic designs to permit determination of 10^{-10}–10^{-8} percent trace impurities (*55B*). Chance et al. (*8B*) have designed a time-sharing multichannel spectrophotometer, reflectometer, and fluorometer of great versatility in biochemical measurements. Other high precision fluorometers (*52B, 53B*) have been designed for measurements of very weak fluorescent emissions from biological molecules (*50B, 68B, 76B*) with quanta correction (*21B*). Several sample cell and/or sample chamber designs have been proposed for multiple sample cuvettes (*65B, 70B–72B*) and low temperature measurements (*73B*). Special light sources are described for calibration (*80B*), improved stability (*5B*), and lifetime measurements (*39B, 82B*). Pulsed dye lasers provide improved sensitivity over alternate excitation sources and several designs are discussed for fluorescence lifetime measurements (*1B, 29B, 44B, 51B, 62B*). The value of measuring emission lifetimes in the picosecond to nanosecond range has led to suitable instrumentation using pulsed laser sources (*20B, 26B, 32B*) and other techniques (*4B, 6B, 63B*) for measuring fast optical signals (*43B*).

Computerization has been adopted for such purposes as on-line fluorimetric data acquisition (*25B, 27B, 64B*); anisotropy measurements (*3B*); real time correction of spectra in photon counting measurements (*81B*); controlled epi-illumination microspectrofluorimeter (*54B*); and time-resolved, component-resolved phosphorescence spectra (*79B*).

Improvements in instrumentation for phosphorimetry have been discussed by Lukasiewicz, Mousa, and Winefordner (*42B*). Other reports describe the use of disc phosphoroscopes (*36B*), high resolution spectrometers (*76B*), and phase sensitive detection methods (*18B, 49B*). Instruments designed for measurements of kinetics of phosphorescence use simple pulsed-source excitation (*9B, 23B, 57B*), adjustable phasing between excitation and emission choppers (*7B*), and a combination of chopped Xe lamp source with electronically gated photomultipliers (*22B*).

A single disc phosphoroscope has been designed for the direct measurement of phosphorescence from separated components on thin-layer chromatograms (*17B*). Fluorescence detection systems have also been designed to quantitate thin-layer chromatograms (*13B*) with the aid of on-line programmable electronic desk computers, and for null point detection of coulometric titrations (*30B*). Chemiluminescence detectors for use with ion-exchange column chromatography have been described (*47B*) as well as other instrumentation for chemiluminescence analysis (*31B*).

The use of fluorescence and phosphorescence in cell biology has been reviewed (*59B–61B*) and was the subject of a conference on quantitative fluorescence techniques applied to cell biology. The published proceedings edited by A. A. Thaer and M. Sernetz include papers on automation of cytofluorometry (*19B*) and microcapillary cuvettes for microscope fluorometry (*67B*). Other developments in methodological and instrumental aspects of microfluorometry (*56B*) include ultrarapid methods (*69B*); rapid multichannel methods for spectral studies of metabolic processes in single living cells (*33B, 34B*); digital microspectrofluorometry (*10B*); use of pulsed tunable lasers for direct measurement of fluorescence decay times in single cells (*62B*); and improved discrimination in the analysis of catecholamines (*38B*).

METHODS AND TECHNIQUES

The potential of phosphorimetry as a spectrochemical method has been reviewed (*1C, 64C, 66C*) and applications of time-resolved (*59C*) and phase-resolved (*23C, 62C, 63C*) phosphorimetry have been described. The observation of room temperature phosphorescence by Schulman and Walling, noted in the last review (*116A*), has been extended toward analytical usefulness (*69C, 103C, 104C*) and toward use in probing dynamic aspects of protein structure (*81C*). Techniques for using phosphorimetry in combination with thin-layer chromatography have been described for the analysis of purine containing compounds (*54C*) and sulfonamides (*22C*). Flow methods combined with phosphorimetry to study photosynthetic systems have been reviewed by Etienne (*19C*). El-Sayed (*17C*) has reviewed applications of phosphorescence–microwave–double-resonance techniques for the study of phosphorescent states of molecules (*14C, 40C, 77C, 106C, 110C, 111C*).

Several developments have extended the capabilities of luminescence spectroscopy in both analytical and physical studies. Derivative luminescence spectrometry (*26C, 88C*) enhances the resolution of minor spectral features and increases specificity of fluorescence spectra from crude oil samples (*26C*). O'Haver has also described a selective modulation procedure for obtaining resolved excitation and/or emission spectra from a mixture of two fluorophores whose spectra overlap too severely for resolution by conventional methods (*67C*). A theory relating rotational Brownian motion to the time autocorrelation function of the intensity of emitted radiation from a system of fluorophores extends the time range over which rotational diffusion may be studied by the technique of fluorescence correlation spectroscopy (*3C*). Applications to studies of lateral diffusion in membranes have been discussed (*72C*). Weber (*101C*) has briefly reviewed theoretical and experimental aspects of polarized fluorescence and several other papers discussing aspects of polarized luminescence may be noted (*2C, 13C, 16C, 95C, 96C*). Fluorescence quantum yield calculations for powders and for solutions measured with spectrofluorometers with or without an integrating sphere are discussed by Billon et al. (*7C*). Other developments include multiple-internal reflection fluorescence spectrometry (*29C, 30C*); fluorescence detected circular dichroism (*93C*); windowless sample cells for front-surface excitation (*55C, 56C, 61C*); tunable laser excitation techniques for fluorometric analysis of product states for evaluation of microscopic rate constants (*109C*); techniques to measure absorption (*44C*) and fluorescence (*53C, 91C*) spectra of transient species, including two photon excitations (*80C*); methods to reduce scatter-peak interference (*4C*), to calculate fluorescence band intensities (*76C*) and band shapes (*10C*); automated fluorometric determinations (*34C, 84C, 85C*); procedures for obtaining corrected spectra (*27C, 87C*); and notes regarding the interpretation of fluores-

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cence parameters (*8C, 82C*).

Techniques for fluorescence lifetime measurements are reviewed by Ware (*99C*), Kokubun (*46C*) and Yoshihara (*108C*). Potential errors in lifetime measurements resulting from photomultiplier tube saturation (*35C*) and from the wavelength dependence of the apparatus response function in deconvolution methods (*97C*) are discussed. A new method (*51C*) using storage ring synchrotron radiation is described for determining very fast fluorescence decay times, shorter than 1 ns, with an accuracy of about 10%. Ware and Lyke (*100C*) describe apparatus and procedures for fluorescence lifetime measurements on saturated hydrocarbons in the vacuum ultraviolet region of the spectrum. Other methods and applications of nanosecond and/or time-resolved fluorescence include photographic systems (*39C*); studies of excited-state reactions (*52C*); vibronic level fluorescence (*5C, 21C*); time-resolved emission from flavine adenine dinucleotide (*98C*); analysis of photochemically unstable compounds (*53C*); and determination of radiative lifetimes of a variety of alkaline earth monohalides (*15C*).

The parameters governing the intensity of laser induced infrared fluorescence have been discussed by Robinson (*79C*) with potential applications (*37C, 38C*) to remote sensing of atmospheric pollutants. The relative merits of continuous wave vs. pulsed laser sources for such remote fluorosensing systems are reviewed by Bristow and De Villiers (*12C*). Other discussions of analytical spectrometry (*41C, 42C, 105C*) have considered signal-to-noise effects (*65C, 68C*); correction for interfilter effects (*60C*); matrix effects (*89C, 92C*); problems in the study of photochemical reactions (*102C*), low temperature luminescence analysis (*94C*); design of a fluorescamine peptide analyzer (*9C*); and fluorometric detectors for use in chromatographic separations (*18C, 83C*). Luminescence methods for determination of aromatics in water (*31C*) have been applied to identification of petroleum contaminants in marine waters (*32C*). A fluorescence technique is described for characterization of surface films on water (*24C*) made from crude oil, lubricating oil, lignosulfonic acid, and samples from a shipping channel. Laser excitation methods have increased the sensitivity of fluorescent measurements of dyes on fabrics by two orders of magnitude (*107C*). Porro and co-workers (*74C*) have presented corrected spectra for 20 polycyclic aromatic compounds and their principal applications in studies of air pollution and food product analysis (*47C*).

There are many monographs on the use of fluorescence and phosphorescence spectroscopy in the study of biochemical problems and processes. New reviews or methods (*6C, 11C, 20C, 57C, 73C*) have appeared but we will emphasize developments in methodological and instrumental aspects of cytofluorometry (*75C*). Many of these developments are reviewed and summarized in the symposium proceedings edited by Thaer and Sernetz. Methods for identifying the influences of organelle interactions and/or metabolite modulations on rates of metabolic pathways using microfluorometry are described by Kohen and co-workers (*45C*). Sernetz has developed a kinetic analysis to study the turnover of fluorogenic substrates in individual fibroblast cells (*86C*). Microfluorometric determinations of histamine (*28C, 49C*) and biogenic amines (*78C*) in single cells have been presented. Corrected fluorescence spectra and rapid recordings of fluorescence intensities from Ehrlich's hyperdiploid ascites turner cells supravitally stained with acridine orange indicate opportunities for automated cytopathology (*25C*).

Papers on methods and applications of thermoluminescence and electroluminescence have also appeared (*33C, 36C, 43C, 48C*).

ORGANIC AND BIOLOGICAL

Hydrocarbons. Although many fundamental and applied papers are surveyed in this section, the wealth of information precludes comprehensive coverage.

Irradiation of aromatic molecules by electron impact was utilized to study various dissociative and radiationless processes in benzene and related compounds (*55D, 56D, 460D, 588D*). Radical formation induced in an x-irradiated organic matrix revealed an interaction between the radicals formed before complete dissociation (*521D*). Matrix-isolated dichlorocarbene displayed an intense red emission which possessed both bending and symmetrical stretching vibrational modes (*617D*). Participation of d orbitals in the excited-state properties of naphthalenthiols was demonstrated (*109D*). Photoluminescence of polycyclic aromatic hydrocarbons was discussed by Hsieh (*268D*) and a number of spectroscopic properties of fluoranthene were measured (*342D*). Solvent and temperature effects were reported for substituted hexatriene (*112D*).

Deuterium isotope effects on the phosphorescence spectra of deuterated benzene, other aromatics, and benzyl radicals were shown to have distinctive positional dependence (*54D, 300D, 361D, 451D, 472D, 663D, 685D, 695D*). Crystal effects were found in the phosphorescence spectra of deuterated benzene isotope fixed in a borazine host crystal (*644D*). The substituent effect on the line width of spectra was examined (*166D*). Relaxation from highly excited vibrational levels in pyrene was examined and a modified model for excimer absorption and emission proposed (*131D*). The effect of oxygen on the radiationless transitions of aromatics in condensed phases is discussed (*60D, 279D*). Competition between intersystem crossing and internal conversion in isolated large molecules (*269D*) and the temperature dependence of hot bands in the phosphorescence spectrum of coronene (*703D*) indicate the complicated nature of large molecule luminescence.

The combination of magnetic and optical techniques reveals a number of interesting details of molecular electronic structure (*28D, 169D, 261D, 303D, 377D, 378D, 379D, 535D, 539D, 707D*). A combination of phosphorescence and EPR measurements was used to investigate the excitation wavelength dependence of the phosphorescence of naphthalene–tetracyanobenzene charge-transfer complexes (*495D*). Luminescence quenching can be achieved by a number of mechanisms. The use of 9,10-diphenylanthracene as a fluorescence quantum yield standard was investi-

gated (*255D*). Fluorescence quenching may depend on the radiation field in excitation and emission (*79D, 295D*). Photochemical quenching may occur (*234D, 495D*). Anthracene fluorescence demonstrated a charge relationship in aqueous micellar solutions (*494D*). Aromatic molecules show both inter- and intramolecular fluorescence quenching in solutions of amines and organic mercurials (*249D, 645D, 662D*). Lloyd used a partly quenched, synchronously excited fluorescence technique to analyze soot, oil, gasoline, and cresote samples (*383D*). The use of quenching in fluorescence analysis is indeed an interesting application of fundamental principles.

Environmental dependence of hydrocarbon luminescence is quite variable. A discussion of solvation structure and relaxation processes is given by Azumi (*36D*). Changes are observed on going from the crystalline to an amorphous state or with increasing viscosity (*399D, 402D*). Temperature (*248D*) and concentration (*140D*) dependence in crystals or low temperature glasses were shown to be considerable. The influence of solvent polarity on fluorescence properties was measured (*138D, 487D*).

A review of electron donor–acceptor complexes in their excited states discusses a number of topics essential to the interpretation of these spectra (*444D*). Solvent effects on excited complexes formed between aromatic hydrocarbons and heterocyclic compounds were observed (*146D*). The charge-transfer reaction between nitrogen and aromatic hydrocarbons produced chemiluminescence (*149D*). A number of inter- and intramolecular charge-transfer complexes were found to luminesce (*284D, 426D, 427D, 688D*). The structure of several charge-transfer complexes was assigned by the method of photoselection (*21D*). A recombination luminescence was observed for the interaction between tetramethyl-*p*-phenylenediamine cation and trapped electrons (*43D, 431D*).

The heavy atom effect is useful in certain analytical applications. An attractive application of this phenomena was made by Seybold and White to the room temperature phosphorescence analysis of substituted naphthalenes (*561D*). Since the fluorescence of certain compounds such as rubrene and carbazole is not quenched, quenchofluorometric analysis may be highly selective in the analysis of these compounds in complexed mixtures (*702D*). The external heavy atom effect may be used to alter photochemical reactions (*238D*). Also, the nature of the compound containing the heavy atom may dramatically affect the emissive properties (*44D, 286D, 448D, 467D, 702D*).

Quasi-linear fluorescence and phosphorescence spectra are being used in a variety of applications such as orientation of molecules in stretched film (*147D*). Quantitative analysis of indole, fluoranthene, phenanthrene, and chrysene by quasi-linear phosphorescence analysis was accomplished by use of an appropriate internal standard (*480D*). Mixtures of polynuclear aromatic hydrocarbons were qualitatively identified by their quasi-linear emission spectra and quantitation was determined by the internal standard technique (*203D, 327D*). Benzo[*a*]pyrene may be determined in biological and environmental samples by use of its quasi-linear fluorescence spectrum and this approach has also been used in onocological studies (*318D*). Anthracene, pyrene, 7,12-dimethylbenzanthracene, perylene, and other aromatic hydrocarbons were determined in solutions and extracts from atmospheric air pollution samples with limits of detection in the range from 10^{-8}–10^{-10} g/ml. (*316D*). Quasi-linear phosphorescence spectra were observed in a solid inorganic matrix (*308D*). Temperature and orientation effects are important in Shpol'skii spectra (*281D, 359D, 420D, 430D, 608D*). The dependence of line width on a number of parameters was investigated (*5D, 6D, 183D*). A number of studies on the nature of the quasi-lines were published (*419D, 429D, 462D*).

Energy transfer may be used to determine the presence of small amounts of benzophenone or analogous compounds in aromatic hydrocarbons (*82D*). A number of papers refer to triplet–triplet energy transfer between carbonyl containing molecules and aromatic hydrocarbons (*7D, 101D, 385D, 609D*).

Structural effects on luminescence are diverse. A dual emission is reported for substituted azulenes (*165D*). Exocyclic substituent effects were discussed by Schulman (*540D*). Photochemical reactants and products were investigated by luminescence spectroscopy (*184D, 421D*). The geometry of polyenes and polyphenyls was determined from fluorescence analysis (*68D, 196D, 454D*). The fluorescence intensities of alkyl and aryl salicylates displayed significant differences (*570D*).

Of the many excellent works on luminescence in the solid-state and exciplex formation, only a few will be mentioned. The luminescence of solid aromatic substances in active nitrogen was studied (*565D*). Luminescence polarization was used as a tool in elucidating the molecular orientation of an impurity in host crystals (*706D*). Modulation excitation spectra gave evidence for triplet excimer formation in highly concentrated solutions of 1,2-benzanthracene and 1,2:3,4-dibenzanthracene (*601D*). Birks derived expressions for the influence of reabsorption and defects on anthracene crystal fluorescence (*67D*).

A combination of thin-layer chromatography and fluorescence permitted differentiation between hydrocarbons of biogenous and petrol origin (*256D*). The fluorescence behavior of several derivatives of chlorobiphenyls was determined (*274D*). Benzo[*a*]pyrene and benzo[*k*]fluoranthrene were extracted from airborne particulates and analyzed by fluorescence (*285D*) and a fluorescent detector for a gas chromatograph (*440D*). Hydrocarbon induced carcinogenesis was studied in biological systems (*317D, 340D*) using fluorescence techniques. Aromatic nitro compounds were estimated by conversion to an amine and coupling to 1,2-naphthoquinone-4-sulfonic acid yielding a fluorescent *o*-diphenol (*48D*).

Heterocycles. The influence of position of the internal heavy atom was shown in azaphenanthrenes (*404D*) and the effect of molecular geometry on intersystem crossing rate constants in a series of aromatic amines discussed (*8D*). The nature of changes in the electronic structure of the ground and excited states upon substitution of heteroatoms into the aromatic framework was outlined (*541D*). Photochemical behavior in heterocyclics has been investigated in several systems (*51D, 463D, 600D, 622D*).

Spectra were reported for a number of substituted pyridines and related compounds (*195D, 265D, 469D, 676D*). The important indole moiety was the subject of a number of papers (*24D, 27D, 231D, 314D*). Fluorescence and phosphorescence properties were presented for *s*-tetrazine in the crystalline state (*260D*). Intra- and intermolecular energy transfer between two unconjugated heteroatom chromophores was observed (*311D, 436D*). Host–guest interactions and excimer formation were shown in crystals containing heteroaromatic molecules (*205D, 682D, 693D*). Magnetic and optical examination were used for several azaromatic molecules (*72D, 251D, 455D, 642D, 705D*). Itoh and Azumi investigated an anomalous change in the fluorescence wavelength distribution of related quinolines as a function of excitation wavelength (*282D, 283D*). Capomacchia and Schulman determined the influence of pH on the absorption and fluorescence properties of quinacrine and 2-methoxy-6-chloro-9-aminoacridine (*110D*). A theoretical discussion is given for the interaction of fluorescence quenchers which do not absorb light (*277D*). Charge transfer (*18D*), inorganic anion (*86D*), amine (*405D*), and radical (*673D*) quenching of fluorescence were reported. Luminescence from an organic charge-transfer salt was investigated (*610D*).

Solvent dependence and reactivity were described for substituted flavines and flavones (*208D, 602D*). Intramolecular interactions between the chromophores in folic acid were investigated (*619D*). Tryptamine and serotonin excitation and fluorescence polarization were measured in glycerol (*592D*). Luminescence of indole was recorded during exposure to ionizing radiation (*593D*). A series of fluorescent whitening agents were tested for potential photocarcinogenesis (*189D, 190D, 191D*). Energy localization is correlated with the photobiology of skin-sensitizing coumarins and psoraleus (*396D*). The localized environment of the tryptophan residue in apoazurin may be influenced by tight packing of the protein as suggested by fluorescence decay and circular polarization evidence (*228D*). Quasi-line spectra were found for *o*-phenanthroline and oxazoles in *n*-alkane matrices (*49D, 465D*). Substituted quinolines and isoquinoline displayed line structure in cyclohexane glasses

at 77 K (*287D, 288D*). Aromatic thiophenes may be identified by their quasi-linear fluorescence and phosphorescence spectra (*10D, 11D*). Proton exchange equilibrium and pK values were determined from fluorescence spectra of nitrogen heterocycles (*108D, 186D, 516D, 519D, 542D*).

Identification of pralidoxime salts was based on an alkaline hydrolysis fluorescence reaction (*185D*). Subnanogram quantities of benzo homologs of quinoline and acridine were analyzed in samples of creosotes, gasoline, sump oils, wood splinters, soot particles, and pitch with high-pressure liquid and microcolumn chromatography with fluorimetric detection (*382D*). A rapid fluorescence test was developed for 4-amino-3,5,6-trichloropicolinic acid (*502D*). N'-Methylnicotinamide and nicotinamide in serum were determined by derivatization with acetophenone and measuring the resulting fluorescence (*129D*). Uroporphyrin and coproporphyrin in urine were quantitated by separation on an anion-exchange resin followed by fluorometric assay (*584D*).

Oxygenated Molecules. Absorption and emission characteristics were determined for hexachloracetone (*350D*) and methyglyoxal (*466D*). Phenolate ions showed a marked decrease in fluorescence quantum yield at excitation energies corresponding to wavelengths where the S_1 and S_2 levels overlap (*338D*). Internal heavy atom effects on the phosphorescence of halogenated 9,10-anthraquinones (*529D*) and the external heavy atom effects on substituted aceto- and benzophenone were reported (*224D*). The magnetic microwave double-resonance technique was used to observe a second-order Stark effect in the spectrum of p-benzoquinone (*567D*) and to identify the lowest triplet level of p-methoxy- and p-thiomethoxyacetophenone as a $^3(\pi,\pi^*)$ (*603D*) in a host crystal of 1,4-dibromobenzene at 1.6 K (*567D*). Solvent effects on absorption and emission spectra were examined for benz[*de*]anthracen-7-ones (*61D*). Phosphorescence in 3,4,5,6,7,8-hexahydronaphthalen-2(1H)-one was found to be predominantly of impurity origin and its quenching effect on ketones determined to arise from an excited-state complex formed between this compound and the ketone (*173D*). The temperature dependence of the phosphorescence spectra of aromatic carbonyl molecules was discussed (*397D*). Excitation of camphorquinone in the S_2 state resulted in a higher phosphorescence quantum yield than excitation into the S_1 state (*362D*). The triplet state of flavone was identified as being predominantly (π,π^*) with some admixture of (n,π^*) character (*497D*). The phenomena of dual phosphorescence observed in aromatic carbonyls may be explained by a model which considers the close proximity of the two emitting states (*24D; 127D, 128D, 301D, 466D*). A related anomaly results from monomer and dimer phosphorescence of p-methylacetophenone in a nonpolar solvent (*612D*). Quenching by amines (*70D*) and solvent dependence (*178D*) were reported for benzophenone phosphorescence. Absorption and luminescence characteristics were discussed for benzaldehyde (*418D*), benzophenone and several phenyl pyridyl ketones (*71D*), and xanthione (*107D*). Hydrogen bonding effects on the emissive properties of 1- and 2-naphthaldehyde (*329D*) and the geometric dependence of the excited states in α-dicarbonyls were described (*33D*). Collisionally induced (*641D*) and quenching (*201D, 527D*) of biacetyl phosphorescence were investigated. Triplet levels in uv stabilizers were investigated to determine their efficiency to quench carbonyl groups in photooxidized polymers (*53D*). Alkylamine quenching of alkyl ketone fluorescence was suggested to occur by a charge-transfer mechanism (*142D*). Fluorescence and phosphorescence lifetimes and quantum yields were reported for o-quinonediazides (*391D*).

Four methods were evaluated for the determination of excited-state pK_a measurements for anthraquinones and related compounds (*509D, 510D*) and the pH-dependent spectra of m- and p-hydroxybenzoic acids (*479D*) reported. Prompt fluorescence and delayed fluorescence in phenylcarbonyl compounds were separated by time-resolved emission spectroscopy (*17A*). The behavior of 2-naphthol fluorescence was described (*332D*). Aldehyde derivatives of carboxylic acid anhydrides were synthesized for their possible analysis of fluorescence derivatives (*450D*). Aromatic aldehydes were determined by using the fluorogenic 1,2-diaminonaphthalene (*461D, 699D*). Serum triglycerides were analyzed by fluorometry (*678D*) and a comparison of nephelometric and fluorometric methods reported (*42D*). Reducing sugars were determined by reaction with ethylenediamine sulfate to produce a fluorescence product in neutral or weakly alkaline solutions (*262D*).

Amines. The effect of molecular geometry on the emissive properties of diphenylamine and related compounds was reported (*271D*). Structural effects on the photophysical processes were measured in saturated amines (*247D*). Delayed luminescence was observed in rigid matrices of aromatic amines (*69D*). High energy recombination luminescence of L-tryptophan was studied in low temperature matrices (*433D*). Intramolecular effects on arylamine fluorescence were reported (*599D*). Methyl and ring deuteration effects on the phosphorescence lifetime of N,N,N',N'-tetramethyl-p-phenylenediamine were discussed (*695D*).

Fluorescamine continues to be widely used as a reagent for detection of amines. Triethylamine was used to stabilize the fluorescent products of the fluorescamine–amino acid reaction on thin-layer chromatograms (*182D, 569D*). An impurity was removed from HCl which previously limited the sensitivity of primary amine analysis (*544D*). Fluorescamine has alternately been used as a colorimetric reagent of primary amines (*628D*). Several papers deal with the application of fluorescamine to the analysis of primary amines (*2D, 122D, 181D, 330D, 452D, 453D*).

Procedures were described for the analysis of 5-hydroxytryptamine in platelets and tissue (*202D, 458D, 615D, 709D*). A column chromatographic method was used for indole separation in the analysis of serotonin and tryptamine (*124D, 233D*). An improved method was introduced for the determination of 5-hydroxytryptophan in plasma (*571D*). An error in the fluorometric determination of tryptophan was corrected (*75D*). A microfluorometric method was applied to the analysis of dopamine in nerve terminals (*167D*). A discussion of 5-hydroxytryptophan in the brain and its analysis by fluorometry was reviewed (*31D*). The tryptophan metabolite N'-formylkynurenine in urine was quantitatively analyzed by a method depending on the formation of the enol form in basic solution (*104D*).

Automation of the fluorometric analysis of histamine and refinements were discussed (*518D, 581D*). An automated continuous-flow system for the extraction and fluorometric determination of histamine was developed (*580D*). Peptides with NH_2-terminal histidine were analyzed by a modification of the o-phthalaldehyde reaction (*245D*). A discussion of problems relating to the determination of histamine and improvements in its assay were reported (*246D, 386D*).

A semiautomated method is described for the analysis of catecholamines in serum and urine and an automated system was developed for urine specimens (*25D, 667D*) and brain tissue (*684D*). Several papers dealt with the analysis of dopa, adrenaline, and noradrenaline in urine (*500D, 546D, 547D, 637D, 659D*). A review by Rorsman, Rosengren, and Rosengren dealt with the analysis of dopa and catechol amines in melanocytes (*515D*). Catecholamines were determined in nerve tissue and brain (*9D, 40D, 355D, 534D, 591D*). Errors in catechol amine assays due to quench effects were discussed (*548D*). Solvent extraction proved to be a simple method to separate the trihydroxyindole products of noradrenaline and adrenaline (*549D*). Methods were introduced for the assay of small plasma samples (*105D, 155D*). Calibration of a fluorometer for the determination of catechol amines was described (*464D*). Laszlo discussed the concentration dependent changes of fluorescence in the formaldehyde-induced fluorescence method for noradrenaline and 5-hydroxytryptamine (*366D*). The formaldehyde fluorescent method for biogenic amines was reviewed by Jonsson (*291D*). Dibasic potassium phosphate was found to cause the standard curve in the dopamine analysis to deviate from linearity (*226D*).

Ambrose reported improvements of the analysis of tyrosine in serum (*23D*). An automated fluorometric method was developed for the screening of tyrosinemia (*227D*). A microassay for homovanillic acid in cerebrospinal fluid was automated (*507D*). Fluorescence from iodine-containing amino acids was reported (*236D*). 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole was used to quantitate microquantities of

aliphatic amines and nitrosamines (*333D, 334D*). Marton and Lee reported the fluorometric analysis of polyamines after separation by high-pressure liquid chromatography (*400D*). Hydrazine in plasma was determined by forming a fluorescent derivative with dimethylaminobenzalazine (*653D*).

Proteins. A number of reviews of interest in protein analysis have appeared including discussions of protein luminescence (*141D, 302D, 343D, 558D, 671D, 672D*), phosphorescence (*309D*), and fluorometric assays (*324D*). Chapman and Dodd reviewed the use of spectroscopic techniques in the elucidation of membrane structure (*116D*). Wahl presented the application of fluorescence anisotropy (*665D*). and Brewer and Passwater introduced an interesting approach to the analysis of cell types using fluorescence and phosphorescence (*93D*). Mathematical analyses were proposed for fluorescence polarization (*409D, 639D*) and decay (*229D*). Binding sites in phosphorylase b were studied using differences in fluorescence decay (*632D*). Instrumentation introduced specifically for protein analysis included a flow cell for studies of conformational changes (*47D*), computer-controlled fluorescence polarization (*34D*), and a multifunctional luminescence spectrophotometer (*310D*). A related review by Penzer discussed the conformation and interaction of peptides and proteins in solution (*428D*).

The luminescence characteristics of tryptophan and tyrosyl residues are of fundamental importance and are the subject of many investigations including the influence of microenvironments (*63D, 78D, 597D*), optical detection of magnetic resonance (*658D, 710D*), quenching of tryptophyl fluorescence characteristics of ribosomal proteins (*209D*), quenching of tyrosyl and tryptophyl fluorescence by inorganic ions (*19D, 120D*), and determination of tryptophan content (*524D*).

A number of physicochemical approaches to the study of proteins were presented such as the application of multisite titration analysis to protein charge-transfer complexes (*150D*), energy transfer (*211D, 213D*), fluorescence quenching and ^{1}H NMR (*103D*), circular polarization of luminescence (*533D, 596D*), room temperature phosphorescence (*526D*), and the temperature dependence of protein fluorescence (*415D*). A report by Kuntz analyzes the mechanisms of protein inactivation by irradiation (*356D*).

Fluorescence quenching was used to study macromolecular conformation (*179D*), binding constants (*326D*), fluorophore accessibility to the solvent (*92D*), and drug-protein association constants (*445D*). Nontryptophan fluorescence was used to characterize the appearance of an age-dependent polypeptide (*588D*). Chen has investigated relaxation processes in the binding of bilirubin to albumin (*121D*) and Somners has characterized albumins by their fluorescence properties (*586D*). "Albumin-Titratable Bilirubin" has been determined by a fluorometric technique (*413D*).

Studies were reported on thyroglobulin fluorescence (*237D, 633D*) and the effect of x radiation on fluorescence characteristics (*690D*). Phase transitions in cytochrome b_5–lipid complexes were measured by the temperature dependence of complex fluorescence (*163D*). Fluorescence spectra were described for β-lactoglobulin AB (*307D*) and elastin and elastin complexes (*46D*). Transferrin in serum was determined by forming a terbium complex and measuring the emission of terbium upon transferrin excitation (*424D*). A microfluorometric assay was introduced for retinol-binding protein in blood plasma (*218D*).

Fluorescamine has been extensively used for the fluorometric determination of amino acids, peptides, and proteins in picomolar concentrations. Procedures have been published for analysis of polyacrylamide gel scans (*171D, 503D, 595D, 621D*), proteins isolated from membranes (*577D*), proteins from cell fractions and homogenates (*648D*), protein in duodenal juice (*536D*), peptides on thin-layer cellulose (*623D*), and peptides in column chromatographic fractions (*449D*). Separation of low molecular weight amines by gel filtration eliminated interfering material in a simplified protein assay (*80D*). Fluorescamine was used in the simultaneous colorimetric and fluorometric analysis of amino acids (*180D*). Chen presented a detailed study of the fluorescence properties of fluorescamine-protein conjugates and the implications of these data for protein studies (*119D*). Fluorescamine was used as a histochemical reagent for the demonstration of peptide hormone secreting cells (*363D*).

An improved synthesis was introduced for fluorescein isothiocyanate, another widely used fluorescent conjugate (*579D*). Fluorescence spectra of protein (*650D*) and heterocyclic isothiocyanates were reported (*401D*). Hemoglobins A, F, and S were identified by a fluorescein labeled antibody technique (*253D*). A modified procedure was presented for labeling of proteins with dansyl chloride (*325D*). *N*-Bromosuccinamide was used to modify the tryptophan fluorescence in papain (*387D*). Other fluorogenic reagents used in the fluorometric determination of proteins were 1-nitroso-2-naphthol (*677D*) and *o*-phthaldialdehyde (*244D*). 4-Methyl-7-(substituted amino)coumarins were used for investigating hydrophobic regions in proteins (*4D*). A new fluorescence histochemical method for endocrine cells (*478D*) and new fluorescent mercurials and acidic fluorochromes for histochemical use (*137D*) were introduced. Eosin Y was used for the fluorometric determination of urine protein (*496D*). Fluorescence and other spectroscopic properties were reported for Schiff bases prepared from pyridoxal and amino compounds (*506D*).

Enzymes. Reviews of some of the aspects of fluorometric and photometric enzyme determinations include the conditions for optimizing accuracy in this approach (*339D*) and the use of fluorescent probes in proteinases (*200D*). Guilbault discussed the use of immobilized enzymes in enzyme electrodes and in the fluorescence monitoring of solid surfaces (*232D*). A new microspectrofluorometer was developed for scanning the NADPH fluorescence spectrum in a single cell (*341D*). The effect of inhibitor binding to an endonuclease was investigated by time-resolved fluorescence spectroscopy (*97D*). Energy transfer measurements were made to determine the distance between an amino acid and the active site of asparate transcarbamylase (*407D*) and to study the localized environment of a dye in the lysozyme–eosin complex (*50D*). Similarities between human and rat lysozyme were investigated with fluorescence and circular dichroism (*438D*). Circularly polarized fluorescence spectra were reported for a number of dehydrogenases (*531D*). Fluorescence polarization of FAD complexed to D-amino acid oxidase was dependent on the monomeric and dimeric forms of the enzyme (*687D*). A curve fitting procedure was introduced to calculate the association constant, number of binding sites, and theoretical fluorescence titration end point of an enzyme–ligand interaction (*172D*). Luminescence spectra of carboxypeptidase B indicated a possiblle interaction between a tyrosyl residue and the Zn atom (*562D*). Some of the properties of alcohol dehydrogenase investigated were fluorescence quenching by *p*-chloromercuriphenylsulfonate (*614D*), energy transfer between subunits (*618D*), and the dissociation constants of complexes with ligands (*349D*). Luminescence spectra of coenzyme–enzyme complexes were reported for NAD (*177D*), NADH (*73D*), FAD (*188D*), and pyridoxal phosphate (*651D*). The effect of inhibitors on L-glutamate dehydrogenase (*223D*), aspartate aminotransferase (*102D*), and *E. coli* phosphofructokinase (*381D*) was followed by fluorescence. A long-lived luciferase–flavin intermediate was isolated and identified by its fluorescence characteristics (*252D*). The effect of zinc on carbonic anhydrase (*258D*), reactivity of immobilized enzymes (*364D*), and the effect of gramicidin D in chlorplasts (*376D*) were studied using fluorescence. The rate of β-naphthyl triphosphate hydrolysis by heavy meromyosin was determined from changes in fluorescence intensity (*292D*). Residual fluorescence has been introduced as an index of purity for NADH (*267D*). Fluorescent markers were used in enzyme purification (*206D*), cellular reactions (*545D*), energy coupling (*587D*), and identification of bacterial enzymes (*390D*).

A generalized approach to enzyme quantitation is based on the addition of a nonfluorescent but fluorogenic substrate and measuring the resulting fluorescence. Examples of this approach were reported for arylamidase with phenylalanyl-β-naphthylamide (*76D*), plasminogen, plasmin, and streptokinase with α-*N*-methyl-α-*N*-tosyl-L-lysine β-naphthol ester (*59D*), α-L-glucosidase with 4-methylumbelliferyl α-L-fucoside (*514D, 683D*), acrosin with 4-methylumbelliferyl *p*-quanidinobenzoate (*98D*), leucine amino-

peptidase with L-leucyl-β-naphthylamide (*636D*), cyclic nucleotide phosphodiesterase with 1,N^6-etheno-2-aza-adenosine 3′,5′-monophosphate (*631D*), hexosaminidase A with 4-methylumbelliferyl-β-D-glucosaminide (*522D*), lipase with monodecanoylfluorescein (*331D*), and methylumbelliferyl-α-galactosidase with 4-methylumbelliferyl-α-galactoside (*95D*). Conversion of the fluorescence substrate quinine to the nonfluorescent product xanthine was used in the assay of quanine aminohydrolase (*65D*). Glucose was determined by enzyme-induced chemiluminescence (*87D, 88D*) and malate dehydrogenase was measured by bioluminescence (*456D*). Detection of pyridine fluorescence was the basis of fluorometric determinations of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, α-hydroxybutyrate dehydrogenase (*511D*), creatine (*368D*), galactokinase (*313D*), and dehydrogenase activity in cells (*204D*). A fluorometric procedure for the determination of δ-aminolevulinate dehydratase activity in erythrocytes by measuring the production of uroporphyrin was described (*113D*).

Uric acid in serum was determined by a coupled reaction with uricase and peroxidase to form fluorescent 2,2′-dihydroxy-3,3′-dimethoxybiphenyl-5,5′-diacetic acid (*354D*). Fluorescamine was applied to the determination of dipeptidase (*483D*), *N*-acetylmuramic acid L-alanine amidase, and lysozyme activity (*428D*). Automated fluorescence procedures were published for glucose (*553D*), creatine kinase (*32D*), amylase (*408D*), and cholesterol (*199D*). Fluorescence assays were reported for reduced and oxidized glutathione (*242D*) acetate in tissue extracts (*239D*). Grove and Levy published a method for analysis of enzyme complexes on polyacrylamide gels (*230D*). A compound of biological origin possessed fluorescence properties quite similar to NADH and might be a possible interferent in microfluorometric studies (*392D*). A method was introduced for the measurement of NADH fluorescence in intact brain (*411D*). The influence of substrate composition on the binding of 8-anilinonaphthalenesulfonate to diphosphopyridine-linked isocitrate dehydrogenase was followed by fluorescence (*176D*). A fluorometric enzymatic method was developed for the analysis of total cholesterol in serum (*270D*). The use of semisolid surfaces in enzymatic analysis is an interesting technique being applied by Guilbault and coworkers (*319D, 512D, 513D*).

Nucleotides and Nucleic Acids. The text edited by Steiner presents a number of principles and techniques applicable to the analysis of luminescence properties of nucleotides and nucleic acids (*107A*). A microspectrophotometer was constructed for the analysis of oligonucleotides in column eluents (*225D*) and nucleotides in single cells (*341D*). Fluorescence of flavins (*686D*) and flavin complexes (*590D*) were reviewed. Triplet-triplet absorption processes (*52D*) and quenching of polyadenylic acid phosphorescence by manganese ions (*606D*) were discussed for polyadenylic acid. The fluorescence of purine and pyrimidine bases of the nucleic acid was studied at room temperature (*664D, 665D*) and lower temperatures (*161D, 493D, 656D*). The external heavy atom effect was reported for pyrimidines in frozen aqueous solutions (*1D*). The effect of hydrogen bonding between purine–pyrimidine base pairs on fluorescence was determined (*664D*). Luminescence properties were published for 4-thiouridine (*563D*) and 4-amino-2-methoxypyrimidine (*605D*). Inorganic probes were discussed in relationship to their utility for the phosphorimetric determination of nucleosides (*90D*). Wilson published a thesis on the polarized fluorescence of dinucleotides (*679D*). Fluorescent derivatives were prepared for substituted adenosine 5′-monophosphates (*370D*), a cyclic AMP analog (*552D*), cytosine-containing compounds (*551D*), and adenine (*475D*). A new cytotoxic fluorescent nucleoside 1,N^6-etheno-2-azaadenosine was synthesized (*630D*). Fluorescence and phosphorescence data were presented for thiopurines (*360D*) and oligocytidylic and polycytidylic acids (*654D*). Fluorescence lifetimes and quantum yields were measured on chloroacetaldehyde-modified dinucleoside phosphates (*626D*). Luminescence processes were investigated for polyadenylic acid (*52D, 606D*) and intramolecular complexes of flavine adenine dinucleotide (*590D*).

Corrected luminescence spectra for DNA and DNA bases (*143D*) and prompt and delayed fluorescence of DNA adsorbates (*476D*) were recorded. Phosphorescence of brewers' yeast $tRNA^{phe}$ was studied by optical detection of magnetic resonance (*264D*). The effect of silver ion on DNA (*504D*) and silver and mercury ions on the acranil–DNA complex luminescence properties (*576D*) was presented. The fluorescence of Tb(III) and Eu(III) was enhanced on addition to *E. coli* tRNA (*312D*). Quinacrine–DNA (*85D, 367D*), quinacrine mustard (*559D*), and DNA–ethidium bromide (*207D, 266D*) complex fluorescence was investigated. Imakubo reviewed studies of the fluorescence of DNA–dye complexes (*275D*).

Fluorometric analyses were reported for adenine (*220D, 697D*), adenosine triphosphate (*575D*), 2′,3′-cyclic adenosine monophosphate 3′-phosphohydrolase (*384D*), and mixture of riboflavin 5′-phosphate and flavin adenine dinucleotide (*670D*). DNA and RNA were determined fluorometrically by use of ethidium bromide complexes (*57D, 77D, 168D*) and phosphorometrically by complexation with silver ions (*568D*). The binding of mithramycin to double-stranded DNA was the basis of a rapid fluorescence assay for DNA (*259D*). The binding of ethidium bromide to DNA was studied by fluorescence yield changes as a function of temperature (*435D*).

Fluorescence analyses of chromosomes (*434D*) and 3-dimensional structures of ribosomes (*106D*) were reviewed. Berberine sulfate (*315D, 437D*) and 5-bromodeoxyuridine (*74D, 164D*) were used as fluorophores in chromosome analysis. Fluorescence spectra were reported for polynucleotide peptide complexes isolated from algae (*499D*), and a fluorescence method was developed for isolating mutants of *C. elegans* (*39D*).

Fluorescent Probes. Fluorescent compounds bound to proteins and other polymers are influenced by their microenvironments and provide convenient probes to study conformational changes in macromolecules (*123D, 298D, 441D, 490D*). Conti has reviewed the use of fluorescent probes in nerve membranes (*132D*). Azzi described the use of 1-anilinonaphthalene-8-sulfonic acid in the study of mitochondrial membranes in his survey of fluorescent probes (*38D*). A discussion of the application of fluorescence techniques to the study of model membranes was presented by Yguerabide (*692D*). Phosphorescence has been used to characterize polymers containing ketone and naphthalene groups by measuring changes in emission as a function of temperature over the range 77–300 K (*585D*). Tachiya presented a description of the kinetics of the quenching of luminescent probes in micelles using a generating function (*607D*). The general purpose fluorescent probe 1-anilino-8-naphthalenesulfonate has been used widely (*64D, 175D, 221D, 613D, 646D*). Singly and doubly labeled dithymidine phosphates were prepared in order to investigate intercalation, solvation, and energy transfer in nucleic acids (*629D*). Conformational and Mg^{+2}-binding properties of isoleucine specific tRNA (*E. coli*) were studied with a fluorescent naphthoxyl group coupled to the free amino group of isoleucine (*528D*). Three different patterns of fluorescence were observed in acridine–DNA complexes (*675D*). 1,3-Dialkylindoles were prepared and characterized and 11-(3-hexyl-1-indolyl)undecyltrimethylammonium bromide was incorporated into micelles of cationic surfactants (*538D*). Dimethylaminonaphthalene-5-sulfonamide dissolved in micelles was used to study microviscosities, transition temperatures, and degrees of order in the hydrocarbon–water interface (*572D*). The critical micelle concentration of decaethylene glycol nonylphenyl ether in water was determined by measuring the fluorescence intensity of 4-methyl-7-anilinocoumarin (*3D*).

The use of fluorescent probes for studying membranes was reviewed by Dobretsov (*160D*). Quasiline structure was found for pyrene monomer imbedded in a membrane (*647D*). The nature of the information derived from measurements of the fluorescence properties of chromophores externally introduced into membranes was discussed (*501D*). Dansyl chloride (*94D, 323D*) and 1-anilino-8-naphthalenesulfate (*423D*) were used for fluorescent labeling of membranes. The effects of metal ions on membranes were investigated (*37D, 235D*). A rare earth ion was used to probe metal ion–nerve interactions (*35D*) and cation complexation (*136D*). Erythrocytes (*484D, 578D*) and lympho-

cytes (*394D, 486D, 560D*) were tagged with fluorescent probes to study membrane properties. Dyes were used to stain (*20D*) and conjugate (*157D, 276D*) membranes of malignant and transformed cells. Procedures were described to affinity label human hemoglobin (*660D*) and to label fluorochromes with radioactive iodine (*473D*).

Polymers. Optical (*594D*) and luminescence (*194D, 336D*) characteristics are reviewed in relationship to the properties of polymers. Stretched film was used to orient polymers with localized symmetry for fluorescence and phosphorescence polarization measurements (*148D*). Tagging of a polymer with a fluorescent molecule was accomplished by a selective staining technique (*674D*) and by insertion of a fluorophore into the polymer chain (*640D*). Excimer fluorescence was found to be sensitive to conformational changes in polymers containing aromatic monomers (*144D, 145D, 668D*). Allen has published several works dealing with excited-state properties of polymers in photooxidative processes (*12D–16D*) and with their luminescence characteristics (*17D*). Intramolecular mobility of polymers was studied by polarized luminescence (*29D, 30D, 99D, 351D, 425D*) and conformational analysis presented for a benzophenone-based polymer (*680D*). Phosphorescence was used to study the stabilizing effect of metal chelates (*250D*), the degradation products of polystyrene (*335D*), and energy transfer in doped styrene and polystyrene (*657D*). Polarized fluorescence measurements using a polyacrylate bead were introduced which may be useful as a binder for fluorescent dyes (*691D*). Intrinsic fluorescence of functional groups was used in polymer characterization (*604D*). In the case of primary amine containing polymers fluorescamine was used toward the same end (*537D*). Relaxation processes in polymer solutions of anthrylmethylmethacrylate–4-vinylpyridine were measured by fluorescence quenching (*328D*).

Dyes. Dyes are referred to elsewhere as labels and stains, and as involved in associative types of interactions from both basic and analytical viewpoints. In this section we cite some of the works relating to fundamental properties of dye molecules which might be of importance in analytical studies.

Solvent effects on the fluorescence of 1-(dimethyamino)-5-naphthalenesulfonic acid (*375D*) and pyronine-G (*443D*) were discussed and the concept of inversion of excited states supported (*375D*). An excitation wavelength-dependent phosphorescence in dyes (*41D, 347D*) and the influence of molecular parameters on laser properties were reported (*517D*). Heavy atom substitution was shown to have a position dependence in effecting intersystem crossing (*347D*). This also influenced the rate of photoconversion of geometrical isomers of cyanine dyes proportional to the magnitude of the spin-orbit coupling factor (*133D*). Quasiline emission spectra were observed for polymethine dyes (*416D*). Rhodamine 6G was an efficient photosensitizer for photoreactions of *N*-vinylcarbazole (*139D*). Eosin complexes with amines were found to have a decreased fluorescence intensity and a change in the spectral distribution from that found with eosin (*125D*). Associative phenomena are common in dye solutions (*84D, 214D*). The polarization properties of rhodamine B were unexplainable using a simple exciton theory (*293D*). The absorption and luminescence properties of monomeric and dimeric species of rhodamine B and sulforhodamine B were reported (*114D*). Luminescence spectra were reported for rhodamine 6ZH (*345D, 353D, 505D*) and rhodamine B (*81D*) and rhodamine 6G (*358D*) in aqueous and alcoholic solvents, and for rhodamine 6G in the vapor phase (*523D*). The excited-state properties of phthalocyanines were studied in single crystals (*304D, 305D*), thin films (*696D*), and solutions (*346D, 417D*). Deuteration effects on the phosphorescence spectra of xanthene dyes (*470D*) and exciton emission in pyronine B and fuchsine were presented (*471D*). Polarization measurements were made on the dication form of acridine dye (*661D*). Procion yellow M-4RS was covalently linked to subcellular organelles of rat brain and used as a viscosity-dependent fluorescent marker (*187D*). The effect of sodium dodecyl sulfate concentration on the absorption and luminescence of acridine orange and other organic dyes was reported (*406D*). A detailed study of the properties of calcein blue indicates that, although it can be used as an indicator, it is too unstable for use as a reagent for the direct fluorometric determination of calcium (*272D*). Nickel and zinc dithiocarbamates were found to increase the fluorescence intensity of several dyes by a factor of 2–3 (*414D*). Forbes and Urbach investigated the effects of fluorescent whitening agents on photocarcinogenesis (*189D–191D*).

Immunofluorescence. A review of the physicochemical and immunological properties of fluorescent labeled antibodies is given by Poetschke (*491D*). An outline of the preparation and properties of reagents employed in diagnostic immunochemistry and a description of their application were presented (*66D*). Parker et al. reviewed the quantitation of antibody–antigen reactions with fluorescence polarization (*477D*). A new broad band interference filter was introduced for immunofluorescence applications (*372D*). Sephadex G-25 Superfine beads were stained with fluorescein isothiocyanate and tetramethyl rhodamine isothiocyanate in order to obtain standards for immunofluorescence measurements (*240D*). Circularly polarized fluorescence was used to study conformational changes in antibodies and antibody fragments (*532D*) and dansyl–anti (dansyl) antibody complexes (*530D*). Two approaches were presented for the removal of unwanted stains (*26D, 290D*).

Only a few of the many applications of immunofluorescence are presented in this section. The immunofluorescent immunoperoxidase and hemadsorption cell-counting techniques were quantitatively evaluated for assessing parallel dose-response curves and were found comparable in sensitivity, precision, and reproducibility (*243D*). Peroxidase labeled antiserums in IgA, IgG, and IgM were used to study the distribution of immunoglobulins and immunoglobulin containing cells in duodenum (*100D*). Hemoglobulins A, S, and F were identified in individual erythrocytes by specific, isolated fluorescent-labeled antibodies (*254D*). Material from liver biopsy was surveyed for hepatitis-B antigen with an immunofluorescent method (*296D*). Vitamin D dependent Ca-binding protein was located using a fluorescein isothiocyanate conjugated antibody (*380D*). Polypeptide hormones in tissues were reported to react maximally with fluorescein-labeled antiserum when the tissues were freeze dried and then fixed by a diethyl pyrocarbonate vapor (*481D*). Formalin fixation sharpened the immunofluorescent images of neurophysin in the hypothalamus (*174D*).

Agriculture. Chen has presented a thesis on the use of phosphorimetric methods for pesticide analysis (*118D*). An apparatus for the measurement of ultraweak biochemiluminescence of biological materials was checked in studies of green and etiolated plants and its application to the agricultural and food industry discussed (*582D*).

As little as 100 pg of aflatoxin B_1 was detected using a combination of TLC plates and time-resolved, wavelength-resolved laser-induced fluorescence (*62D*). Spectral effects were studied for aflatoxins B_1, B_2, G_1, and G_2 in solvents of varying polarity (*117D*). Absorption and fluorescence were applied to the analysis of aflatoxins B_1 and G_1 (*616D*). The distribution of aflatoxin in contaminated corn was measured using a simple fluorescence method (*89D, 554D, 573D*). Florisil minicolumns were used to separate aflatoxins for fluorescence analysis (*652D*). The quantitative determination of aflatoxins in nutmeg was accomplished with a fluorodensitometric approach (*58D*). The reaction of aflatoxin with trifluoroacetic acid was used in an aflatoxin assay (*498D*).

Dansyl derivatives were used for the determination of hydroxybiphenyls, carbamates, and herbicides (*197D, 371D, 389D*). Dimethoate and malathion in in situ samples of lettuce were determined by TLC and fluorometry. Naturally fluorescent pesticides were detected on silica gel plates before and after heat treatment (*393D*). A fluorometric assay for sevin content in water was developed (*432D*). 4-Chloro-7-nitrobenzo-2,1,3-oxadiazole was used as a coupling agent for the determination of dithio- and thiolcarbamates (*643D*). Coumaphos and coroxon in eggs were detected by in situ fluorometry (*701D*). The fungicide 5-fluorocytosine was determined in serum by a rapid, quantitative fluorometric method (*508D*). Monosodium glutamate was determined in foods using fluorescamine as a label (*134D*).

Chemiluminescence was observed from fungi (*689D*) following treatment with alkaline H_2O_2. Identification of sco-

pleten in cotton plants and its possible importance in byssinosis were discussed (*666D*). The proteolytic activities of α-chymotrypsin, trypsin, pepsin, and bromelain were determined by their ability to effect the release of a fluorescent agent bound to internal hydrophobic sites in intact protein substrates (*589D*). A rhythmic change in the rate of excretion of fluorescent components from cells of oat and soybean roots was observed (*564D*). The fluorometric assay of riboflavin in foods was shown to be influenced by the presence of reducing sugars (*457D*). Fluorescent phenolic compounds were isolated from cranberries and identified (*126D*). DeSaldarriage studied the luminescence of flavonoids isolated from sweet orange oil (*152D*). The refining of cane sugar was controlled using a corrected fluorescence measurement approach (*111D*). A spectrophotometric method of analysis for 2,4-dihydroxy-1,4(2*H*)-benzoxazin-3-one was found to be superior to a spectrophotofluorometric determination (*550D*). An automated fluorometric assay of thiabendazole was capable of processing sixty specimens per hour (*468D*).

Pharmaceuticals and Steroids. The formation of fluorescent chelates for the determination of tetracyclines, rifomycins, salicylates, ioniozids, and carbohydrates was reviewed (*374D*). High-speed liquid chromatography was used with fluorescence derivative formation for assaying trace amounts of alkaloids and adrenaline (*442D*). A malonic acid–acetic anhydride reagent formed stable fluorophors with alkaloids containing a tertiary amine group and permitted quantitation of cocaine and other samples (*620D*). A sensitive assay was described for the alkaloid ergotamine (*263D*). Fluorescence and phosphorescence spectra were reported for the hallucinogens harmine, harmaline, harmabol, harmane, and norharman (*241D*). A specific fluorimetric assay has been reported for digoxin (*96D*) and a simplified procedure for determination of digitalis alkaloids introduced (*446D*). GLC and fluorimetric assays for procainamide were compared (*198D*). Ultraviolet absorption, fluorescence, and phosphorescence excitation and emission spectra of oxybarbiturates were examined (*216D*). A further simplification in the identification of oxybarbiturates was accomplished by measuring the temperature dependence of fluorescence (*320D, 322D*). King has observed that the previously reported phosphorescence in amylobarbitone was probably due to a trace impurity (*321D*). Dansylated derivatives of barbiturates were separated by thin-layer chromatography and quantitated by in situ fluorometry (*162D*). A fluorimetric procedure for methaqualone was devised based upon reduction with lithium borohydride to yield the fluorescent product dihydromethaqualone (*543D*).

Phenothiazines and related drugs have found widespread usage in psychotherapy and the need for their control continues to rise. Thirteen phenothiazine derivatives were examined phosphorimetrically (*215D*). An automated fluorescence procedure was described for perphenazine in tablets by conversion to a fluorophore with permanganate oxidation (*344D*). A simplified fluorometric procedure was utilized for the determination of butaperazine in plasma (*395D*). A spectrophotometric method for the determination of piperazine was reported (*45D*). Amphetamines containing a primary amine grouping were determined by formation of fluorophores with fluorescamine (*153D*).

A modification has been presented for the assay of the diuretic furosemide in serum or urine (*192D*). Fluorescamine formed a fluorophore with procainamide and served as the basis for a sensitive determination of this local anesthetic (*611D*). The anesthetic dibucaine was investigated to determine the optimal pH conditions for fluorometric analysis (*403D*). Kusnir and Barna determined ampicillin, kanomycin, neomycin, polymyxin B, and streptomycin in concentrations of picomoles per milliliter (*357D*). Sulfonamides were determined by thin-layer chromatography followed by fluorescence-quenching densitometry (*212D*). Absorption and fluorescence spectra were reported for rivanol (*447D*) and phenyl salicylate (*520D*). Arylamines were quantitated by coupling with *N*-(1-naphthyl)ethylenediamine (*599D*). The mechanism for the sodium nitrate–dulcin reaction was investigated (*635D*). 4-Chloro-7-nitrobenzo-2,1,3-oxadiazole formed fluorophors with amphetamines (*649D*) and propoxyphene (*638D*) suitable for the analysis of therapeutic levels. The luminescence characteristics and the effect of halide ions on the fluorescence and phosphorescence were reported for antihistamines (*681D*). Metal ion and barbiturate additive effects on tetracycline fluorescence (*337D*) and the fluorescence of cannabinoids (*158D*) were discussed. A rapid fluorometric method has been reported for the determination of propranolol in serum (*22D*). In a comparison between colorimetric and fluorometric methods for thiamine analysis, Park recommends a fluorometric procedure for lower concentrations and a colorimetric procedure for higher concentrations (*474D*). An automated assay was described for vitamin A in pharmaceutical preparations (*289D*). Foster and Dunn evaluated a radioimmunoassay procedure for cortisol and compared their results with those of an accepted fluorometric method (*193D*). The validity of automated procedures for urinary estrogens was discussed (*273D, 280D*). Lithium hydroxide chromogens of Δ^4-3-keto steroids were measured by using a rotating pellet holder constructed for a commercial spectrofluorimeter (*306D*).

Chemiluminescence and Related Processes. The involvement of excited states in chemi- and electroluminescence was presented by Hercules (*257D*). Glover reviewed the role of chemiluminescence in gas analysis and flame emission spectrometry (*217D*). Several reviews dealt with chemiluminescence and its applications (*91D, 412D, 708D*). Cormier, Wampler, and Hori discussed the chemical requirements for light emission and the mechanism of the light reaction in bioluminescence systems (*135D*). Mechanisms and applications of bioluminescence were reviewed for enzymic and nonenzymic systems (*694D*). Ding has reviewed the role of dioxetane in biochemiluminescence (*156D*). Electrochemiluminescence (*352D*) and its interrelationship with voltammetry were discussed (*525D*).

The reaction of aluminum atoms with molecular ozone in the gas phase produced chemiluminescence (*704D*). A mixture of brominated biphenyls was identified as the main emitting species in the chemiluminescent reaction of several aryl Grignard reagents with molecular oxygen (*83D*). A vibrational dependence of the fluorescence quantum efficiency in the chemiluminescence of electron-transfer reactions was reported (*115D*). γ irradiation of aromatics of hydrocarbon glasses produced a luminescence depending on the recombination of cation–electron and cation–anion pairs (*151D*). Degassing of methyl cyanide solvent used in chemiluminescence reactions resulted in an increase in luminescence intensity by an order of magnitude (*634D*). Chemiluminescence due to formaldehyde in flames and the reaction of atomic hydrogen with tellurium tetrabromide were reported (*398D, 566D*). Electroluminescence in aqueous solutions was demonstrated for a number of systems (*154D, 159D, 373D, 625D*). The variation of rubrene redox potentials in various solvents may be correlated with the quantum efficiency of electrogenerated chemiluminescence (*485D*). Electron-transfer reactions for palladium and platinum porphyrins were investigated using electrogenerated chemiluminescence (*625D*). Chemiluminescence accompanied the reaction of arenesulfonylquinonimines with amines (*624D*). A violet chemiluminescence was produced when succinylfluorescein reacted with oxygen in aprotic solvents (*299D*).

Isacsson and Wettermark reviewed the analytical applications of chemiluminescence (*278D*). The use of a liquid scintillation counter in analytical applications is discussed (*294D*). An instrument for chemiluminescence analysis was tested on the determination of $Fe(CN)_6^{-3}$ by using luminol (*297D*). Seitz and Hercules described the application of trace metal analysis by chemiluminescence (*555D*). Seitz and Neary surveyed the application of chemiluminescence in analysis and the methods used for bioluminescence detection (*556D*). Chemiluminescence was applied to the analysis of gases (*219D, 598D*).

The luminol reaction forms the basis of detection for numerous compounds via chemiluminescent analysis. Seitz and Hercules investigated the properties of the iodine-luminol reaction (*557D*). The chemiluminescence of luminol was used in the detection of 2-substituted ketones (*698D*) and blood glucose (*87D*). Calcium release from aequorin induced by various agents formed the basis for a study of tension development in muscle fibers (*170D*). The mechanism

of action of hemin proteins on the chemiluminescence of luminols was investigated and the basic kinetic equations describing the reaction developed (*422D*). Water-soluble proteins were determined by a chemiluminescence assay and reagent concentration and pH studied (*488D*, *489D*). Ultraviolet-induced chemiluminescence in proteins was discussed (*700D*).

The rise in bioluminescence has been used to assay for ATP (*388D*). Mutant luciferases exhibited red shifts in their luminescence spectra of as much as 12 to 15 nm (*130D*). Analytical applications of bacterial luminescence were reviewed by Gerlo (*210D*). A study was conducted of the reaction of DNA with *N*-acetylethylenimine and it was suggested that chemiluminescence resulted from oxidation of the glycosidic carbon atom (*365D*). Silinsky designed a photometric reaction chamber to investigate the properties of the bioluminescence assay for ATP (*574D*). Bioluminescence was used as an index of plant viability (*439D*). Several publications dealt with bioluminescence in specific systems such as the *Cypridina* luciferin analogs (*222D*), *Chlorella* (*369D*), *Actinomyces lucensis* (*492D*), and *Renilla reniformis* (*669D*).

INORGANIC

Photophysical properties of inorganic complexes with emphasis on features of importance to the photochemist have been reviewed (*39E*). The nature of luminescence centers in alkali halide phosphors (*56E*) was reviewed. The luminal reaction formed the basis for a sensitive detector of metal ions in liquid chromatographic effluents (*47E*, *81E*). Chemiluminescence from the resultant luminol–H_2O_2 reaction catalyzed by transition metal ions was observed when ions were introduced at the parts per billion level. An extensive review of luminescence analysis of metals and nonmetals was presented (*101E*). Townsend described methods for the fluorometric analysis of nonmetals and rare earth mixtures (*116E*). The relationship between chemical studies and fluorescence characteristics was studied for hydrazone derivatives and their complexes (*110E*). Dyatlova and Temkina discussed methods of modifying the structure and stability of complexonates and the resultant effect on their luminescent properties (*32E*). Byler reviewed the fluorescence and phosphorescence properties of inorganic pigments (*17E*).

Aluminum and Beryllium. Morisige has presented an extensive compilation of the fluorescence characteristics of aromatic Schiff base complexes for aluminum, gallium (*78E*), beryllium, and scandium (*79E*) analysis. The optimum conditions for fluorimetric analysis were discussed. Eriochrome red B was used as a fluorochrome to detect beryllium, magnesium, aluminum, indium, gallium, and zinc (*23E*). Phosphorescence spectra at 77 K were recorded for aluminum, gallium, indium, and magnesium acetylacetonates, trifluoroacetylacetonates, and hexafluoroacetylacetonates (*22E*). The reaction of aluminum and oxygen was studied by the method of laser-induced fluorescence (*24E*). 3-Amino-5-sulfosalicylic acid formed intensely fluorescing complexes with aluminum and beryllium and this reaction was used to determine aluminum with a limit of detection of 5 ng/ml of Al (*1E*). Preconcentration of aluminum was achieved by precipitation with oxine and fluorometric analysis performed on the resulting solid complex (*95E*). The presence of volatile beryllium compounds in orchard leaves was detected by the morin fluorometric method (*38E*). Dinaphthoylmethane [1,3-di(2-naphthyl)-1,3-propanedione] was used as a reagent to both coprecipitate and form a luminescent product with beryllium for in situ fluorometric detection of the precipitate (*96E*).

Alkali and Alkaline Earth Metals. Lieu and Handy devised an in situ fluorimetric procedure for the analysis of magnesium, calcium, and strontium (*70E*). Circular dichroism and fluorescence spectroscopy were applied to determine the stoichiometry and formation constants of the carboxylic antibiotic X-537A with alkaline and alkaline earth metals (*27E*). Fluorescence and fluorescence lifetimes were measured for a series of alkaline earth monohalides (*25E*). Molecular fluorescence was reported for calcium and strontium hydroxide and barium chloride using a continuum excitation source (*55E*). Calcium release in muscle fibers was followed by aequorin luminescence (*170D*). Chlortetracyline was used as a fluorescence reagent for calcium because of its stability in alkaline solutions (*64E*) and isocein was introduced as a fluorogenic reagent in calcium determinations (*54E*). Fluorescence quenching was observed when magnesium phthalocyanine complexes formed donor–acceptor complexes with O_2 and related molecules (*41E*). Citrate interference in two fluorometric methods for the determination of magnesium in plasma (*18E*) and a fluorometric method for the determination of magnesium in renal tubular fluid (*11E*) were reported. Fluorescence from molecular sodium was induced by laser excitation (*29E*, *37E*). Absorption and fluorescence spectra were presented for rubidium chloride crystals doped with Pb^{2+} (*89E*). Absorption and phosphorescence spectra of pyrazine complexes with lithium, sodium, potassium, and zinc ions were discussed (*75E*).

Bismuth, Cadmium, Lead, and Mercury. Luminescence was used to study the energy levels of bismuth and lead in polycrystalline host lattices (*7E*). Phosphorescence and photochromism were observed for salicylalaniline chelates of Cd, Hg, Be, and Zn (*62E*). Fluorescence spectra due to free excitons were studied in CdS at 4.2 K (*84E*). Cadmium was separated from interfering metal ions by anion exchange and determined fluorometrically after complexation with calcein (*49E*). Concentrations as low as 2×10^{-8} M of Cd^{+2} may be detected by this procedure. Lead in concentrations of 5–2000 ng/ml was determined by coprecipitation with calcium oxalate, ignition to CaO·Pb phosphor, and measurement of its luminescence (*97E*). Since $(PbCl_4)^{-2}$ ion fluoresces more intensely on cellulose, a fluorescent ring-oven technique was developed based on the selective solubility of lead in ammonium acetate (*107E*). This approach provided a simple, reasonably rapid procedure adaptable for air pollution studies.

Gallium, Indium, and Thallium. Al, Ga, In, Sc, and Mg may be determined by their fluorescence when complexed with an azoaryl ligand. *o,o'*-Dihydroxyazobenzene, *o,p,o'*-trihydroxybenzene, and related compounds were used for the fluorometric determination of Al and Ga and optimum conditions were reported for the determination of Al and Ga with 2-(2,4-dihydroxyphenylazo)-1-hydroxybenzene and 3-(2-hydroxy-4-methoxyphenylazo)-4-hydroxybenzenesulfonic acids (*50E*). The determination of Ga in Al was accomplished by extraction of a Ga complex with 8-quinolinethiol into an organic ketone and measuring the resultant fluorescence at 505 nm. The complex is stable for 2 h and microgram quantities of Ga may be determined (*121E*). The luminescence of thallium salts was studied in solution (*76E*) and as an impurity introduced into a crystalline lattice (*85E*, *90E*, *91E*).

Cobalt, Chromium, Manganese, and Platinum. DeArmond has reviewed the electronic properties of Cr(III), Ir(III), Rh(III), and Ru(II) complexes and their relationship to nonradiative transitions (*26E*). An anomalous Stokes shift was observed in the phosphorescence spectra of *cis*-Na[Cr(iminodiacetate)$_2$] and of *trans*-Na[Cr(methyliminodiacetate)$_2$] (*102E*). The magnetically induced, circular emission spectrum of Cr(III) in hexagonal guanidinium aluminum sulfate hexahydrate was measured (*77E*). A program has been set up to adapt the centrifugal fast analyzer to trace inorganic analysis and determinations have included sulfate, phosphate, zinc, and selenium in natural waters. As a part of this program the determination of chromium in natural waters was developed based on the catalysis of the oxidation of luminol by hydrogen peroxide (*9E*). Fe(II) and Co(II) were found to quench the phosphorescence of the tris(ethylenediamine)chromium(III) complex (*120E*). Luminescence spectroscopic properties were reported for the hexacyano chromium(III) (*19E*, *99E*) and hexaaminechromium(III) hexacyanocobaltate(III) (*60E*). 10-Methylacridinium chloride fluorescence was quenched by a series of Cr(III) complexes (*21E*). Phosphorescence was observed for bis(2,6-pyridinedicarboxylato) (*51E*), 1,10-phenanthroline and 2,2'-bipyridine (*59E*), and acidoamine (*67E*) and oxygen coordinated (*86E*) chromium(III) complexes. Radiative and radiationless processes were studied for Cr(III) doped in magnesium oxide (*20E*) and ytrrium aluminate (*124E*).

Tris(2,2'-bipyridine)ruthenium(II) phosphorescence was quenched by Co(III) and Ru(III) complexes (*80E*), Cr(III)

complexes (*6E*), and Fe(III), Fe(II), and Tl(III) ions (*69E*) in solution. Multiple emissions were observed from heterochelated complexes of iridium(III) (*123E*). Flynn and Demas described the synthesis and luminescence of the tris(2,2′-bipyridine)iridium(III) ion (*40E*). Fluorescence spectra were determined at 77 K for Cd, Cu, and Pd complexes of dihydroporphyrins (*36E*). A phosphorescence decay study was reported for bis and tris complexes of Rh(III) with 2,2′-dipyridyl-1,10-phenanthroline (*45E*). Schenk reviewed the fluorescence characteristics of manganese containing halites and calcites (*100E*).

Lanthanides (Rare Earths) and Scandium. The influence of solvent on the rate of energy transfer between a ketone and lanthanide was studied (*35E*). Piperidinium tetrakis(rare earth 1,3 diketonates) were synthesized for La, Pr, Nd, and Eu and their fluorescence and infrared spectra reported (*57E*). The temperature dependence of electron transfer for the excited Eu(II) species in acidic solution was studied (*15E*). Room temperature absorption and luminescence of Eu^{+3} were investigated in crystals of $Ba_3(VO_4)_2$ and $Cd_3(VO_4)_2$ (*63E*). Doping of rare earth salts with europium was used to study site symmetry and to aid in identification of energy levels (*71E*). The dependence of fluorescence quantum yield on excitation wavelength was studied for solutions of rare earths and particularly for europium (*108E*). Stability constants for the Eu(III) complex with *o*-benzoyl benzoate were calculated from fluorescence intensity measurements (*112E*). Dysprosium, holmium and neodynium were found to quench europium complexes of dibenzoylmethane (*114E*). A new analytical approach was used for determining Eu in lanthanides. This method coprecipitates Eu into a lanthanum host lattice and excites Eu by energy transfer (*73E*).

Unsaturated iron-binding capacity was determined based on the enhancement of Tb fluorescence upon binding to empty transferrin binding sites (*30E*). Absorption and fluorescence spectra were measured for Tb in $Tb_3Al_5O_{12}$ (*58E*). Chemiluminescence was observed from Dy and Tb solutions in sulfuric acid (*13E*). The circularly polarized luminescence of Tb complexes was found to be quite sensitive to ligand environment (*72E*). A mixed complex of Tb with acetylacetone and EDTA was used to determine Tb in lanthanum oxide with a sensitivity of 2×10^{-4} % (*113E*). A procedure for the determination of 0.1–10 ng of Tb_4O_7 and Dy_2O_3 in 10 ml using complexes with EDTA and 1,2-dihydroxybenzene-3,5-disulfonic acid was described (*114E, 115E*). Excitation and emission spectra were recorded for Pr, Nd, Sm, Eu, Tb, Dy, Ho, Er, Tm, and Yb in LaOCl (*33E*), Ce and Pr in LaF_3 (*34E*), Gd in CaO (*53E*), Nd in yttrium aluminum garnet (*106E*), and Pr in $LaAlO_3$ (*28E*). Anisic acid β-hydroxynaphtholhydrazide forms a fluorescing complex with Sc in acetone–water solvents which may be used to determine Sc concentrations greater than 3×10^{-6} % (*31E*).

Other Metals. Hafnium complexes with morin in 1 N sulfuric acid and upon addition of methyl alcohol it is possible to detect 25 ng of Hf in 5 ml of solution (*93E*). In $HClO_4$, the Hf complex of quercetin fluoresces without interference from Zn and forms the basis for the fluorometric determination of Hf with a limit of detection of 10 ng/ml (*65E*). 8-Quinolinol-5-sulfonic acid was used to mask Cu, Ni, Al, Fe, and U interferences and as an indicator in the zinc–ferrocyanide titration (*4E*).

Fluorescence spectra were recorded for copper(I) complexes with pyridinecarboxylic acids (*42E*) and hexamethylenetetramine (*46E*). 2-Hydroxy-1-naphthaldehyde forms a fluorescent complex with Sc and a nonfluorescent complex with Cu to form the basis for the determination of copper(I) by fluorescence titration (*111E*). Tungsten may be determined by formation of a fluorescent complex with morin with a sensitivity of 15 ng of W (*93E*). Ligand–ligand interactions were observed as changes in fluorescence in Zn complexes with 1,10-phenanthroline and bipyridyl (*83E*) and β-diketonato complexes of Be, Al, and Zn (*82E*). Lamola and co-workers developed an assay for zinc protoporphyrin in whole blood which could be useful as a screening method for chronic lead poisoning (*68E*). Photochromism and luminescence characteristics were reported in the zinc salicylalaniline chelate (*61E*). Triphenyltin content in potatoes was determined fluorometrically after complex formation with 3-hydroxyflavone. A cryostat for temperature-dependent studies was used for measuring fluorescence of solid ammonium uranyl triacetate (*109E*). Electrochemiluminescence was reported for uranyl ion in perchloric (*14E*) and sulfuric (*88E*) acids. Quenching of uranyl luminescence by aliphatic alcohols was found during pulse photolysis (*105E*). Spectra (*87E, 125E*) and lifetimes (*87E*) were presented for complexes of the uranyl ion. 3-Hydroxyflavone formed a fluorescent complex used in the determination of triphenyltin (*117E*).

Nonmetals. Fluorescence was observed from the NO_2 moiety upon laser excitation (*16E*). Schwarz and Okabe developed a technique for NO (*104E*) and SO_2 (*103E*) detection based on the measurement of fluorescence intensity after excitation with a Zn discharge lamp. Utilizing this approach the detector response was linear from 15 ppb to 7 ppm for NO and from 8.6 ppb to 1.8 ppm for SO_2. Luminescence characteristics were reported for SO_2 (*10E, 12E, 74E*) and a discussion of the triplet–singlet transition was presented (*119E*). Axelrod has reported a method capable of detecting subnanogram quantities of nitrite (*2E*). The laser-excited resonance fluorescence of I_2 was studied (*92E*). Solvent effects on the extraction efficiency and the fluorescence intensity were investigated for the determination of Te with rhodamine dyes (*43E*). 2,4,4-Trihydroxybenzophenone formed a highly fluorescent chelate with Te which permitted the determination of nanogram amounts of Te (*8E*). Fluorometric detection of parts per billion of Se in water (*94E*) and in plant materials (*44E*) with the reagent 2,3-diaminonaphthalene was reported. Dimethylaminobenzalizine is a fluorogenic reagent for hydrazine and formed the basis of the fluorescence measurement of hydrazine in plasma (*118E*). A fluorescence quenching analysis of sulfide took advantage of sulfide inhibition on the oxidation of thiamine to fluorescent thiochrome (*52E*). The analysis of hydrogen peroxide was determined with the reagent 2′,7′-dichlorofluorescein (*5E*). 8-Quinolinol-5-sulfonic acid was used as a masking agent for interferences as well as an indicator in the determination of phosphate (*3E*). A new fluorometric method for the determination of urinary phosphate used the quenching effect of phosphate on the fluorescence of the Mg chelate of 3-hydroxy-3′,4′-dimethoxyflavone (*48E*). The fluorescence quenching in aromatic hydrocarbons by inorganic ions is discussed (*122E*). Luminescence, potentiometric, and thermal properties were reported in a study of refractory phosphates (*98E*). Excitation and emission spectra were determined for a series of halides containing cyanide ion impurities (*66E*).

MISCELLANEOUS AND ADDENDA

This section includes materials which seemed inappropriate in other sections or were late additions.

Reviews on thermoluminescence (*52F*) and bioluminescence (*33F*) were reported. Spectral and luminescence properties were used to provide a classification of excited states (*57F*). Ortho effects are discussed for triarylphosphines (*51F*). Luminescence and circular dichroism spectra were measured for uranyl complexes (*8F*) and aromatics in cholesteric phases (*40F*). Fluorescence was observed from the second excited singlet state of thiophosgene (*43F*) and in methylglyoxal (*15F*). A time-dependent theory for polarized luminescence was proposed (*38F*) and polarized fluorescence spectra reported for *all-trans*-retinol and 1,8-diphenyl-1,3,5,7-octatetraene (*32F*). Fluorescence spectroscopy was used to study surfaces (*19F*) and surface films (*21F*). The thermodynamic validity of light efficiencies was discussed (*13F*). Measurements were made on the influence of deuterated solvents on quenching efficiencies (*4F*) and an anomalous heavy atom effect reported for cytidine (*1F*).

The important porphyrin moiety was the basis for many luminescence studies of which a few are mentioned in this review. Fluorescence spectra of hematoporphyrin (*59F*), tetraarylporphyrins (*49F*), etioporphyrins (*71F, 72F*), metal complexes of dihydroporphyrins (*18F*), zinc porphine (*9F*), and protoporphyrin IX dimethyl ester (*10F*) were studied. Quasiline spectra of porphyrins (*58F*) and metal complexes of etioporphyrin (*27F*) and porphines in organic crystals (*36F*) were reported. Energy transfer was

observed between coupled porphyrins (*54F*). Photooxidation of tetraphenylporphine and its derivatives was stimulated by amines and resulted in changes in their fluorescence spectra (*33F*). Phosphorescence spectra were measured for metal porphyrins (*23F–26F, 30F, 62F, 63F*). Dimerization of porphyrins in methylcyclohexane was followed by fluorescence (*73F*). Radiationless processes (*29F*), charge localization effects (*28F*), and polarized fluorescence (*41F, 74F*) were studied for metal porphyrins. A detailed study was recently reported on the use of erythrocyte protoporphyrin in the detection of iron deficiency (*41F*).

Laser action was reported for 4-methylumbelliferone (*6F*). Luminescence of hydrocarbons present in cigarette smoke was measured (*60F*). Bloodstains of various ages were distinguished by their luminescence (*64F*), and a combination of Raman and fluorescence scattering from plasma specimens was correlated to pathological disturbances (*39F*). A thesis was published by Young on the fluorescence of normal urine (*70F*). Fluorometric determinations were reported for cosmetic stabilizers (*56F*), uric acid (*67F*), catechol amines (*69F*) in plasma and urine, and GABA in cerebrospinal fluid (*20F*). Absorption, fluorescence, and phosphorescence of estrogens (*68F*), the fluorometric determinations of cortisol (*47F, 53F*), progesterone (*17F*), and estriol (*66F*), and a pellet holder for steroid analysis (*35F*) were studied. Luminescence quenching was used for the determination of traces of water in organic solvents (*65F*). Ion-exchange chromatography was used to increase the specificity of the fluorescence determination of folic acid (*48F*) and fluorescamine increased the sensitivity of the thin-layer procedure for primary amines (*50F*). Fluorometric determinations were reported for thiamine disulfate in blood and urine (*44F*), vitamin B_6 in blood (*16F*), and uronic acids (*31F*). Peroxide formation in fatty acids and phospholipids were followed (*45F*) and the amount of fat was determined (*37F*) fluorometrically. Laser excitation fluorimetry was capable of determining formaldehyde in air at concentrations of less than 1 ppm (*5F*). Oil spills were identified by fluorescence and confirmed by GLC (*14F*).

Luminescence spectra were recorded for bilirubin in hydrocarbon glasses (*7F*). Fluorescence titration data were used to determine binding constants for bilirubin–albumin complexes (*11F*). 8-Anilinonaphthalene 1-sulfonate was used as a fluorescent probe for drug–microsomal (*2F*) and steroid–liposome (*55F*) interactions. Cellular processes were investigated by using native fluorescence (*46F*) and fluorescence from a fluorogenic reagent (*61F*) or stain (*22F*). Fluorescence, phosphorescence, and phosphorescence lifetimes were measured for an allergen extracted from *Ascaris suum* (*3F*).

This review is largely a compilation of the literature of molecular fluorescence and phosphorescence spectroscopy indexed by chemical abstracts during the period from December 1973 to December 1975. Only minimal attempts have been made to assess the relative merits of the contributions. It is our hope that this effort will facilitate the location of pertinent materials in this extensive literature.

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Infrared Spectrometry[1]

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This review covers publications cited in Chemical Abstracts (CA), volumes 80–83 (1974–75), through the December 29, 1975 issue.

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