THE PHOTOSENSITIVITY OF DROSOPHILA'S CIRCADIAN TIME SENSE. A.T. Winfree, Department of Biological Sciences, Purdue University, W. Lafayette, Ind.

Like the overwhelming majority of studied plant and animal species, Drosophila's circadian time-sense or internal "clock" is acutely sensitive to visible light. In populations exposed to light - even if only a single flash - then left in continual darkness, adult flies emerge from their pupal cases in daily bursts 6 hours wide for 8 to 20 days, depending on temperature. Behavioral experiments with this circadian rhythm indicate that the individual fly (in larval, pupal, or adult stages) harbors an internal physiological oscillator, or "clock" with very nearly 24 hours period (1). This oscillator has an experimentally adjustable amplitude and phase, including a zero-amplitude state of indeterminate phase (2).

Against this periodic backdrop, one can quantify the oscillator's photosensitivity by examining its rephasing as a function of total energy in a brief exposure to light... with the following results:

a) The photoreceptor is probably not a carotenoid and is probably not involved in vision. Its absorption spectrum is unknown; but the action spectrum for resetting is rather undistinguished (1).

b) Photosensitivity near 450 nm increases ten to twenty-fold during the first 3 days of darkness after prolonged exposure to bright light. Subsequent exposure exceeding 10^7 $ergs/cm^2$ seems necessary to reverse this 'dark adaptation' (3). This might reflect bleaching of a pigment with a rather small extinction coefficient (see below), followed by slow resynthesis.

c) The rhythm's response to light (when dark-adapted) saturates below 1000 $ergs/cm^2$. At this energy no more than one pigment molecule in ten could have absorbed a photon, even assuming the greatest plausible extinction coefficient, $\sim 10^5$ liter/mole-cm. Thus it seems unlikely that light affects the circadian oscillation directly, by photolysis of a periodically accumulating substance.

d) There is little reason to suppose the photoreceptor itself is influenced by the circadian oscillation. Dark-adaptation after prolonged illumination follows roughly the same course with the clock rephased at standard amplitude or reset to zero amplitude.

On these grounds, photoreceptor kinetics and its effect on the circadian oscillator may be tentatively modelled as follows. P is photoreceptor concentration in arbitrary units and I is light intensity in $ergs/cm^2$ sec. Time, t, is measured in seconds.

$$\frac{dP}{dt} = 2 \cdot 10^{-6} (1 - P) - 10^{-7} IP$$

The rate constant 10^{-7} corresponds to a rather undistinguished molar absorption cross-section of $2\frac{1}{2} \cdot 10^6$ $cm^2/mole$, or an extinction coefficient of 10^3 liters/mole-cm., supposing 10% of the light reaches P through cuticle, etc. It could be greater, if less light penetrates to the photoreceptor or if many photons are absorbed between bleaching absorptions.

P photo-breakdown products are postulated to catalyze both synthesis and first order breakdown of a substance x, reacting in an oscillatory way with another substance y:

$$\frac{dx}{dt} = 7.3 \cdot 10^{-5}\ y + IP(1 - x)/300$$

$$\frac{dy}{dt} = -\ 7.3 \cdot 10^{-5}\ x$$

This model was tailored to fit the results of 1000 resetting experiments to within the precision of phase measurement. Three testable predictions emerge from this scheme:

a) Pupae reared in continual darkness with clocks not functioning (at equilibrium, at zero amplitude) should be inducible to normal rhythmicity by exposure to as little as 100 ergs/cm^2 of blue light, but not by much less than that. This was confirmed experimentally (3).

b) Such induced rhythms should exhibit constant high sensitivity to subsequent rephasing light-pulses (without dark adaptation, since P is unbleached). This has not been tested.

c) Continuous exposure to as little as $\frac{1}{100}$ ergs/cm^2 sec of blue light (much less than full moonlight) has been found to damp out circadian rhythmicity within a week, as expected from the above equations (ms. in preparation).

In conclusion: The available data on photo-induction, -rephasing, and -suppression of Drosophila's circadian time sense are compatible with a simple kinetic model which places the photoreceptor external to the clock process. Attempts to locate and identify the photoreceptor (perhaps in the insect brain) might be guided by difference spectrophotometry, and by these indications that the pigment's recovery after bleaching is uncommonly slow.

References

1. Skopik, S.D. and Pittendrigh, C.S. (1967) Proc. Nat. Acad. Sci. 58, 1862-1869.

Konopka, R.J. and Benzer, S. (1971) Proc. Nat. Acad. Sci. 68, 2112-2116.

Zimmerman, W.F. and Ives, D. (1971) in Biochronometry, ed. M. Menaker, National Academy of Sciences, Washington D.C., pp. 381-391.

2. Winfree, A.T. (1971) in Biochronometry, ed. M. Menaker, National Academy of Sciences, Washington D.C., pp. 81-106.

Winfree, A.T. (1973) J. Comp. Physiol. in press.

3. Winfree, A.T. (1972) J. Comp. Physiol. 77, 418-434.

Winfree, A.T. (1972) J. Theoret. Biol. 35, 159-189.

SPECTRAL SENSITIVITY OF CIRCADIAN RHYTHMS.

Ruth Halaban. Department of Biological Sciences, State University of New York at Albany, Albany, N.Y. 12222.

There are two major purposes for elucidating the action spectrum for biological clocks: 1. To identify the photoreceptor and to follow up the effect of its excited state on the endogenous circadian rhythm. 2. To show the relationship between circadian rhythms and photoperiodism and test the Bünning's hypothesis (3).

Action spectrum: Determinations of action spectrum were done on various organisms and on different aspects of the circadian rhythm such as free-running periodicity, phase shifting, initiation or inhibition of the rhythm. Unfortunately, in most cases action spectrum analysis was carried out on one aspect of the circadian rhythm for the organism under study with the assumption that it represents the full scope of the action spectrum response. However, in some cases where more than one particular aspect of the circadian rhythm was examined, this assumption proved to be wrong. Action spectrum of monochromatic light was carried out for the phase shifting of Gonyaulax (11), Chlorela-less Paramaecium (5), Drosophila (8) and for the damping of the oscillation of Neurospora (17). In other studies combination of broad-band filters and different light sources were used for action spectrum analysis. In most organisms blue (410-530 nm) and red (590-700 nm) had an effect on the rhythm. There is no correlation between the presence of the photosynthetic apparatus and the responsiveness to red light. In some studies blue and red light had different effect on various aspects of the circadian rhythm. For example: free-running periodicity was longer under continuous blue light and shorter under continuous red light (9); the pattern of phase shifting was also different. red affected advances (9) and blue affected delays (9). Phytochrome involvement in circadian rhythms was positively demonstrated in Phaseolus (4) and Lemna (12) while no involvement was observed in Coleus (9) Gonyaulax (11) and Paramaecium(5). Phase shifting of a circadian rhythm can be also elicited by U.V. irradiation (5,19). In conclusion: a. Taking into acount all the action spectra results, there is no indication for the presence of a particular pigment which is solely responsible for the absorbtion of light for the circadian rhythm. b. Some studies indicate that the different responses of the circadian rhythm can be elicited by illumination with different light qualities. c. Studies with U.V. light demonstrated the involvement of nucleic acids in the circadian clock.

Circadian rhythms and photoperiodism: The use of different light qualities for the specific control of circadian rhythm and photoperiodic response can help elucidate the intriguing question of whether a unified master clock controls both daily and annual activities (3). However, in very few experiments were both daily and annual rhythms studied in the same organism. Pectinophora diapause was induced by monochromatic light of 600 nm which did not entrained the overt circadian rhythm (16). It was therefore concluded (14)

that the two are under the control of different mechanisms. On the other hand results from experiments with birds in which also different light qualities were used tend to support the Bunning's hypothesis (15). It is possible that different organisms evolved different mechanisms for time measurement, however the use of monochromatic light could be extended to study this problem in more detail.

Light effects and clock mechanism: At first glance it seemed disappointing that action spectrum experiments did not reveal a lot of information about the mechanism of circadian oscillation. However, effects similar to those generated by light can be induced by other treatments such as temperature (1, 7, 21) and metabolic inhibitors (6). One can therefore infer that different light qualities affect the operation of the clock in a similar way, which is mainly through an effect on macromolecular synthesis.

Attempts were also made to follow up the subsequent reactions after excitation with monochromatic light. For example: There are evidences that phytochrome conversion affects changes in membrane permeability (ref. 18 for general review) and recently it was demonstrated to have enzymatic activity (20). Detailed speculation cannot be made as for the involvement of light in the operation of the clock since nothing as yet is known about the molecular nature of the circadian rhythm. Advances in this direction were made by the isolation of clock mutants (2, 9, 13). Hopefully, the future availability of mutants altered in their response to light will help illuminate the nature of the interaction between the two.

REFERENCES

1. Brinkman, K. 1970. In Biochronometry. M. Menaker, ed. p. 567
2. Bruce, V. G. 1972. Genetics, 70:537
3. Bunning, E. 1936. Ber. Deut. Botan. Ges., 54:590
4. Bunning, E. and I. Moser. 1966. Planta, 69:101
5. Ehret, C. F. 1960. Cold Spring Harbor Symp. Quant. Biol., 25:149
6. Feldman, J. F. 1967. Proc. Nat. Acad. Sci. U.S., 57:1080
7. Feldman, J. F. and S. B. Stevens. In Behaviour of Microorganisms, J. Adler, Ed. in press.
8. Frank, K. D. and W. F. Zimmerman. 1969. Science, 163:688
9. Halaban, R. 1969. Plant Physiol., 44:973
10. Halaban, R. and J. F. Feldman. 1972. Plant Physiol., abst., p. 63
11. Hastings, J. W. and B. M. Sweeney. 1969. J. Gen. Physiol., 43:697
12. Hillman, W. S. 1971. Plant Physiol., 48:770
13. Konopka, R. and S. Benzer. 1971. Proc. Nat. Acad. Sci. U.S., 68: 2112
14. Lee, A. D. 1971. In Biochronometry. M. Menaker, ed. p. 372
15. Menaker, M. and A. Eskin. 1967. Science, 157:1187
16. Pittendrigh, C. S., et.al., 1970. Proc. Nat. Acad. Sci. U.S., 66:758
17. Sargent, M. L. and W. R. Briggs. 1967. Plant Physiol., 42:1504
18. Shropshire, W. Jr. 1972. In Photophysiology, v. 4, A. S. Geise, ed. 34
19. Sweeney, B. M. 1963. Plant Physiol., 38:704
20. Tazuka, T. and Yamamoto, Y. 1972. Plant Physiol., 50:458
21. Zimmerman, W. F., et.al., 1968. J. Insect. Physiol., 14:669

CIRCADIAN RHYTHMS IN INSECTS: THEIR RELEVANCE TO PHOTOPERIODISM.

J. W. Truman, The Biological Laboratories, Harvard University, Cambridge, Massachusetts

A central problem in the study of photoperiodism is how organisms measure changes in day length. In insects models of the photoperiodic mechanism are based either on a circadian oscillator or on an "hour-glass."

The physiological basis of several circadian rhythms in the silkmoth, Antheraea pernyi, were studied and compared to the photoperiodic response in the same species. Transplantation and lesion experiments indicated that the eclosion and flight activity rhythms are controlled by "clocks" located in the cerebral lobe area of the brain. In both cases entrainment of the rhythm is mediated through photoreceptors in the moth brain and not through the eyes. The secretion of the prothoracicotropic hormone (PTTH) in larvae is also controlled by a circadian clock. As with the other two rhythms, the removal of the eyes does not significantly interfere with entrainment of the PTTH secretion rhythm. In the pupal stage PTTH secretion is photoperiodically regulated. This photoperiodic mechanism is likewise confined to the cerebral lobes and photoreception is extra-optic (Williams, 1969. Symp. Soc. Exp. Biol. 23: 285). Thus, in its location and mode of photoreception, the photoperiodic response of A. pernyi shows superficial resemblance to its circadian rhythms.

An analysis of the eclosion response of A. pernyi showed that the free-running cycle could be envisioned as being divided into 2 distinct phases--a short initial synchronization period and a long dark decay period (Truman, 1972. Z. vergl. Physiol. 76:32). The synchronization period shows an apparent photoreversibility in that a light interruption during this time brings the clock back to the beginning of the cycle. Light interruptions during the dark decay period appear only to change the time-course of the remainder of the cycle. These relationships result in an hour-glass behavior of the Pernyi clock during the majority of photoperiod regimens: lights-off signals the beginning of a clock cycle, then lights-on alters the kinetics of the cycle such that it terminates at some point late in the photophase. Since adult eclosion is triggered at the end of a clock cycle, it shows a phase relationship which is a function of the entire photoperiod and which is generated by an interaction of the lights-on and lights-off signals with the clock. The behavior of the flight-activity rhythm is also consistent with this model of the clock. The onset of activity occurs 6-7 hrs after lights-off in all photoperiods from 4L:20D to 17L:7D. This relationship is expected from a rhythm which is triggered early in the circadian cycle. Under these photoperiods the clock cycle is initiated by lights-off and activity is then triggered before the cycle is altered by lights-on.

Over the range of photoperiods tested the activity and eclosion rhythms undergo a 6 to 8 hour shift with respect to one another. The fact that events triggered at the beginning and end of a circadian cycle can assume different temporal relationships which are dependent upon photoperiod provides a simple modification of a coincidence model for photoperiodic time measurement. The interaction of two such events generates the typical response observed under complete photoperiods and also is consistent with data obtained using skeleton photoperiods.

MAM-B1

REDUCTONE EFFECT IN E.COLI SUBMITTED TO PHOTODYNAMIC ACTION WITH METHYLENE BLUE. L.R.Caldas, S.Menezes*and R.Alcantara Gomes*, Instituto de Biofisica da UFRJ and Comissão Nacional de Energia Nuclear, Rio de Janeiro, Brazil .

Previous papers (1,2,3) on the effect of reductone (enol tartronic aldehyde) on UV irradiated bacteria have led to the assumption that reductone acts by preventing repair. Reductone has no effect when cells are X - or gamma irradiated. Using methylene blue as the active dye it is shown that reductone is able to increase photosensitivity of cells after photodynamic treatment. This effect is enhanced when bacteria are grown in glucose containing media. We shall discuss the action mechanism of reductone considering levels of energy deposition in cells (specific types of damage produced by ionising radiation, UV and photodynamic treatment) and genetic capabilities of some strains of E.coli concerning repair.

References :

(1) Caldas, L.R., Hasson Voloch, A., Mayr,L.M. and Alcantara Gomes, R., An. Acad. Bras. Ci., 1968,40, (2), 243-247.

(2) Alcantara Gomes, R., Mayr, L.M., de Souza, C.M. and Caldas, L.R. , Int.J.Rad.Biol., 1970, 18 (5), 439-449.

(3) Leitão, A.C., Caldas, L.R. and Alcantara Gomes,R., Compt.Rend. Acad. Sci., 1971 (273), 411-413.

This work was supported by the Banco Nacional do Desenvolvimento Economico (Contract FUNTEC-143), Comissão Nacional de Energia Nuclear, Conselho Nacional de Pesquisas and Conselho de Pesquisas, UFRJ.

MAM-B2

POSSIBLE METABOLIC CONTROL BY DIRECTED IN-VIVO MOTION. Sydney J. Webb, Department of Bacteriology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Monochromatic light of between 330 and 410 nm absorbed in vivo only by the flavinoid pigments of the respiratory membrane system has been found to sequentially mutate the genes of Escherichia coli. The order of the sequence in which the mutations occur is changed by nutrition. Microwave frequencies of between 40 and 150 GH_z have been found to interfere with the syntheses of protein and DNA. The particular frequencies affecting DNA synthesis form a series in which each successive frequency is separated by a constant frequency. From this constant the radius of the in vivo motion has been calculated. The value of the constant is altered by nutritional factors. It appears that some form of unidirectional spiral motion in vivo controls events in time and space.

THE PHOTOSENSITIZED IMMOBILIZATION OF *A. SALINA* NAUPLII BY POLYNUCLEAR AROMATIC HYDROCARBONS. A CORRELATION OF CARCINOGENIC AND PHOTODYNAMIC ACTIVITIES. David D. Morgan David Warshawsky and Florence Miles.*

The photosensitized (photodynamic) immobilization of the nauplii of *Artemia salina* by polynuclear aromatic hydrocarbons (PNA's) and aza-PNA's was investigated for 41 different ring systems. Aqueous salt water solutions containing a PNA sensitizer (10^{-8} - 10^{-7} M) and the nauplii were incubated in the dark and then irradiated at 366 nm. The immobilization of the nauplii was found to be a linear function in time and to be proceeded, in most cases, by an induction period. Photodynamic activities of the individual PNA's were corrected for the amount of light absorbed by the sensitizer and expressed on a relative scale as the relative rate of photodynamic immobilization (RRPI). Benz[c]acridine was used as a standard and assigned an RRPI of 1.0. The effect of nauplii age, dark incubation period, PNA concentration and possible mechanisms for the immobilization will be discussed. The correlation of RRPI values with the carcinogenic properties of the PNA's will also be presented.

MAM-B4

PHOTOOXIDATION OF SNAKE VENOM ENZYMES SENSITIZED BY DYES. R. Straight, P. Garn*, and J. Glenn*, Venom Research Laboratory, Research in Medicine, Veterans Administration Hospital, Salt Lake City, Utah.

Photooxidation sensitized by dyes (SPO) has been used to detoxify lethal snake venoms. We have used SPO to prepare venom toxoids for vaccines and antivenins for snakebite therapy. In order to determine optimum conditions for SPO of venoms we have studied the effect of SPO on five enzymes commonly found in snake venoms. Venom samples were collected from live specimens of Crotalus atrox, Crotalus adamanteus, Bothrops atrox, and Naja naja. The samples were frozen and lyophilized and were assayed for esterase, protease, amino acid oxidase, phosphodiesterase and phospholipase A activity. The venoms were fractionated by polyacrylamide gel electrophoresis (DEP) and the gels were sliced into 1.6 mm slices. Enzymatic activities were determined on an aqueous extract of each slice such that each enzymatic activity could be correlated with a particular protein band before and after SPO. Photooxidation was carried-out in a Warburg apparatus at 25°C containing methylene blue, eosin Y or riboflavin, (1×10^{-5}M); phosphate buffer (0.01 M, pH 7.5) and venom (10 mg protein per ml.) for 0, 15, 30, and 60 minutes. Oxygen uptake, enzymatic activity and lethal toxicity (mouse LD_{50}) were measured. In general SPO decreased the lethal toxicity and inactivated the enzymes assayed. However, depending on the sensitizer used and the type of venom being photooxidized enzyme activity was observed to increase continuously or to increase and then decrease with time of illumination particularly in the case of phospholipase A and phosphodiesterase. Also there was a progressive loss of resolution of protein bands and immunoprecipitin bands on DEP with increasing illumination time. Supported by VA Medical Research, grant no. 01-8187-103.

DOSE DEPENDENT CHANGES IN THE SEDIMENTATION CHARACTERISTICS OF BACTERIAL DNA PRODUCED, IN VIVO, BY NEAR ULTRA-VIOLET IRRADIATION (365 nm) AND 8-METHOXYPSORALEN. M.J. Ashwood-Smith and Elizabeth Grant*. Department of Biological Sciences, University of Victoria, Victoria, B.C., Canada. Supported by National Research Council of Canada Grant No. A6206.

Near ultra-violet irradiation (320-380 nm) in the presence of 8-methoxypsoralen (8 MOP) causes mutations and death in a number of cellular systems. We wish to report a dose dependent alteration in the normal molecular weight profile of bacterial DNA as analysed by alkaline sucrose gradient centrifugation when E. coli is irradiated (365 nm) in the presence of 8 MOP (40/μg/ml). Irradiation with black light gave survival and mutation induction values as follows:-Survival values are followed by mutant yield per 10^6 survivors in parenthesis:-500 ergs/mm^2=60% (1.5), 1000 ergs/mm^2=25% (4.8), 1500 ergs/mm^2=5%(9), 2000 ergs/mm^2=0.3% (> 60). Mutants are revertants to tryptophan independence. The amount of DNA, characterised, by a normal sedimentation profile, disappeared as a function of the irradiation dose such that 40% remained after 500 ergs/mm^2, 20% after 1000 ergs/mm^2, 10% after 1500 ergs/mm^2 and 4% after 2000 ergs/mm^2. This disappearance was quantitatively accounted for by a corresponding increase in DNA recoverable as a very rapidly sedimenting "complex". Neither protein nor RNA was associated in any appreciable amounts with this rapidly sedimenting material. Irradiation of E. coli B in the presence of glucose resulted in approximately three times as much energy being required to produce the same degree of death, mutation and the disappearance of DNA characterised by normal sedimentation properties. These results suggest that some of the 8 MOP photosensitized damage can be rapidly repaired if energy is immediately available. The genetic nature of this mechanism is being investigated.

MAM-B6

TRYPTOPHAN PHOTOPRODUCT EFFECTS ON GENETIC RECOMBINATION IN BACTERIA. F. Landa* and A. Eisenstark, Division of Biological Science, University of Missouri, Columbia, Missouri.

Exposure of L-tryptophan to blacklight (365nm) produces a photoproduct(s) which is toxic to recombinationless (rec) strains of bacteria. The effect of this photoproduct on various microbial recombination systems was investigated by treating rec cells with (a) irradiated and (b) non-irradiated L-tryptophan. The photoproduct increases the number of recombinants upon transduction of Salmonella typhimurium with P22 phage, and induced P22 phage production in lysogenic strains of S. typhimurium. The photoproduct decreases the number of recombinants formed in Escherichia coli K12 conjugation and in T4 phage crosses. The photoproduct has no effect on transformation in Bacillus subtilis. A model will be presented that may account for the altered recombination frequencies upon treatment of cells and DNA with tryptophan photoproduct.

PHOTORECONSTITUTION OF BIOLOGICALLY-USEFUL COLLAGEN GELS.

E. B. Masurovsky and E. R. Peterson*, Albert Einstein College of Medicine, Bronx, N.Y.

Collagen gels reconstituted from acid extracts of rat-tail tendon by certain chemical procedures, e.g., exposure to ammonia vapor, have served as admirably suitable substrates for the cultivation of a variety of cells and tissues(Ehrmann and Gey,1956), including neural tissues (Bornstein,1958). Gelation by these means, however, cannot be innocuously conducted in the environs of living cells. We(Masurovsky and Peterson, 1972) demonstrated that collagen extracts(*vide supra*) when mixed with ethanolic riboflavin solution, or riboflavin-5'-phosphate($\geqslant$0.05%) aqueous solution in a ratio of $\sim$5:1 v/v, and spread on glass or plastic surfaces, could be reconstituted by exposure to visible light for $\sim$5-10 minutes into gels suitable for the cultivation of cells constituting peripheral nervous system, central nervous system, muscle, skin, and other tissues. Photochemical reactions, presumably involving localized free radical activity, are thought to participate in the conversion of these collagen extracts into gels. Such gels have a resilient, porous structure that readily accommodates to cell movements and permits out-growing cells and/or cellular processes to burrow and develop within the gel matrix. These gels may be formed fairly near living cells,permitting the transfer of tissue culture "modules"(Masurovsky et al.,1971) for special experimental purposes, and suggesting a variety of possible medical, surgical, and prosthetic applications. Supported by an Alfred P. Sloan Foundation grant and by Grant NS-08770 from the NIH.

MAM-B8

ENHANCEMENT OF SELENIUM PHOTOVOLTAISM BY TREATMENT WITH PORPHYRIN DERIVATIVES.

A. D. Adler and V. Greenfield*, New England Institute, Ridgefield, Conn. 06877

By treating commercially available selenium solar cells with coatings of Langmuir trough films of various porphins, chlorins, and metallo-derivatives and acid salts of these materials enhanced photovoltages and photocurrents are both obtained. The overall increased power output is 2 to 10 fold over the untreated cell depending on the applied load. While the dark resistance of the cell is decreased, it also appears that the photoemissivity is increased. Preliminary experiments show a positive temperature coefficient for the power output. Further studies on the nature of this surface effect and its extension are under investigation.

MAM-B9

The Photosensitized Oxidation of Alpha-Lipoic Acid.

R. W. Murray, F. E. Stary*, and S. L. Jindal.* Department of Chemistry, University of Missouri-St. Louis, St. Louis, Mo. 63121.

Alpha-lipoic acid has been identified as a growth factor for many bacteria and protozoa, as a coenzyme in oxidative decarboxylation reactions, and also has been suggested as playing a role in the primary quantum conversion act of photosynthesis. When α-lipoic acid (protogen A) is isolated from natural sources it is reported to be accompanied by a monooxide which has been given the name β-lipoic acid (Protogen B). We have subjected d,ℓ α-lipoic acid to methylene blue sensitized photooxidation. The oxidation products include two thiolsulfonates and four thiolsulfinates. The two thiolsulfonates arise from double oxidation at each of the sulfur atoms in the dithiolane ring. The thiolsulfinates are the cis and trans monooxides arising from oxidation at each of the sulfur atoms. The thiolsulfinate isomers are not separated by ordinary tlc methods. Their presence is deduced from the shifted nmr spectrum using tris (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-d_6-4,6-octanedione-d_3)-europium(III), Eu-FOD-d_{27}, as the shift reagent. The mechanism of the oxidation, which is believed to involve singlet oxygen, will be discussed.

MAM-B10

RED BLOOD CELL LYSIS INDUCED BY A PRODUCT OF SINGLET OXYGEN AND CHOLESTEROL.

T. Yamane* and A. A. Lamola, Bell Laboratories, Murray Hill, New Jersey.

Incorporation of small amounts of the main product of attack of singlet oxygen upon cholesterol, cholesterol-5α-hydroperoxide, into the membranes of normal red blood cell (rbc) causes them to hemolyze (1). The hemolysis has characteristics very similar to the light-induced hemolysis exhibited by the rbc of patients with erythropoietic protoporphyria (2). The hemolysis induced by the cholesterol hydroperoxide is inhibited by the presence of small amounts of β-carotene or α-tocopherol in the membranes of the rbc.

---

(1) A. A. Lamola, T. Yamane and A. M. Trozzolo, Science 179, 1131 (1973).
(2) B. D. Goldstein and L. C. Harber, J. Clin. Invest. 51, 892 (1972).

CHLOROPLAST STRUCTURE AND DEVELOPMENT. L. Bogorad, The Biological Laboratories, Harvard University, Cambridge, Mass. 02138.

The prevailing view of the structure of photosynthetic lamellae at the photosynthetic level -- i.e. with regard to photon absorption and energy migration -- has not changed fundamentally since the pioneering penetrating experiments of Duysens in the early 1950's. In the intervening years, the existence of two photosystems has been firmly established. Attempts to discover their component parts to study interactions between them with regard to energy transfer, electron transport and phosphorylation have increased rapidly. Over this same 20-year period there have been many technological changes in electron microscopy and interpretations of electron microscope data have generated a number of views of the organization of photosynthetic lamellae. The most attractive current view of structure, based largely on the freeze-etch technique, is that the photosynthetic lamellae consist of a hydrophilic-edged lipid matrix in which protein or lipoprotein aggregates are embedded.

Chloroplasts develop completely in darkness in some plants. Among these are algae (e.g. Nitella) in which mature chloroplasts increase in number by division of mature plastids. In most higher plants, small proplastids are present in meristematic cells. In seedlings grown in darkness, these develop into more complex membrane-containing etioplasts. The etioplasts mature into chloroplasts when the tissues are illuminated. The most conspicuous changes during maturation are greening (i.e. chlorophyll formation and accumulation), detectable by eye; the dissolution of the paracrystalline prolamellar body and the assembly of photosynthetic membranes, visible with the electron microscope; and the acquisition of the photosynthetic capacity, assayable by many techniques.

Among the components needed for photosynthesis, etioplasts of maize contain coupling factor for photosynthetic phosphorylation and several membrane polypeptides which are also present in mature photosynthetic lamellae. During light-induced plastid development additional polypeptides are introduced into the membrane and its lipid composition changes. On the other hand, some polypetpides of the etioplast prolamellar body are undetectable in preparations of chloroplast thylakoids. Etioplast membrane preparations are incapable of carrying on light-independent acid-to-base phosphorylation or of responding osmotically. The development of the capacity to carry on acid-to-base phosphorylation is directly correlated with the development by the membranes of the capacity to respond osmotically but appears to be independent of chlorophyll accumulation. The kinetics of chlorophyll accumulation in darkness after etiolated maize leaves have been illuminated briefly are distinctly different from the kinetics of the acquisition of acid-to-base phosphorylation capacity. However, the latter is correlated with the appearance of a 46,000 dalton polypeptide in the lamellae.

Studies of plastid development in other species carried out in other laboratories will also be summarized.

References:

Branton, D. Membrane structure. Ann. Rev. Plant Physiol. 20: 209 (1969).

Forger, J.M., III and L. Bogorad. Acid to base phosphorylation and membrane integrity in plastids in greening maize. Biochim. Biophys. Acta 226: 383 (1971).

Forger, J.M., III and L. Bogorad. Development of photosynthetic membranes. In preparation.

Lockshin, A., R.H. Falk, L. Bogorad, and C.L.F. Woodcock. The coupling factor for photosynthetic phosphorylation from plastids of light- and dark-grown maize. Biochim. Biophys. Acta 226: 366 (1971).

(Supported in part by a research grant from the National Science Found.)

ORGANIZATION OF PROTEINS WITHIN THE CHLOROPLAST MEMBRANE by S. M. Klein and L. P. Vernon, Ph.D., Brigham Young University, Provo, Utah.

Chloroplast membranes represent a biological system of definite structure. We endeavored to determine the dimensional locations of the various proteins comprising these membranes and their derived fragments. Whole spinach chloroplasts, their Triton X-100 fragments TSF 1, containing photosystem I (PS I), and TSF 2, containing photosystem II (PS II) activity, and the particles obtained by differential centrifugation of a chloroplast suspension passed through a French pressure cell, were investigated by SDS-acrylamide gel electrophoresis. The chloroplasts were reacted with S^{35}-labeled p-diazonium benzenesulfonic acid (DABS), and the label distribution followed. It was observed that TSF 1 was approximately ten times more labeled than TSF 2. Upon further resolution of the proteins by SDS-acrylamide gel electrophoresis, most radioactivity was recovered in proteins with molecular weights of about 20 kilo daltons. The protein distribution patterns of the $DABS^{35}$ labeled fragments, however, differed drastically from those which were characteristic of TSF-1 and TSF-2 from unlabeled membranes, impairing the assignment of radioactivity to definite proteins. At this point we can conclude only that PS I is located at the outside of the membrane.

MAM-C3

EMISSION KINETICS OF PHOTOSYSTEM I AND PHOTOSYSTEM II FLUORESCENCE ON A PICOSECOND TIME SCALE. M. Seibert, GTE Laboratories Inc., Waltham, Mass. and R.R. Alfano* Physics Dept., City College of New York, New York, N.Y.

Fluorescence emission kinetics of isolated spinach chloroplasts have been observed with an instrument resolution time of 10 psec using a frequency doubled, mode-locked Nd:glass laser and an optical Kerr gate. Emission kinetics at 685 nm displayed two maxima, the first 15 psec and the second 90 psec after the flash. The intervening minimum occurred between 40 and 50 psec. Equations describing the following model systems were derived, solved and fitted by computer to the above data: (1) one absorbing species which transfers energy to a second fluorescing species, (2) one absorbing species which both fluoresces and transfers energy to a second fluorescing species, (3) two dependent absorbing species, only one of which fluoresces, (4) two independent species which both absorb and fluoresce, (5) two independent absorbing species, one of which fluoresces and the other which transfers energy to a third fluorescing species. Of the above models, only (4) and (5) give the observed two peaks. This evidence indicates that two independent (on this time scale) fluorescing species are involved. Since an upper limit of 70 psec has been estimated previously for the lifetime of Photosystem I fluorescence[1] and that of Photosystem II is known to be several hundred psec (at low actinic intensity), the first peak is interpreted as being fluorescence from Photosystem I ($\tau \leq 10$ psec) and the second from Photosystem II ($\tau = 200$ psec).

[1]Borisov and Il'ina (1972) Biokhimiya (Engl. Transl.) 30:6, 693.

ESR SIGNAL II: THE EFFECT OF VARIOUS CHEMICAL AND PHYSICAL TREATMENTS.

M. C. Beinfeld, Department of Pharmacology, Washington University Medical School, St. Louis, Missouri.

There has been a recent rebirth of interest in ESR signal II in photosynthesis. The kinetic response of ESR signal II to light has been measured in chloroplasts and algae (1, 2) and was found to be sufficiently fast to be consistent with the rate of electron transport. Some chemical and physical treatments which have a profound effect on photosynthetic electron transfer also dramatically alter signal II. U. V. irradiation progressively destroys signal II in parallel with the destruction of oxygen evolution. Tris (0.8M) buffer treatment inhibits oxygen evolution but greatly stimulates signal II. High concentrations of DCMU, HOQNO, ioxynil, and o-phenanthroline inhibit the light-response of signal II. At high DCMU or HOQNO concentrations in addition to inhibition of light-responsive signal II, a gradual loss of dark-stable signal II is seen as a result of illumination. These data along with others will be discussed with regard to whether signal II is acting on the "oxidizing" or "reducing" side of photosystem II.

1. M. C. Beinfeld. (1972). A fast kinetic component of ESR signal II in Photosynthesis. New York Academy of Sciences, Proceedings (in the press).
2. G. T. Babcock and K. Sauer (1973). Flask kinetic studies of the slow decaying, g=2.0046, EPR signal in spinach chloroplasts. Biophysical Society Abstract WPM-H4.

MAM-C5

THE RELATION OF SIGNAL 2 TO ELECTRON TRANSPORT IN PHOTOSYSTEM 2 IN ESR STUDIES OF SPINACH CHLOROPLASTS

by J.T. Warden, Jr.* and J.R. Bolton, Department of Chemistry, University of Western Ontario, London, Canada.

The broad, structured resonance, Signal 2, attributed to Photosystem 2 in ESR studies of photosynthetic organelles has often been a subject of speculation. Although this species has tentatively been assigned to a derivative of plastoquinone, no functional role for this species has yet been ascertained. We have applied the technique of flash photolysis-ESR to this system and we have detected a fast-rising (<1 msec) free radical transient in Jensen-Bassham type spinach chloroplasts which has the same spectral profile as Signal 2. The decay time is ~6-10 sec. This flash transient is formed in approximately equimolar concentration to that of Signal 1. We have studied the behaviour of Signal 2 both with steady-state light and with the flashlamp under a variety of conditions including addition of various redox components and inhibitors. We will discuss the results in terms of a model for the function of the Signal 2 species.

MECHANISM FOR LIGHT INDUCED LOWERING OF FLUORESCENCE INTENSITY IN PHOTOSYNTHETIC ORGANISMS AT LOW TEMPERATURE. R. JACOBS & S. BRODY, DEPT. OF BIOLOGY, NEW YORK UNIVERSITY, N.Y., N.Y.

Low temperature studies *in vivo* simplify the observation of primary processes by suppressing secondary diffusion controlled reactions. Irradiation of photosynthetic material at 77° K results in a lowering of fluorescence intensity, referred to as F.L. F.L. is reversible upon warming. Log fluorescence intensity plotted as a function of log time of irradiation gives straight lines with different slopes for each fluorescence band (685, 695, 735mm). F.L. was studied as a function of concentration of various exogenously agents (e.g. electron donors, acceptors, inhibitors, etc.) Electron acceptors and donors tested have different effects on F.L. of each fluorescence band. F.L. of 685 is abolished by DCMU, it seems that F.L. 695 and F.L. 685 result from interdependent processes. Fluorescence polarization studies indicate orientatinnal changes occur in chlorophyll molecules associated with energy transfer in System II. F.L. in green plants and photosynthetic bacteria appears to arise from the combination of several mechanisms: formation of a quencher on the electron donor side of System II; formation of a quencher at System I; the quenchers do not diffuse or react further at low temperature; a decrease in transfer efficiency from System II to bulk Chl.

MAM-C7

THE TIME COURSE OF LIGHT EMISSION OF *SCENEDESMUS OBLIQUUS*, Chr. Holzapfel and A. Haug (intr. by E. Kuntz), MSU/AEC-PRL, East Lansing, Mich. USA.
The time course of delayed light emission and that of prompt fluorescence was measured during the period of light adaptation of the cells exposed to the stimulus light in a photon counting apparatus. Generally the delayed light emission was more affected by a change in experimental conditions as compared to prompt fluorescence.

1. Illuminating normally grown wildtype cells with higher light intensities (>70 mW/cm^2), the intensity of delayed light increases to a maximum in the first few minutes, then decreases to a steady state level after about an hour. The maximum is missing at lower exciting light intensities.
2. In dark adapted wildtype cells at high light intensities, the time course of delayed light emission is similar to that one described above, apart from a lag period within the very first minutes of light adaptation preceding the increase in emission intensity. That lag period is characterized by a relative minimum of the intensity lying higher than the intensity of the steady state level. The length and the profile of the lag period depends on the time of dark adaptation. With increasing dark adaptation time the maximum of the delayed light intensity occurs later.
3. The intensity of the delayed light for mutant 8 shows only a steady decrease during the time course.
4. The time course of prompt fluorescence practically does not show any difference for wildtype, mutant 8, or mutant 11 cells.
5. For the light adapted wildtype cells the intensity of delayed light and that of prompt fluorescence increases linearly with increasing intensity of the exciting light below the saturation point in the light curve. The increase is less than linear above the saturation point (about 10 mW/cm^2).

Supported by MSU/AEC Contract No. AT-(11-1)-1338.

QUANTITATIVE DETERMINATION OF IN VIVO PIGMENT ORIENTATION BY LIGHT SCATTERING. R. Van Metter, C. E. Swenberg, and N. Geacintov, Department of Physics and Chemistry, New York University, New York, N. Y.

A general microscopic formalism for calculating the Mie scattering has been developed which is applicable to an interpretation of anisotropies in the light scattering of oriented biological samples. The utility of this approach is illustrated by explicit application to the anisotropic scattering of light from magnetically oriented Chlorella cell suspensions, which has been recently determined by Van Nostrand (1). The relative concentrations of the different spectroscopic forms of chlorophyll a in vivo (2) were used to construct theoretically the wavelength dependence of the light scattering from Chlorella suspensions. Comparisons of this curve and the experimentally determined anisotropies in the light scattering were utilized to estimate the relative orientation of the different spectroscopic forms of chlorophyll. The analysis indicated that the short wavelength absorbing from (Ca 667) has the lowest degree of orientation. The relationship between our microscopic formalism and the theory of selective light scattering (for unoriented samples) developed by Latimer and Rabinowitch is demonstrated (3).

(1) F. W. Van Nostrand Jr., PhD. Thesis 1972, N.Y.U., unpublished.
(2) C. S. French, J. S. Brown, and M. C. Lawrence, Plant Physiology (1972) 49, 421.
(3) P. Latimer and E. Rabinowitch, Arch. Bioch. 84, 428 (1959).

MAM-C9

A SCANNING DOUBLE BEAM SPECTROPHOTOMETER. Geoffrey Hind and Jerry Rapp*, Biology Department, Brookhaven National Laboratory, Upton, New York 11973.

A double beam spectrophotometer, capable of operation in either the split beam or dual wavelength mode, has been equipped with a scanning drive and interfaced to a PDP-11 computer. Difference spectra with a resolution of $3 \cdot 10^{-5}$ absorbance units are obtained by subtraction of two arrays corresponding to two scans taken under different steady state conditions, for example, light minus dark or reduced minus oxidized. The difference spectra are then further processed in a CDC 6600 computer to decompose the spectral envelopes in terms of known absorbing species. In this way, steady state difference spectra from isolated chloroplasts can be quantitatively analyzed under different conditions of illumination, uncoupling or inhibition.

Application of this instrument to elucidation of electron transport reactions in photosynthesis, and to the study of other photobiological systems will be described. (Supported by NSF grant AG-279 and by the U.S.A.E.C.)

ON THE STATE 1 - STATE 2 PHENOMENON OF PHOTOSYNTHESIS

R. T. Wang & J. Myers, Dept. of Botany & Zoology, Univ. of Texas, Austin, Texas 78712.

Transition from 710 to 650 nm light is followed by a rapid chromatic transient in O_2 evolution explained as adjustment in fraction (q) of System II open traps. Following a transient, the quantum yield of oxygen at 650 nm slowly (minutes) rises. The same phenomena also occurs after transition from 650 to 710 nm light. Bonaventura and Myers (1969) explained the slow phenomena in terms of changes in α, the fraction of absorbed quanta delivered to photosystem II: for any chosen wavelength α becomes maximum in state 1 after 710 nm and minimum in state 2 after 650 nm. The hypothesis (also used by Duysens and by Murata) predicts that the Joliot plot for rate O_2 evolution vs q should have two different curves, one for state 1 and one for state 2. Further, the ratio of rates observed at any value of q should be constant and equal to the ratio of α's for the two states.

Recently Delrieu sought but failed to find the two predicted curves and ascribed the state 1-state 2 phenomenon to changes in the apparent equilibrium constant between centers for the two photosystems. We have reexamined the problem in Chlorella. We could reproduce Delrieu's results when "state 1" is generated by darkness or by very low intensities of 710 nm. However, intensities of 710 nm sufficient to give maximum quantum yield produce a different state and give the predicted two different Joliot curves for state 1 and state 2.

THE SCOPE OF PHOTOBIOLOGY. J. D. Spikes. Department of Biology, University of Utah, Salt Lake City, Utah

1. Introduction. Light and life

2. Photophysics and photochemistry in relation to photobiology
   a. the nature and properties of light; wavelength and energy conventions
   b. the absorption of light by polyatomic molecules; excited states
   c. photochemistry
   d. absorption and action spectra

3. Photobiologically active light in the environment
   a. sources: sun, moon, stars, bioluminescence
   b. sunlight: at the top of the atmosphere, absorption and scattering in the atmosphere, energy-wavelength relations at the earth's surface
   c. cycles of light in nature: diurnal, annual, lunar

4. Plant photobiology
   a. introduction, "stoichiometric" and "trigger" photoprocesses
   b. chlorophyll synthesis
   c. photosynthesis: overall reactions in different plant groups, photosynthetic pigments and organelles, current ideas on mechanism
   d. photomovements in parts of non-motile plants
   e. photomovements in motile plants
   f. red-far red light responses in plants: phytochrome and the photoperiodic control of flowering, seed germination, vegetative growth, etc.
   g. miscellaneous photoresponses: protoplasmic viscosity and streaming, membrane permeability and ion uptake, enzyme "activation," dormancy, autumn coloration, circadian responses, control of sporulation, sensitivity to infection and toxic agents, etc.

5. Animal photobiology
   a. introduction: biological importance of photoreception in animals
   b. photoreception in animals: reception without organized multicellular eyes, major types of eyes, fine structure and taxonomic distribution of photoreceptor cell types, visual pigments and the photochemistry of vision
   c. non-oriented responses to light: photoreceptor development, iris responses, circadian responses, color changes, photoperiodic responses (reproduction, migration, metabolism, etc.)
6. Biological effects of ultraviolet (UV) radiation
   a. UV in the environment
   b. UV effects on amino acids and proteins
   c. UV effects on nucleic acids
   d. UV effects at the cellular level: repair of UV damage
   e. UV effects on organisms
7. Photosensitization phenomena in biology (photodynamic action)
   a. types of photosensitization reactions
   b. mechanisms of photodynamic action
   c. example of photodynamic effects
8. Bioluminescence
9. References

   a. Clayton, R.K., Light and Living Matter: Vol. 1, The Physical Part; Vol. 2, The Biological Part. McGraw-Hill Book Co., New York (1971). This is an excellent brief introduction to the interactions of light with matter and to the major areas of photobiology.
   b. Giese, A.C. (editor). Photophysiology, Vols. 1-7 (1964-1972). Academic Press, New York. Each volume contains a set of fine reviews of topics in photobiology.
   c. Smith, K.C. and P.C. Hanawalt. Molecular Photobiology. Academic Press, Inc., New York (1969). A very readable survey of the biochemical and biological effects of ultraviolet radiation; recovery processes are stressed.

THE ULTRAVIOLET WORLD OF INSECTS. Timothy H. Goldsmith, Department of Biology, Yale University, New Haven, Connecticut

The solar spectrum at the surface of the earth is limited at the short wavelength end by several mm of ozone in the upper atmosphere. This filter attenuates wavelengths shorter than 320 nm, and by 290 nm there is very little energy reaching the biosphere[1]. As is true for other vertebrates, our visible spectrum ends at about 390-400 nm, not because the visual pigments are insensitive to near ultraviolet light(300-400 nm), but because the vertebrate lens absorbs these wavelengths before they reach the retina. Many arthropods, on the other hand, utilize the near uv in vision.

The reason that vertebrates discard the near uv with yellow lenses and other adaptations such as the macula lutea is probably for the relief of chromatic aberration[2]. In this view, the capacity to evolve a color-corrected lens is limited, and there has been strong selection for the maintenance of visual acuity. The expedient solution has been to restrict the spectral band reaching the retina by filtering out those wavelengths where the aberration problem is worse and, coincidentally, the total available flux is least.

Arthropod eyes, being constructed on a different optical principle, do not face the same problem. Acuity is determined by the interommatidial and acceptance angles of the retinulae, and there is no advantage to be gained by removing the near uv in the dioptic structures. Consequently many arthropods--and most particularly the insects--see in the uv. [3]

Vision in the uv involves two specializations. First, the cornea is transparent at all wavelengths longer than the 280 nm absorption band of its constituent proteins. Secondly, the retinas contain visual pigments with principal (chromophoric) absorption maxima in the near uv (340-380nm). Those species such as crabs or lobsters that spend much of their time in aquatic environments where the natural uv is attenuated by suspended particles tend to lack uv-absorbing visual pigments. In addition, their corneas are frequently pale yellow from near uv-absorbing materials of an unidentified nature [4].

The uv-absorbing visual pigments are rhodopsins, with vitamin A aldehyde (retinaldehyde) as chromophore. In at least some species a uv pigment is one of several spectral classes of rhodopsin present in the retina [3,5]. Single unit recordings provide a method of examining the spectral responses of individual retinular (photoreceptor) cells. Recent evidence involving dye injection of single cells with identified spectral properties indicates that the different classes of visual pigment can be sorted into different photoreceptor cells in the same ommatidium, providing the anatomical basis at the receptor level for color vision[6].

Behavioral evidence of several kinds also points to color vision involving the near uv as a distinct spectral region. The phototaxis as well as the spontaneous alighting preferences of insects are frequently (but not always) preferentially driven by uv rather than other colors. Honeybees can be trained to make color discriminations in color matching experiments analagous to those employed in psychophysical studies

of human color vision. Near uv has a high degree of saturation for bees, as does violet in the human visible spectrum. Moreover, mixtures of uv and yellow generate for bees new, non-spectral colors analagous to purples.[7-9]

Ultraviolet cues are important components of the visual world of insects. Flower colors frequently involve patterns of differential uv reflectance that can be appreciated by insect pollinators but not by the unaided human eye. The wings of butterflies also certain patches of high reflectance which flag prospective mates. In flight these signals can be quite conspicuous to other members of the species, but remain unseen by vertebrates. And polarization patterns of natural sky light, involving near uv and violet, are used by many arthropods in navigation.[7-9]

(1) Green, A. E. S. (ed.). The middle ultraviolet: its science and technology. Wilet, New York, 1966.

(2) Wald, G., Eye and Camera. Sci. Amer., Aug. 1950, pp. 22-41.

(3) Goldsmith, T. H. and Bernard, G. D. The visual system of insects. In "The Physiology of Insecta," 2nd ed., M. Rockstein, editor. Academic Press (1973).

(4) Goldsmith, T. H. and Fernandez, H. R. Comparative studies of crustacean spectral sensitivity. Z. vergl. Physiol. 60:156-175 (1968).

(5) Goldsmith, T. H. The natural history of invertebrate visual pigments. In The Photochemistry of Vision, ed. H. J. A. Dartnall, Chap. 17 Handbook of Sensory Physiology, Vol. VII, Springer-Verlag:685-719(1972).

(6) Mote, M. I. and Goldsmith, T. H. Compound eyes:localization of two color receptors in the same ommatidium. Science 171: 1254-1255(1971).

(7) Daumer, K. Reizmetrische Untersuchung des Farbensehens der Bienen. Z. vergl. Physiol. 38: 413-478 (1956).

(8) Mazokhin-Porshnyakov, G. A. Insect vision. Plenum Press, New York, 1969 (Translated from Russian).

(9) von Frisch, K. The dance language and orientation of bees. Belknap Press, Cambridge, Mass., 1967.

STRUCTURE OF VISUAL PIGMENT MEMBRANES. C. R. Worthington, Departments of Biological Sciences and Physics, Carnegie-Mellon University, Pittsburgh, Pa.

The molecular structure of rod and cone outer segments and rhabdomeres have been studied by birefringence and electron microscopy but only retinal rod outer segments have been studied by X-ray diffraction. Retinal rod photoreceptors are an intriguing system for study by X-ray diffraction for they have an elegant multilayered structure. The lamellar repeat is the disc-to-disc distance inside the photoreceptor. However, the X-ray experiment is not easy and definitive X-ray patterns were not obtained until 1969. Gras and Worthington (Proc. Nat. Acad. Sci. 63, 233, 1969) obtained patterns from intact frog, rat, cattle and monkey retinas and Blaurock and Wilkins (Nature 223, 906, 1969) obtained a similar pattern from the frog retina. Both groups assigned a bilayer profile to the disc membrane. However, there was some disagreement in that we stressed the asymmetry of the membrane profile. A feature of our 1969 model was the assignment of the rhodopsin molecules to the membrane layer facing the intradisc space (Worthington, Fed. Proc. 30, 57, 1971).

Electron microscopy and X-ray diffraction studies on isolated disc membrane preparations from frog retina led to the molecular localization of the rhodopsin molecules (Blasie, Worthington and Dewey, J. Mol. Biol. 39, 407, 1969). The planar area of $4900A^2$ associated with each rhodopsin molecule was obtained in this study. Liebman (Biophys. J. 2, 161, 1962) had estimated that the concentration of rhodopsin within the frog retinal rod was 2.5mM. From these measurements, it was argued that the rhodopsin molecules were on one side of the disc membrane. Our X-ray analysis using model-building considerations indicated that the rhodopsins were on the inside but further study was desirable. Other techniques and, in particular, freeze etching studies will also help in providing a definitive answer on the location of the rhodopsin molecules in the disc membranes.

The original 1969 X-ray analysis was based on h = 8 orders of diffraction. The 1969 data was obtained using comparatively long exposure times of about one day. During this time small changes within the retinal rod structure did occur. Our present data was obtained using exposure times of only one or two hours. The sharpness of the diffraction reflections indicate that changes did not occur in this shorter period. A total of h = 20 orders of diffraction from intact frog retina have been recorded but only h = 12 orders have been used in the analysis. Work is in progress to include these higher orders and so improve the resolution of the analysis. Our 1969 X-ray analysis has been verified using direct methods of structure analysis (Worthington and Gras, Biophys. Soc. Abstracts p. 255a, 1972). A Fourier synthesis of the frog retinal rod structure at a resolution of 13A has been obtained and will be described.

Structural comparisons with nerve myelin can be made. At the present time the electron density profile of the nerve myelin membrane is known at a resolution of 7A (McIntosh and Worthington, Biophys. Soc. Abstracts p. 91a, 1973). Similarities and differences between the profiles of nerve myelin and the disc membrane will be discussed. One feature is that both show a narrow low density region of about 20A or less in width.

On the basis of thickness of the disc membrane it is argued that the disc membrane contains a discontinuous lipid bilayer. That is, the rhodopsin molecules effectively replace a number of lipid molecules from a hypothetical continuous lipid bilayer (Worthington, Current Topics in

Bioenergetics Vol. V, 1973). This result is a necessary consequence of the 1969 X-ray analyses on the intact retinal rod structure.

Recently an electron density profile of the sarcoplasmic reticulum (SR) membrane has been obtained at a resolution of 17A (Liu and Worthington, Biophysics Soc. Abstracts p. 91a, 1973). The SR membrane fragments occur as vesicles and, in the centrifuge, these vesicles can be flattened to resemble the disc configuration. The disc membrane and the SR membrane have very similar electron density profiles. In each case the X-ray analyses indicate that the major part of the protein component is on the inside of the vesicle. The membranes also have a similar function in that the SR membrane releases calcium due to an action potential whereas the disc structure releases calcium due to the action of light (Hagins, Ann. Rev. of Biophys. and Bioeng. Vol. I, 1972). It would appear as if the same kind of mechanism for calcium release is involved in both membranes.

LOCALIZATION AND FUNCTION OF RHODOPSINS IN TWO PHOTORECEPTOR MEMBRANES
W. Stoeckenius, Cardiovascular Research Institute, University of California, San Francisco, California.

Bacteriorhodopsin is a rhodopsin-like protein found in the purple membrane of Halobacterium halobium. It contains 1 mole of retinal per mole of protein bound as a Schiff base to a lysine residue. The purple membrane forms as distinct patches in the surface membrane of H. halobium cells, and these patches contain 25% lipid and bacteriorhodopsin as the only protein (1, 2). A similar situation exists in the photoreceptor membranes of the retina, except that, in the case of rod outer segments, differentiated membrane area has been segregated from the surface membrane in the form of the disks in the interior space of the rods; the lipid to protein ratio in the disk membranes is higher than in the purple membrane, and other proteins are present in small amounts.

Freeze-fracturing and freeze-etching electron microscopy reveal the localization of proteins in membranes. Membranes fracture along an interior plane, and proteins penetrating the hydrophobic interior are revealed as particles on the fracture faces. This is demonstrated on a model system consisting of synthetic phospholipid bilayers with or without incorporated proteins. While bilayers consisting only of lipids or lipid bilayers with protein bound to the surface show smooth fracture faces, rhodopsin-containing lipid bilayers show particles on the fracture faces. The model system has a symmetric distribution of rhodopsin with equal numbers of particles on the complementary membrane fracture faces; the natural membranes are highly asymmetric. In both the purple membrane and the disk membranes after fracturing, all the particles are found on the cytoplasmic side of the membrane with the corresponding depressions on the opposite face. The proteins may actually span the width of the membrane. The outer membrane of the retinal rod shows particles identical in appearance to the particles in the disks; they are also located on its cytoplasmic side. Whereas the distribution of rhodopsin in the plane of the disk membranes and the outer membranes of the rods appears random, bacteriorhodopsin forms a planar hexagonal lattice in the purple membrane. In both membranes the rhodopsins appear to change the permeability of the membrane when they absorb light. In the rod outer segments this is thought to be a passive change of permeability (3); in the case of the purple membrane, an ion pump seems to be involved.

Similar to rhodopsin, bacteriorhodopsin - in the isolated membrane as well as in intact bacteria - can be bleached by light. The absorption maximum shifts from 560 nm to 415 nm but, unlike rhodopsin, bacteriorhodopsin in the dark, within a few milliseconds, returns spontaneously to the long wavelength form. This return to the long wavelength form can be retarded if the isolated membrane is treated with ether. Continuous illumination will then drive practically all of the pigment into the bleached state, and it returns to the unbleached state only when the light is turned off. In this system pH measurements show that bleaching of bacteriorhodopsin causes a release of protons which are bound again when the pigment reverts to the 560 nm form in the dark.

If suspensions of halobacteria containing the purple membrane are deprived of metabolic energy sources by prolonged starvation in salt solutions and then illuminated with light of a wavelength absorbed by the purple membrane, an acidification of the medium is observed. This occurs under aerobic as well as anaerobic conditions. The pH of the suspending medium returns to the dark-level when the light is turned off. The effect is much larger and develops much more slowly than the release of protons during bleaching of the isolated purple membrane; it is dependent on light intensity. Uncouplers such as CCCP, FCCP, DNP - which are known to increase

the permeability of membranes to protons - abolish the light-induced pH effect or accelerate the return of the pH to the dark-level when added at the end of the illumination period. Apparently in the light the cells generate a proton gradient across the cell membrane, and this process is mediated by the purple membrane. In the apparent absence of other energy sources, the simplest mechanism to explain this effect is to assume that illumination causes a rapid cycling of the pigment through the bleached and unbleached states and that the accompanying release and uptake of protons in the bleaching cycle of bacteriorhodopsin occurs as a vectorial process across the membrane; protons are released on the outer membrane surface and are taken up on the cytoplasmic side. This would require an asymmetric orientation of bacteriorhodopsin across the membrane, which has been demonstrated to exist in the freeze-fracture preparations. The electrochemical gradient thus generated is presumably used to drive other metabolic processes, either directly or via ATP synthesis, as may be true generally not only for mitochondria but also for other procaryotic organisms (4). Halobacterium halobium cell envelopes contain an ATP-ase similar to mitochondrial ATP-ase and the membrane bound ATP-ases of other procaryotic cells (5). Illumination increases the ATP content of the cells. When bacteria are suspended in salt solutions and kept under anaerobic conditions, their ATP level falls to very low values; if they are then exposed to light of wavelength absorbed by the purple membrane and if they contain purple membrane, their ATP content rises tenfold or more to values comparable to those under aerobic conditions with substrate present. This effect is abolished by CCCP but not by KCN.

Some other observations also fit the hypothesis that halobacteria, which occur naturally in an environment with a high radiation density, can use light as an alternative energy source and that the purple membrane is the phototransducer. The synthesis of purple membrane is triggered by low oxygen tension in the growth medium and can be completely suppressed if the medium is saturated with air. Light stimulates the synthesis of purple membrane by H. halobium cells (6). Illumination of respiring bacteria with light absorbed by the purple membrane reduces the oxygen consumption of the cells. Halobacteria are motile and we have observed phototactic responses of the cells.

It thus appears that the rod outer segment disks and the purple membrane are rather similar in their structure and chemical composition but differ in their function. The disk membrane acts as a photosensor and may operate by inducing passive changes in membrane permeability. The purple membrane appears to be a phototransducer which operates as a light-driven proton pump.

Unpublished results reported here have been obtained in collaboration with Drs. Arlette Danon, D. Oesterhelt, R. A. Cone and W. L. Hubbell. The work was supported by NHLI Program Project Grant HL06285 and NASA Life Scientist Grant NGL 05-025-014.

1. Oesterhelt, D. and W. Stoeckenius, Nature New Biol. 233, 149 (1971).
2. Blaurock, A. E. and W. Stoeckenius, Nature New Biol. 233, 152 (1971).
3. Hagins, W. A., Ann. Rev. Biophys. Bioengineer. 1, 131 (1972).
4. Harold, F. M., Bacteriol. Rev. 36, 172 (1972).
5. De, K., H. Passow, W. Stoeckenius and M. White, Pflugers Arch. Ges. Physiol. 289, S R.15 (1966).
6. Danon, Arlette and W. Stoeckenius, NASA Symposium on Extreme Environments. Mechanisms of Microbial Adaption; June 26-28, 1972; Ames Research Center, Moffett Field, California; page 25.

THE PHOTOSYNTHETIC MEMBRANE. R. B. Park, Department of Botany, University of California, Berkeley, California.

The photosynthetic membrane, as it exists in prokaryotic or eukaryotic cells, represents an elaboration of a limiting organelle or cell membrane. This elaborated membrane contains the photosynthetic pigments and, unlike the membranes of animal photoreceptors, stores an appreciable portion of the radiant energy absorbed by it as usable chemical potential. In higher plant chloroplasts, as well as chloroplasts of some algae, the elaborated membrane system is divided into two major types: unappressed stroma lamellae and stacked grana lamellae. Work in a number of laboratories during the past five years has shown these two types of membrane can be physically separated and studied with respect to photochemical function and chemical composition. Grana lamellae possess both photosystems while stroma lamellae possess only photosystem 1. Evidence from quantum yield studies, as well as studies of variable fluorescence indicate that approximately half the chlorophyll in stroma lamellae is inactive and does not appear to represent a damaged photosystem 2. The possible developmental relationship between stroma and grana lamellae as studied in pulse labeling experiments will be discussed. The major colorless lipids are very similar in concentration in both membrane types, the major lipid differences lying in chlorophyll composition. On a membrane-protein basis, there is about 1.4 times as much chlorophyll in grana lamellae as in stroma lamellae. Also, the chlorophyll a/b ratio of stroma lamellae is about 6 whereas that of grana lamellae is about 2.4 in field grown spinach. The peptide compositions of both membranes are also quite similar when assayed by SDS gel electrophoresis. Minor, but consistent, differences in peptide composition are observed. Apparently subtle differences are quite sufficient to bring about both the large functional and morphological changes one finds between the two membranes.

EXCISION REPAIR OF BASE DAMAGE. Ronald D. Ley. Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439.

The observations that ultraviolet (UV)-irradiated bacteria could preferentially release pyrimidine dimers from their DNA and that UV-irradiated bacteria incorporate bromouracil (BrUra) into parental DNA resulted in the following proposed model for the excision repair of pyrimidine dimers[1,2]: 1) A UV-specific endonuclease produces an incision near the pyrimidine dimer; 2) exonuclease activity results in the release of the dimer and a limited number of adjacent nucleotides; 3) the resulting gap is filled in by repair replication using the complementary strand as a template; and 4) the resynthesized region is joined to the contiguous parental strand. Evidence to support the occurrence of the above steps during the reconstruction of damaged DNA has been obtained experimentally *in vivo*, and in some cases, *in vitro*.

Velocity sedimentation studies on DNA from UV-irradiated, excision proficient (uvr+) strains of *Escherichia coli* indicate that during post-UV incubation single-strand breaks appear in the dimer-containing DNA[3]. This restricted degradation of cellular DNA was not observed in a UV-sensitive, repair deficient mutant (uvrA) which also is unable to selectively remove pyrimidine dimers from its DNA[4]. Such *in vivo* experiments as these indicate that an endonuclease which acts specifically on dimer-containing DNA exists in repair proficient strains of bacteria. The actual existence of a UV-specific endonuclease has been substantiated by the purification of an enzyme from extracts of *micrococcus luteus* which exhibits an endonucleolytic activity towards irradiated DNA; no activity was observed with unirradiated DNA or irradiated DNA that had been photoreactivated to remove pyrimidine dimers *in situ*[5,6]. Similarly, DNA sedimentation studies indicate that a UV-specific endonuclease also occurs in normal human fibroblasts[7]. In addition, extracts of HeLa cells have been observed to degrade UV-irradiated DNA more rapidly than unirradiated DNA[8].

Experimental evidence for the involvement of an exonuclease in the selective release of pyrimidine dimers from UV-irradiated DNA has also been obtained. An exonuclease purified from extracts of *M. luteus* hydrolyzes irradiated and unirradiated denatured DNA, but will degrade native DNA only after UV-irradiation and treatment with the UV-specific endonuclease[5]. Unlike the hydrolysis of denatured DNA, the degradation of irradiated native DNA treated with UV-specific endonuclease is limited and ceases after the release of the dimer and a few additional nucleotides. Thus, based on *in vitro* studies, it appears that at least two enzymes, a UV-specific endonuclease and a UV-specific exonuclease, are required for the selective release of pyrimidine dimers from DNA.

Completion of repair following dimer excision would involve DNA repair synthesis to fill in the repair-induced, single-strand gap and subsequent single-strand joining (i.e.--steps 3 and 4 of the proposed model). The observed incorporation of the thymidine analogue bromodeoxyuridine (BrdUrd) into parental DNA of UV-irradiated *E. coli*[9] and mammalian cells[10] may occur as a result of the postulated repair synthesis. On the basis of quantitative measurements of BrdUrd incorporation into parental DNA in association with, and presumably as a result of, the excision of pyrimidine dimers, estimates of the size of the repaired regions have been made. Repaired regions in the DNA of UV-irradiated mammalian cells appear to be rather uniform in size and represent the resynthesis of a single-strand region ∿100 nucleotides in

length[10]. In *E. coli*, however, the repaired regions appear to be heterogeneous in size with some regions being at least as long as 1500 nucleotides[9]. A model to explain the size heterogeneity of repaired regions, and data in its support, has been presented[11]. In reference to this model, the following events are proposed: 1) DNA polymerase I is responsible for the short repaired regions in *E. coli* DNA; 2) large repaired regions result from a *rec*-mediated repair synthesis; and 3) both enzyme systems normally compete for excision sites.

To effect the completion of excision repair, a DNA ligase such as that isolated from *E. coli*[12] could presumably carry out the single-strand joining of repaired regions to contiguous parental DNA.

The excision mode of repair appears to be ubiquitous in nature and has been observed in numerous procaryotic and eucaryotic organisms. In addition, excision repair does not seem to be restricted to UV-induced base damage, but may also function in the repair of base damage which results from ionizing radiation[13], and exposure to such chemicals as nitrogen mustard, mitomycin C, and psoralen[14].

References

1. P. Howard-Flanders, *Annu. Rev. Biochem.* 37:175, 1968.
2. R. B. Setlow, *Progr. Nucl. Acid Res. Mol. Biol.* 8:257, 1968.
3. R. B. Setlow, *in "Regulation of Nucleic Acid and Protein Biosynthesis"* (V. V. Koningsberger and L. Bosch, eds.), p. 51 Elsevier, Amsterdam, 1967.
4. T. Kato, *J. Bacteriol.* 112:1237, 1972.
5. S. R. Kushner, J. C. Kaplan, H. Ono, and L. Grossman, *Biochemistry* 10:3325, 1971.
6. W. L. Carrier and R. B. Setlow, *J. Bacteriol.* 102:176, 1970.
7. R. B. Setlow, J. D. Regan, J. German, and W. L. Carrier, *PNAS* 64:1035, 1969.
8. D. H. Burt and T. P. Brent, *Biochem. Biophys. Res. Commun.* 43:1382, 1971.
9. P. K. Cooper and P. C. Hanawalt, *J. Mol. Biol.* 67:1, 1972.
10. J. E. Cleaver, *in* "Advances in Radiation Biology" (J. T. Lett, M. R. Zelle and H. Adler, eds.) Vol. 4, Academic Press, 1973 (in press).
11. P. K. Cooper and P. C. Hanawalt, *PNAS* 69:1156, 1972.
12. M. Gellert, J. W. Little, C. K. Oshinsky and S. B. Zimmerman, *CSHSQB* 33:21, 1968.
13. P. V. Hariharan and P. A. Cerutti, *J. Mol. Biol.* 66:65, 1972.
14. R. S. Cole, *J. Bacteriol.* 197:846, 1971.

POSTREPLICATION REPAIR OF NUCLEOTIDE DAMAGE. W. Dean Rupp, Yale University School of Medicine, New Haven, Connecticut, 06510, U.S.A.

From the survival of UV-irradiated excision-defective E. coli, it is seen that these cells contain about 50 pyrimidine dimers per genome after a UV dose that reduces the survival to 37%. This suggests that these cells are able to bypass unexcised photoproducts in some way. A specific repair process involving recombination is implicated since excision-defective strains that are also mutant at the recA locus are killed by a UV dose that produces only one or two pyrimidine dimers per genome (1).

Physical studies of the DNA synthesized in UV-irradiated excision-defective cells demonstrate several unusual properties of the newly synthesized DNA that are quite different from the DNA made in unirradiated cells (2, 3). The new strands synthesized during a ten-minute labeling period have approximately as many gaps as there are pyrimidine dimers in the template strand. The average size of these gaps is estimated to be about 1500 nucleotides, a size that is similar to the length of the Okazaki fragments that are normal intermediates during DNA replication. The most likely interpretation of these observations is that DNA synthesis proceeds up to a pyrimidine dimer which blocks further continuous synthesis. Further polymerization is then begun at the initiation site for the next Okazake fragment. When cells are incubated for longer periods of time (50 minutes), the gaps are filled in and the new strands are converted to long continuous strands when the strain is $recA^{+}$ but not when it is $recA^{-}$ (4, 5). Experiments utilizing density labels have shown that strand exchanges between old and new strands are rare in unirradiated cells, but quite common in the UV-irradiated cells (6). Our interpretation of these results is that a recombinational event may be required to insert the correct base sequence in the gap opposite the dimer in the template strand, thus insuring that the information in a dimer-containing sequence is not lost. The accompanying schematic diagram of these events presents a simplified version of this model.

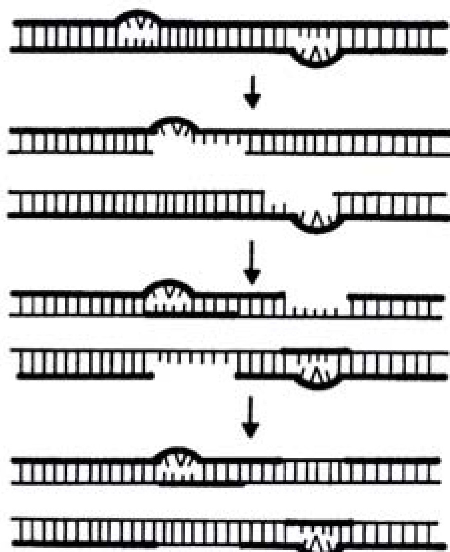
Since excision repair occurs in many organisms, we can ask whether postreplication repair is also widespread. In Haemophilus influenzae, low molecular weight strands are synthesized in UV-irradiated cells and at least one recombination gene is required for successful postreplication repair in this organism (7). Various laboratories have also reported the synthesis of short new strands in UV-irradiated mammalian cells, and the subsequent lengthening of these into long continuous strands. It might be expected that postreplication repair plays an important role in the survival of mammalian cells since dimer excision is quite slow and much DNA is replicated at times when many unexcised photoproducts remain in the DNA. One of the most detailed studies on mammalian cells demonstrates the incorporation of new nucleotides into the gaps during postreplication repair (8). No experiments designed to detect UV-induced strand exchanges have been reported in organisms other than E. coli. Unpublished experiments of Zipser and Rupp show that UV-induced exchanges in L5178Y mouse lymphoma cells are not observed with the present methods, but these techniques are not sufficiently sensitive to detect short exchanges that would be sufficient to introduce the proper nucleotides into the region opposite the template strand dimers.

One feature of this repair process is that there are no enzymes that are required for the recognition of specific photoproducts. Any type of nucleotide alteration that blocks continuous DNA polymerization is expected to produce new strands with gaps which will then be substrates for postreplication repair.

Repair involving recombination may also occur before replication in certain cases where both DNA strands are damaged. This occurs in DNA cross-linked by psoralen plus 350 nm light (9) and may also account for the X-ray sensitivity of Rec^- strains of E. coli.

REFERENCES

1. Howard-Flanders & Boyce. Rad. Res. Sup. 6, 156 (1966).
2. Rupp & Howard-Flanders. J. Mol. Biol. 31, 291 (1968).
3. Iyer & Rupp. Biochim. Biophys. Acta, 228, 117 (1971).
4. Smith & Meun. J. Mol. Biol. 51, 459 (1970).
5. Rupp, Zipser, Wilde, Reno & Howard-Flanders. In: "Time and Dose Relationships in Radiobiology as Applied to Radiotherapy". Eds. Bond, Suit & Marcini, Brookhaven Nat. Lab. p. 1 (1970).
6. Rupp, Wilde, Reno & Howard-Flanders. J. Mol. Biol. 61, 25 (1971).
7. Leclerc & J. Setlow. J. Bacteriol. 110, 930 (1972).
8. Lehman. J. Mol. Biol. 66, 319 (1972).
9. Cole. Proc. Nat. Acad. Sci. Wash. (1973) In press.



MECHANISMS FOR THE REPAIR OF DNA CHAIN BREAKS. David A. Youngs, Department of Radiology, Stanford University School of Medicine, Stanford, California 94305.

A single-strand break may be formed by ionizing radiation at any one of several locations in the sugar-phosphate backbone of DNA. The chemical nature of the break will be different at each of these locations, and as a result, the relative ease with which repair can occur will be different. The simplest type of break should require only polynucleotide ligase for repair. Other types of breaks may require modification of chain ends and/or excision of damaged bases, and DNA repair resynthesis before ligase can perform the final sealing reaction. After UV irradiation, single-strand breaks are produced enzymatically by the uvr gene products. This incision step is followed by excision of damaged bases, DNA repair resynthesis and resealing of the strand.[1,2] Much progress recently has been made in analysing the single-strand break repair processes. In E. coli K-12, the repair of single-strand breaks induced by X-radiation has been subdivided into three processes[3,4] which have been termed Types I, II, and III, corresponding to the relative speeds at which they proceed. Type I repair[3] is complete in less than one minute after X-irradiation, even at 0°C. This process may require only polynucleotide ligase action. The Type I process acts preferentially on breaks produced under anoxic conditions. It repairs ∿3-times as many DNA single-strand breaks after X-irradiation under anoxic as under aerobic conditions. Type I repair in E. coli can be inhibited by heat or cold shock treatment or by NEM or PCMB when present during X-irradiation. The Type II process[4,5] repairs ∿90% of the breaks remaining after Type I repair is complete, whether the breaks were produced by X-irradiation under aerobic or anoxic conditions. This process occurs rapidly in buffer or growth medium with a half-time of ∿1 min at 37°C. In wild-type cells this process is largely dependent on the presence of DNA polymerase I. Studies with polA strains which lack this enzyme indicate that DNA polymerase III can act in the Type II repair process at a rate which is ∿5-times slower than that observed in wild-type cells. It seems likely that Type II repair acts on a class of breaks which requires modification and/or excision of damaged bases before the repair resynthesis step. Type III repair requires growth medium, is complete in 40-60 min at 37°C[4,5], and is deficient in recA, recB, recC[6], and exrA[5] mutants of E. coli K-12. The Type III process repairs with the same efficiency breaks which are produced by irradiation under either aerobic or anoxic conditions. This was the type of repair first observed by McGrath and Williams.[7] The Type III process does not require DNA polymerase I[5] but is dependent on normal DNA synthesis since it is absent in the thermosensitive dnaB[8] and dnaE mutants at the restrictive temperature. Type III repair can be irreversibly inhibited by post-irradiation amino acid starvation or by post-irradiation treatment with inhibitors such as chloramphenicol, dinitrophenol, quinacrine, and acriflavine. By correlating the yield of DNA single- and double-strand breaks with the D_0 values for cell survival, an estimate of the contribution of chain breaks to the lethal effect of X-radiation can be made. For the polA exrA strain a maximum of 49% of the lethal events could be due to unrepaired single-strand breaks, 8% to double-strand breaks, and the remainder, a minimum of 43%, to some other lesion, possibly base damage.[5] There is evidence suggesting that X-ray-induced base damage may be repaired by a process similar to the excision repair process acting on UV-induced lesions.[9] The excision repair of UV-induced lesions and the repair of X-ray-induced single-strand breaks may involve similar steps after the initial break is produced. Thus, systems analogous to the Type II and III repair processes should be active after UV irradiation. Strains which are deficient in

Type II (polA) and III (recA, recB, and exrA) repair are also partially deficient in the excision repair of UV-induced damage.[1,10,11] In addition, the excision repair process can operationally be divided into two branches, one which can occur in buffer (similar to Type II repair) and another which requires growth medium (similar to Type III repair).[12] The exrA strain is deficient in the medium-dependent Type III repair and also lacks the medium-dependent branch of the excision repair process.[12] Thus, there appears to be good analogy between the Type II and III processes for the repair of X-ray-induced DNA single-strand breaks and the branches of the excision repair process.

---

[1]Setlow, R.B., and J.K. Setlow. 1972. Ann. Rev. Biophys. Bioengr. 1:293.
[2]Howard-Flanders, P. 1968. Ann. Rev. Biochem. 37:175.
[3]Town, C.D., K.C. Smith and H.S. Kaplan. 1972. Radiat. Res. 52:99.
[4]Town, C.D., K.C. Smith and H.S. Kaplan. 1973. Submitted to Radiat. Res.
[5]Youngs, D.A., and K.C. Smith. 1973. J. Bacteriol. 114, No. 1.
[6]Kapp, D.S., and K.C. Smith. 1970. J. Bacteriol. 103:49.
[7]McGrath, R.A., and R.W. Williams. 1966. Nature 212:534.
[8]Fangman, W.L., and M. Russel. 1971. Mol. Gen. Genet. 110:332.
[9]Setlow, R.B., and W.L. Carrier. 1973. Nature New Biol. 241:172.
[10]Cooper, P.K., and P. Hanawalt. 1972. Proc. Natl. Acad. Sci. U.S. 69:1156.
[11]Youngs, D.A., and K.C. Smith. 1973. These abstracts.
[12]Van der Schueren, E., D.A. Youngs, and K.C. Smith (in preparation).

BEHAVIORAL AND BIOCHEMICAL RESPONSES TO UV RADIATION. A. C. Giese, Department of Biological Sciences, Stanford University, Stanford, Ca.

UV covering the span 15nm to 390nm might be subdivided into the vacuum UV 15 to 200nm, far UV 200 to 310nm and near UV 310-390nm. Space UV coming directly from the sun includes the entire UV span, but the ozone layer in the atmosphere at about 20-40km absorbs all the vacuum UV and most of the far UV, therefore sunlight at the surface of the earth includes only 287-390nm, and is thus mainly near UV. The sunlight far UV, while of low intensity and variable, depending upon latitude, season of year, time of day and atmospheric conditions, has many biochemical effects upon cells and the behavior of organisms is generally such as to minimize exposure to far UV.

Higher animals tend to shun sunlight, but whether the UV component or the heat (and evaporative loss, especially in deserts) elicit this behavior has not been determined. In some cases higher plants orient their leaves to minimize exposure, and some minimize chloroplast exposure by appropriate movements inside the cell. However, some lower organisms show positive responses to low intensity far UV, changing to negative responses on increase in intensity. Many organisms avoid bright sunlight, and in some cases far UV may be the determining cue, but whether UV determines the position and movement of plankton in the sea is not known. Animals have adaptations which reduce far UV exposure of the skin: pigment, hair, feathers, scales, shells, etc. Plants also minimize entry of far UV by cuticle, although protective coverings are less feasible in plants which must have light for photosynthesis.

Plants also possess a cellular far-UV absorbing pigment (flavone). Action spectra for killing and mutation by far UV resemble absorption by nucleic acids which are considered the main cellular receptors of far UV. The major far UV-induced cellular lesion is the cyclobutane pyrimidine dimer (mainly thymidine dimer) in cell DNA. Such dimers interfere with replication leading to sterilization or mistakes (mutants?). That pyrimidine dimers are not the only UV photoproducts is indicated by the failure to achieve complete recovery by illumination (see below) and increased effectiveness of far UV action on animal cells when irradiation occurs at a higher temperature, or the same UV dose is flashed, permitting completion of secondary reactions. The nature of these secondary reactions has, however, not been analyzed. Action spectra for immobilization of ciliary activity and sensitization to heat resemble protein absorption, suggesting protein as the receptor. UV links DNA and protein, but the action spectrum for this effect has not been determined. An action spectrum may be misleading, as in sunburn and tanning, where it measures the success with which UV wavelengths penetrate the screen of dead squamous epithelium to reach the vulnerable prickle cells, not the absorption by a receptor molecule. UV-induced biochemical changes in the skin include the change from trans- to cis-urocanic acid; the latter absorbs UV 290nm strongly, thus serving a useful protective function. The prickle cells in the epidermis are killed by far UV, presumably following lysis of their lysosomes; whether the products of the lysosome breakdown or urocanic acid cause the engorgement of blood vessels in the dermis to produce erythema (reddening of the skin) is not known. Erythema is followed by tanning, melanin being introduced into the cells of the epidermis by melanocytes which in an unknown way are stimulated to activity by far UV.

UV-induced DNA damage is subject to repair either by photoreactivation (PR) or dark repair (DR). In PR the photoenzyme which is widely distributed in cells, though not present in cells of mammals, combines selectively with UV-DNA (not native DNA) and absorbs visible light, splitting

the dimers. "Cut and patch" DR may occur; an endonuclease excising about a dozen nucleotides including the dimer; under action of the DNA-polymerase the piece of DNA is replaced complementary to the intact strand and bound to the DNA ends by a ligase. Or, replication may occur around a dimer and in postreplicative repair the missing portion is then replaced by a mechanism much like cut and patch. Cells without effective repair mechanisms are much more sensitive to far UV than those which have them; this is most spectacularly shown in humans with xeroderma pigmentosum in which a single exposure of human skin to far UV may result in cancer.

Near UV is important in vitamin D production in vertebrate skin. Near UV also has deleterious effects, but the doses required are of a different order of magnitude from effective far UV doses. However, in the presence of natural sensitizers, the near UV may be as damaging as far UV. Some natural sensitizers require oxygen, that is, they promote photooxidations, e.g., the hypericins (from plants) and the porphyrins (products of a deranged porphyrin metabolism), while others, the furocoumarins (produced by several families of plants), do not. The hypericins and porphyrins affect cell membranes while the furocoumarins form adducts with cellular DNA. Consequences, behavioral and biochemical, will be discussed.

Life has been exposed to sunlight UV radiation since its beginning. In fact, because the primeval atmosphere probably had little oxygen, the land was abiotic and was occupied by living things only after the development of oxygen-producing photosynthetic organisms, when enough oxygen accumulated to form the ozone screen. Repair mechanisms were even more essential to early life than now; perhaps the prevalence of such mechanisms is a heritage from this period of much greater UV hazard.

References

Plant responses: Caldwell (higher plants) Photophysiology 6(1971): 131-77. Halldal and Taube (algae) Photophysiology 7(1972):163-188. Virgin (cells and chloroplasts) Photophysiology 1(1964):273-303.

UV effects on animal cells: Giese (general) Photophysiology 2(1964): 203-245. Painter (mammalian cells) Photophysiology 5(1970):169-189.

Mutation: Zetterberg (general) Photophysiology 2(1964):203-245. Giese (action spectra) Photochemistry and Photobiology 8 (1968): 527-546. Witkin (mechanism) Ann. Rev. Genetics 3 (1969):525-552.

Photosensitization: Giese (general) Photophysiology 6(1971):77-129.

Sunburn: Johnson, Daniels and Magnus, Photophysiology 4(1968):139-202.

Cancer: Epstein, Photophysiology 5(1970):235-273.

Repair mechanisms: Hanawalt (Microorganisms) Photophysiology 4(1968): 203-251. Cook (photoreactivation in animal cells) Photophysiology 5(1970):191-233. Smith (recombination repair) Photophysiology 6 (1971):209-278. Smith and Hanawalt, 1969, Molecular Photobiology, Academic Press.

A SURVEY OF BIOLOGICAL AND PHYSICAL SCHEMES FOR ENERGY CONVERSION.

Roderick K. Clayton, Cornell University, Ithaca, New York, 14850.

Various schemes for large scale energy conversion, some involving biological processes, will be surveyed. The following topics will be examined briefly.

1. Fossil fuels: Can we exploit known reserves with more economy and less pollution?
2. Wastes; their exploitation to make methane or to grow algae. Can thermal pollution be turned to good use here?
3. Nuclear energy and the question of thermal pollution.
4. Other terrestrial sources of energy: tides, ocean currents, winds, and geothermal energy.
5. Solar energy.
   a. Direct heating.
   b. Growth of grain and hence of cattle and chickens. Growth of algae, aided by nutrient wastes.
   c. Photosynthetic production of hydrogen from water.
   d. Solar batteries: Inorganic, organic; cells patterned on photosynthetic models.

Intrinsic physical limitations on the efficiency of solar energy conversion will be discussed.

References:

Single-Cell Protein. R. I. Mateles and S. R. Tannenbaum, Eds. M.I.T. Press, Cambridge, Mass., 1968.

Summary Report of the Cornell Workshop on Energy and the Environment. Superintendent of Documents, U. S. Government Printing Office, Wash., D. C., 1972.

Solar Cells: Outlook for Improved Efficiency. Ad hoc panel on solar cell efficiency, National Research Council. Space Science Board, 2101 Constitution Avenue, Wash., D. C., 1972.

BIOPHOTOLYSIS OF WATER TO HYDROGEN AND OXYGEN.

L. O. Krampitz, Department of Microbiology, Case Western Reserve Univ., Cleveland, Ohio.

The research to be reported is concerned with the biophotolysis of water to hydrogen and oxygen by coupling the activity of the bacterial enzyme, hydrogenase, with the reducing power created by photosystems I and II of the photosynthetic apparatus. It is generally accepted that the two systems operating in tandem are capable of placing an electron from the oxygen-water couple at +0.8 volts to a potential as low as -0.7 volts, pH 7.0. Since at this pH the hydrogen electrode has a potential of -0.42 volts it is thermodynamically feasible to employ the electrons obtained by the two photosynthetic systems to obtain hydrogen.

After the discovery that ferredoxin was involved in pyruvate oxidation and hydrogen formation by some anaerobic bacteria (1) and that a ferredoxin from plant material was identical to the photosynthetic pyridine nucleotide reductase discovered by San Pietro and colleagues, Arnon and colleagues (2) showed that photosystem I could be coupled with a bacterial hydrogenase to form hydrogen with ascorbate or cysteine as the electron donor.

Our efforts have been directed toward bringing photosystem II into operation in conjunction with photosystem I in order that water may be the electron donor. The viologen dyes, which are 4,4' dipyridylium salts, have been used as electron acceptors for photosystems I and II with water as the electron donor, or with only photosystem I employing ascorbate, cysteine and other reductants as the electron donor (3)(4)(5). Many of these dyes have an oxidation-reduction potential as low or lower than the hydrogen electrode. Peck and Gest (6) were able to obtain substantial quantities of hydrogen from reduced 1,1'-dimethyl 4,4' dipyridylium dichloride (methyl viologen, E_0' -0.45V) catalysed by bacterial hydrogenases.

An experimental difficulty employing the viologen dyes as oxidants for photosystems I and II is that the reduced forms are extremely autoxidizable, and measures must be taken to trap the liberated photosynthetic oxygen. To test the feasibility of obtaining hydrogen from water by photosystems I and II, we employed the blue-green alga <u>Gloeocapsa alpicola</u> with methyl viologen as the electron acceptor. We then coupled the reduced viologen with a hydrogenase preparation obtained from <u>Escherichia coli</u> to obtain hydrogen. To trap the evolved oxygen a double trap was employed, <u>i.e.</u>, glucose oxidase and glucose in addition to catalase and ethanol. With an experimental volume of 150 milliliters, ten µmoles of reduced methyl viologen were obtained and 4.2 µmoles of hydrogen were evolved when the reduced viologen was added to the hydrogenase preparation. Monuron and diuron inhibited the formation of reduced methyl viologen, indicating that the electrons originated from water.

With the cooperation of Dr. George Olah, Professor of Organic Chemistry at our institution, we have embarked upon a program of synthesizing 4,4' dipyridylium salts with substitutions of electron

withdrawing or donating groups on the 1, 1' quaternary nitrogens. Seven compounds have been prepared and their ability when reduced to form hydrogen in the presence of hydrogenase was compared to 1, 1' dibenzyl 4, 4' dipyridylium dichloride (benzyl viologen).

		Relative Activity
Benzyl viologen	=	100
R_1 = 4-fluorobenzyl	=	60
R_2 = 3-fluorobenzyl	=	95
R_3 = 2, 4-dichlorobenzyl	=	25
R_4 = 3-methylbenzyl	=	30
R_5 = 7-carboxylbenzyl	=	80
R_6 = 4-nitrobenzyl	=	30
R_7 = 3, 4-dimethylbenzyl	=	35

In order to obtain information regarding the autoxidation of the reduced species of each compound (free radical) electron spin resonance studies have been performed. These results will be reported.

Results of experiments designed to obtain electron acceptor compounds other than the viologen dyes which are precursors for the hydrogenase reaction will be reported.

1. Mortenson, L.E., Valentine, R.C. and Carnahan, J.E. Biochem. Biophys. Res. Comm. 7, 448 (1962).

2. Tagawa, K. and Arnon, D.I. Nature 195, 537 (1962).

3. Zweig, G. and Avron, M. Biochem. Biophys. Res. Comm. 19, 397, (1965).

4. Kok, B., Rurainski, J.R., and Owens, O.V. Biochim. Biophys. Acta 109, 347 (1965).

5. Black, C.C. Biochim, Biophys. Acta 120, 332 (1966).

6. Peck, H.D. Jr. and Gest, H.J. Bact. 71, 70 (1956).

Supported by the National Science Foundation Grant GI-34992X.

ENGINEERING SYSTEMS FOR BIOLOGICAL ENERGY CONVERSION PROCESSES. Lloyd Herwig, National Science Foundation (RANN)

THE CONTROL OF THREE BRANCHES OF THE EXCISION REPAIR PROCESS IN ESCHERICHIA COLI K-12 BY THE polA, dnaE, AND exrA GENES.

David A. Youngs and Kendric C. Smith, Department of Radiology, Stanford University School of Medicine, Stanford, California 94305.

We have examined the ability of the polA, exrA, polA exrA, and polA dnaE strains of E. coli K-12 to repair single-strand breaks produced in the DNA by the excision repair process after UV irradiation. The polA[1,2] and exrA mutants of E. coli K-12 are partially deficient in the repair of incision breaks. The polA exrA strain was more deficient in the repair of incision breaks than a closely related polA exr+ strain, indicating that the effects of the polA and exrA mutations on this process are at least partially independent. However, the polA exrA strain was not completely deficient in repair of incision breaks, suggesting the existence of still another branch of excision repair which is independent of both the polA and exrA gene products. Results with a polA dnaE strain (the dnaE mutation results in production of thermosensitive DNA polymerase III) indicate that the repair of incision breaks which occurs in a polA strain is largely dependent on DNA polymerase III activity. These results suggest that the exrA branch of excision repair may also require DNA polymerase III. Thus the available evidence suggests that a minimum of three branches may be present in the excision repair process in a wild-type cell: (1) a DNA polymerase I dependent pathway, (2) an exrA gene dependent pathway, and (3) a DNA polymerase III dependent pathway. Evidence for a rec-gene dependent pathway of excision repair, which is independent of polA1, has also been reported[3].

[1]Paterson, M.C., J.M. Boyle, and R.B. Setlow. 1971. J. Bacteriol. 107:61.
[2]Kanner, L., and P. Hanawalt. 1970. Biochem. Biophys. Res. Commun. 39:149.
[3]Cooper, P.K., and P. Hanawalt. 1972. Proc. Nat. Acad. Sci. USA 69:1156.

TAM-C2

SENSITIZATION OF ESCHERICHIA COLI K-12 CELLS TO UV IRRADIATION BY 2,4-DINITROPHENOL.

Emmanuel Van der Schueren and Kendric C. Smith, Department of Radiology, Stanford University School of Medicine, Stanford, California 94305.

UV-irradiated E. coli K-12 wild-type cells were sensitized by a post-irradiation treatment with 10^{-2} M 2,4-dinitrophenol (DNP). This sensitization was not found in uvrB cells, suggesting that DNP interferes with the excision repair system. Earlier data from survival[1] and sedimentation experiments[2,3] have implicated DNA polymerase I in excision repair. More recently additional pathways of excision repair dependent on recA recB[4] and exrA[5] have been proposed. The polA1 strain was sensitized to the same extent as the wild-type strain by DNP but the survival of an exrA strain after UV irradiation was not changed by incubation in DNP. Thus, DNP treatment seems to interfere with the postulated exrA gene-dependent part of the excision process. Sedimentation studies confirm the hypothesis that DNP interferes with the repair of incision breaks in DNA produced by the excision repair process. Recombination deficient strains (recA, recB and recA recB) were protected by a DNP treatment after irradiation. This protective effect was dependent on the presence of functional uvr genes and was absent in recA uvrB and recB uvrB strains.

[1]Monk, M., Peacey, M. and Gross, J.D. (1971) J. Mol. Biol. 58:623.
[2]Kanner, L. and Hanawalt, P. (1970) Biochem. Biophys. Res. Commun. 39:149.
[3]Paterson, M.C., Boyle, J.M. and Setlow, R.B. (1971) J. Bacteriol. 107:61.
[4]Cooper, P.K. and Hanawalt, P. (1972) Proc. Natl. Acad. Sci. U.S.A. 69: 1156.
[5]Youngs, D.A. and Smith, K.C. J. Bacteriol. (submitted).

MEMBRANE CHANGES IN UV-IRRADIATED E. COLI B/r CELLS THAT DIE OF RESPIRATORY FAILURE.

P. A. Swenson and R. L. Schenley, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Ionic and non-ionic detergents have little effect on respiring bacteria but if the cultures are poisoned with KCN, rapid lysis, as indicated by turbidity losses, takes place. Ultraviolet (UV) radiations cause *Escherichia coli* cells grown in minimal medium with glycerol as a carbon source to cease respiring and growing about an hour after irradiation. We tested the effect of the non-ionic detergent Triton X-100, on growth and plasma membrane dissolution (both measured by turbidity changes), respiration, and viability of unirradiated and irradiated *E. coli* B/r cells. When added to cells immediately after irradiation (520 ergs/mm^2 at 254 nm), a decrease in turbidity occurred only when respiration was about to cease; when added after cessation of respiration, the turbidity loss set in immediately. In both cases the turbidity loss was about 60%. At a lower UV dose where cessation of respiration and growth is only transitory, detergent treatment caused a turbidity loss at the same time as for the high dose, but the loss was followed by an increase; the initial loss in turbidity we interpret as due to dissolution of plasma membranes of non-respiring cells and the increase to the growth of the respiring cells in the population. In all cases with irradiated cells, the detergent had only a negligible effect on respiration and viability. We conclude that Triton X-100 causes dissolution of plasma membranes only of non-respiring cells, i.e., those cells not destined to survive. These results support our previous conclusion that UV causes death through respiratory failure of a fraction of the irradiated population. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

TAM-C4

ACTION SPECTRA FOR THE KILLING OF UV-SENSITIVE SPORES OF BACILLUS SUBTILIS.

N. Munakata*, Lynn Marple*, T.T. Meng* and C.S. Rupert, Inst. for Molecular Biology, University of Texas at Dallas, Dallas, Texas 75230.

Bacterial spores are unique forms of organisms suitable for obtaining photobiological parameters under various experimental conditions, which can also be used to monitor the intensity of environmental radiations. For these purposes, the spores of *B. subtilis* strain, UVSSP-42-1, which have been shown to be deficient in two of the dark repair mechanisms removing UV-induced thyminyl-dihydrothymine from spore DNA, have certain advantages because they are highly UV sensitive, and the UV-dose-survival curves exhibit straight exponential kinetics at any wavelength. We have obtained the action spectra for the killing of these spores, showing here each wavelength in nm and the dose in erg·mm^{-2} (in parentheses) which kills 90% of the initial population: 230(59), 240(50), 265(25), 270(20), 280(32), 290(260), 300(4,300), 313(85,000). Grossly the pattern seems to correspond to the absorption coefficient of thymidine. However, some fine structure is also observed with a peak at 270 nm and a dip around 275 nm, which might be caused by the presence of dipicolinic acid. Spores in which a portion of thymidine residues was substituted by 5-bromodeoxyuridine, showed decreased sensitivity below 285 nm, but higher sensitivity above this wavelength. This also argues that direct UV adsorption by thymidine or by 5-BU leads to the lethal damage in the spore DNA, with quantum yields dependent on the molecular species involved. Supported by NIH Research Grant No. 5 R01 GM 16547 from the National Inst. of General Medical Sciences and the University of Texas at Dallas Research Fund.

RECOVERY IN U.V. SURVIVAL CURVES.* A. E. S. Green, J. H. Miller and E. C. Pollard, (intr. by D. Billen), Dept. of Physics and Astronomy, University of Florida, Gainesville, Florida 32601.

We examine a number of approaches for characterizing recovery phenomena following exposure to ultraviolet radiation. First we assemble a set of experimental ultraviolet radiation-dose response curves under various conditions that influence recovery. These data are examined in relationship to the survival-dose functions of Haynes[1] (H), Green and Burki[2] (GB), and a generalization of GB to include multiple genomes (MGB), each of which attempts to incorporate recovery functions. The comparative analytic features of these recovery functions are examined along with other simple variants. The parameters in these equations are adjusted by a non-linear least square search routine which assigns a relative chi square to the fits. Using this ensemble of fits as a guide and our knowledge of the factors which modify radio-sensitivity in the cases studied, we attempt to converge upon an accurate and physically meaningful recovery function. Finally, we explore various ab-initio characterization based upon current knowledge of recovery mechanisms at the molecular level.

[1]R. H. Haynes, Radiation Research Supplement 6, 1-29 (1966).
[2]A. E. S. Green and John Burki (submitted for publication).

*Supported in part by the United States Atomic Energy Commission.

TAM-C6

TEMPERATURE DEPENDENCE OF ULTRAVIOLET RADIATION SENSITIVITY OF CLEAN, DRY T-1 BACTERIOPHAGE: RELATION TO A HOST-REPAIRABLE COMPONENT OF IONIZING RADIATION DIRECT ACTION. D. J. Fluke, Department of Zoology, Duke University, Durham, N. C.

The direct action of ionizing radiation on T-1 bacteriophage, dried from nutrient broth and irradiated *in vacuo*, results in differing plaque survival on *E. coli B* and *E. coli* B_{S1}. The difference suggests that a component, possibly excitational, of ionizing radiation damage may be susceptible to the host repair systems by which B and B_{S1} host strains differ. The dependence of the difference upon irradiation temperature is not markedly different from that evident for direct action in general, however. It has been possible to dry a fresh T-1 lysate from 1/400 nutrient broth in water, with reasonable stability of plaque survival, and to check directly the radiation sensitivity, dry, for 254 nm ultraviolet radiation and fast electrons. These UV-transparent phage preparations were irradiated *in vacuo* in the same apparatus used for fast electron irradiations. They showed little irradiation temperature dependence in sensitivity toward 254 nm radiation, although differing notably in sensitivity with respect to the two host strains, of course. Parallel experiments with fast electrons on the same phage preparations also showed a substantial decrease in temperature dependence, on either host, for irradiation at 300°K and 77°K. The host-related difference in sensitivity of such direct action, however, increased from about 12% to about 30% of that on *E. coli B*, by the removal of broth from the electron-irradiated preparations. The results overall continue to be consistent with a substantial excitational component of the direct action. Work supported by A.E.C. Contract No. AT(40-1)3631.

DIFFERENT GENETIC FORMS OF XERODERMA PIGMENTOSUM. K.H. Kraemer*, H.G. Coon* and J.H. Robbins. National Cancer Institute, NIH, Bethesda, Md.

We have studied genetic heterogeneity among xeroderma pigmentosum (XP) patients by a cell-fusion method similar to that of De Weerd-Kastelein, Keijzer and Bootsma who have reported the existence of 2 complementation groups of XP patients (Nature, New Biol. 238: 80, 1972). We now report finding 4 complementation groups in a series of 7 patients whose cells have impaired initial rates of UV-induced tritiated thymidine (^{3}HTdR) incorporation. Using killed Sendai virus, heterokaryons were made by fusing fibroblasts from all the possible pairings of the XP patients. The cells were irradiated with 150 ergs/mm^2 from a germicidal lamp, incubated at 38° with ^{3}HTdR for 3 hr., washed, and processed for autoradiography. Analysis of the number of grains over the nuclei in binucleate cells revealed that each XP cell line could be assigned to one of 4 complementation groups. Heterokaryons formed by fusing cell lines from any 2 groups gave 70-100% of the UV-induced ^{3}HTdR incorporation of normal donors' unfused fibroblasts, whereas heterokaryons formed by fusing cells within the same group gave no more incorporation per nucleus than was found in the same cells unfused. The 4 complementation groups were found to represent 4 classes of repair rates: unfused cells of group A (2 patients) had $<$ 2% of the normal rate of incorporation; group B (1 patient), 3-7%; group C (2 patients), 15-25%; and group D (2 patients), 25-55%. Preliminary experiments with cell lines from 2 additional patients indicate that they are in group C. One of the patients in group A reportedly has severe neurological involvement (DeSanctis-Cacchione syndrome) while the other has no reported neurological abnormalities. There are neurological abnormalities in the patients of 2 of the other 3 groups, and each of these 3 groups may prove to have certain of its own distinct clinical features.

TAM-C8

EFFECT OF CELL PHYSIOLOGICAL CONDITION ON MAMMALIAN HOST CELL REACTIVATION. C. D. Lytle, S. G. Benane,* and C. F. Blackman, USDHEW, P.H.S., F.D.A., Bureau of Radiological Health, Division of Biological Effects, Rockville, Maryland 20852 and Environmental Protection Agency, National Environmental Research Center, Research Triangle Park, North Carolina 27711.

Several important aspects of host cell reactivation (HCR) of UV-irradiated virus are known, including the central role of excision repair. Further investigation regarding the generality of HCR and factors which influence it are reported here. From experiments with 18 different human cell cultures, no significant differences were found in HCR ability for UV-irradiated herpes simplex virus among cells from different organs or from individuals of different sex or age. Two aspects of culture *in vitro* "age" were observed: (1) the HCR ability (determined by D_{37}) increased somewhat with passage number; (2) infection of cultures at increasing times after a particular subculture demonstrated a small increase in D_{37} and a marked decrease in the relative level of the second component of the two-component survival curve. A similar depression of the second component was found when depleted, rather than new, medium was used. An experiment with mouse Balb/3T3 cultures indicated that normal, contact-inhibited cells have a one component survival curve, while a transformed culture had an additional, less sensitive component. Taken together, these data indicate that (1) several cell culture conditions have only a small effect on HCR (as measured by the slope of the survival curves), and (2) those conditions which tend to inhibit cell growth depress the relative level of the second component of the survival curve. Thus, some aspects of host cell reactivation are related to cell growth.

ULTRAVIOLET AND X-RAY ENHANCED REACTIVATION OF HERPES SIMPLEX VIRUS IN NORMAL AND MALIGNANT RAT AND HUMAN CELLS. K. B. Hellman, K. Haynes*, C. D. Lytle and L. Bockstahler, DHEW, FDA, Bureau of Radiological Health, Division of Biological Effects, 12709 Twinbrook Parkway, Rockville, Maryland 20852.

The phenomena of ultraviolet and x-ray enhanced reactivation were studied in normal and malignant rat and human cells by measuring the survival of ultraviolet irradiated Herpes simplex virus. The rat cultures consisted of primary embryonic cells and an adenocarcinoma cell line which were derived from the Osborn-Mendel rat, to minimize strain differences. The normal and malignant human cultures were primary embryonic lung and HeLa cells, respectively. Similar levels of host cell reactivation in the two component survival curves were found in both cell cultures of each species. Ultraviolet and x irradiation of malignant rat cells resulted in an enhancement of UV irradiated virus survival. UV irradiation of HeLa cells gave a similar but lower enhancement. The effect of UV irradiating the malignant cells is to change the slope of the second component (larger D_{37}) of the two component virus survival curves without changing the percent of infected cells expressing that component. Although enhanced reactivation of the virus was observed in the malignant cells, no ultraviolet and little, if any, x-ray enhancement was demonstrated in the normal cells. These results indicate that host-cell reactivation and UV or x-ray reactivation may operate independently in mammalian cells.

TAM-C10

CELLULAR REPAIR OF ULTRAVIOLET IRRADIATED ADENOVIRUS-2 IN NORMAL HUMAN FIBROBLASTS. Rufus S. Day, III, Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

A plaque assay for adenovirus-2 on human fibroblast cultures has been developed and used to measure the survival of ultraviolet irradiated virus on 14 human fibroblast cell lines. When 4 Xeroderma Pigmentosum (XP) cell lines were used as viral hosts, the dose to inflict an average of one lethal hit per virus in the viral population (D_{37}) was 85, 150, 620, and 780 ergs/mm^2, while when using 9 normal cell lines as hosts, 2000, 2090, 2010, 2650, 2150, 2200, 2080, 2270, and 1950 ergs/mm^2 were required to inflict the same damage. In the case of an XP cell line which had been judged to have normal repair by others (Burk et al. J. Lab. Clin. Med. 77, 759, 1971; Cleaver, J. Invest. Dermatol. 58, 124, 1972) the adenovirus-2 D_{37} is only 1470 ergs/mm^2, demonstrating a possible repair deficiency in this line. The inactivation curves were repeated between 2 and 13 times on each cell line. All viral survival curves using normal fibroblasts and the "variant" XP line as hosts were linear down to at least a surviving fraction of .001-.0001 on a log survival vs. linear dose plot, while the curves using the 2 most sensitive XP lines showed resistant tails extrapolating back to less than 1% of the viral plaque forming population. The results of three experiments show that the tail is not due to multiplicity reactivation.

PROTECTIVE INTERACTION BETWEEN FLAVIN AND UV-IRRADIATED DNA. D. Roth, Department of Pathology, NYU Medical School, New York, N.Y.

Mechanisms by which flavins were observed to protect DNA against far-UV radiation were investigated. UV-induced strand separation at thymine photolesions in native calf thymus DNA was indicated by a shift toward linearity in the thermal dissociation curve of the intercalative DNA-acriflavine complex. Proportionate changes in fluorescence intensity of the dissociated acridine dye served as specific, quantitative marker for the change in DNA structure produced by thymine photodimerization. Sensitivity was higher than could be achieved by absorption spectrophotometry or viscometric method. We found that $5X10^{-6}$ to $5X10^{-5}$M FAD, in the presence of oxygen, furnished protection which became virtually complete at the higher concentration. The protection was greater than that which could be attributed to a simple filter effect, and suggested the occurence of an interaction between flavin and DNA during irradiation. This agrees with existing evidence that photoexcited FAD readily interacts with nucleotide bases, raising the possibility of an energy sink mechanism whereby potentially injurious photons absorbed by DNA may be transferred to the flavin and dissipated harmlessly. Such a protective mechanism may operate physiologically in the cornea, whose exceptionally high riboflavin content may be a substitute for hair, keratin, and melanin, necessarily absent from corneal epithelium.

TAM-C12

METABOLIC MAPS AND SUBSTRATE IRRADIATION TIME

Ralph L. Sherman, Sorin Comorosan* and Wang H. Yee*
J.M. Richards Laboratory, Grosse Pointe Park, Michigan

A homomorphism exists between classic metabolic maps of intermediary metabolism and maps constructed of the t_m and τ parameters of the respective enzymes. Effective substrate irradiation can produce a ten percent enhancement of the initial velocity of a substrate-enzyme reaction. The effectiveness of the irradiation is wave length, intensity and time dependent. The periodic nature of the time dependency gives rise to the two parameters t_m and τ used in this discussion and defined as follows: $T = t_m + n\,\tau$, $n = 0,1,\cdot,\cdot, 5$ where, T is an effective irradiation time, and t_m and τ are experimentally determined parameters for each specific enzyme (wave length and intensity held constant at their optimum values).

TAM-D1

THE ISOLATED, PERFUSED EYE: A USEFUL TOOL IN PHARMACOLOGY, PHOTOBIOLOGY AND PHYSIOLOGY, Anthony L. Marchese and Alexander H. Friedman, Department of Pharmacology and Therapeutics, Loyola University Stritch School of Medicine, Maywood, Illinois 60153.

Gouras and Hoff (Invest. Ophthal. 9(5)388-399, 1970) demonstrated the feasibility of studying apparently-normal physiological function in the isolated, intact eye perfused through its own arterial circulation by an oxygenated blood substitute. Such a preparation can be stimulated under steady-state conditions by mono- or polychromatic light from a xenon source and its viability monitored by observing the electroretinogram (ERG) and optic nerve potential elicited by pulses of high intensity light throughout the perfusion period. We will discuss the advantages of the preparation in the detection of arterio-venous differences in constituents of the synthetic perfusion fluid and show how the venous effluent can be collected, fractionated, characterized neurochemically and subsequently bioassayed in *in vitro* and *in vivo* preparations. The response of cultures of retinal pigment epithelium (RPE) of mammalian origin to perfusate constituents will be demonstrated utilizing time-lapse photography and electron microscopy. The application of the isolated, perfused eye technique to neuropharmacological studies will be illustrated. (Supported in part by USPHS GRSG and The Wood Foundation)

TAM-D2

ACTION SPECTRUM AND LIGHT ADAPTATION OF THE LIGHT-INDUCED MEMBRANE HYPERPOLARIZATION IN *APLYSIA* L-10 NEURONS. M. C. Andresen*, F. G. Sweeney*, and C. L. Brandt. Biology Dept., California State Univ., San Diego, Cal.

The large autoactive L-10 neuron of the isolated abdominal ganglion of *Aplysia californica* is photosensitive, responding to flashes of light with a dose-dependent membrane hyperpolarization which produces a decrease in frequency or cessation of the normal spiking pattern. The dose of monochromatic light producing a defined "threshold hyperpolarization" was determined at 10nm intervals from 350nm to 700nm on each of more than 20 individual neurons (these neurons survive 18 to 20 hours *in vitro* making possible very long experiments). A composite action spectrum constructed from these data resembles carotenoid absorption with a broad peak centered around 480nm. The exposure dose eliciting a "threshold hyperpolarization" at 480nm averages 3.3×10^{10} Q/mm^2 and is about two log units less than the dose required at 610nm and 360nm. Adaptation of the light-induced membrane hyperpolarization occurs to a series of one-half second light flashes delivered at one minute intervals, the response to each flash successively decreasing and reaching 33% of the initial response on the ninth flash. This light adaptation displays pseudo-first order kinetics. An action spectrum resembling carotenoid absorption and showing a primary peak around 480nm has been derived from the kinetic data on light adaptation determined at 10nm intervals. Dark adaptation is slow and follows a hyperbolic time course reaching half-maximum in about 25 minutes. The monochromatic light used in these studies was isolated from a super-high pressure mercury arc with a B and L monochromator set to a 10nm bandpass. A reduced image of the exit slit (1 x 2mm) was focused on the neuron. Irradiance at the cell surface was measured with a photocell calibrated at each wavelength interval with a calibrated thermopile.

THE EFFECTS OF LIGHT ON THE RESPIRATION OF VERTEBRATE AND INVERTEBRATE RETINAS WITH SPECIAL REFERENCE TO THE EFFECTS OF ACETYLCHOLINE AND GAMMA-AMINOBUTYRIC ACID ON THE FROG RETINA. M. J. Jaffe and E. L. Pautler*, Botany Dept. Ohio Univ., Athens, Ohio & Biophysics Dept. Colorado State Univ., Ft. Collins, Colorado.

Dark adapted retinas of 4 vertebrates (*Lepomis macrochirus*, *Rana pipiens*, *Gallus gallus* and *Rattus rattus*), 1 invertebrate (*Octopus binaculatus*), and the compound eye of the lobster (*Homarus americanus*) were examined by electronic respirometry to test the effect of darkness and 600 rpm flicker light on oxygen uptake. In all cases but 1, respiration in flicker light was significantly lower than in darkness. The reverse was true in the octopus retina. The effects of a number of neurotransmitters was tested on the frog retina. 100 uM acetylcholine (ACh) significantly increased respiration above the dark level, and 100 uM gamma-aminobutyric acid (GABA) decreased O_2 uptake to the level normally found in flicker light. When cholinergic drugs were tested, both 100 uM d-tubocurarine and atropine decreased dark respiration to a level below that found in the light. Because of these data and observations reported by others, we suggest a possible mechanism for the regulation of respiration in darkness and light based on control by ACh and GABA. The flicker light effect on respiration was observed in the presence of 10 mM aspartic acid, but ACh, GABA and atropine had no effect on the electroretinogram or on the PIII component. We therefore conclude that light activated electrical events and light induced respiratory changes are not causally related. They probably occur in parallel and their possible relationship to the visual process is discussed. Supported by the National Science Foundation.

TAM-D4

AN EXPLANATION FOR THE PURKINJE SHIFT IN FISHES. F. W. Munz and W. N. McFarland, Dept. of Biology, Univ. of Oregon, Eugene, Oreg., and Section of Ecology and Systematics, Cornell University, Ithaca, N. Y.

Visual pigments were extracted from the retinae of a large and diverse sample (179 species, in 40 families) of tropical marine fishes. By the method of partial bleaching, most of these were found to be retinal-1 pigments, with their maxima tightly grouped between 489 and 502 nm; but a few have λ_{max} at shorter wavelengths. The spectral position of these pigments does not correlate with diurnal or nocturnal activity patterns, nor do closely related species necessarily have similar pigments. In general, the deeper-living species have rhodopsins with λ_{max} at shorter wavelengths, a correlation with the bluer quality of light in deeper water.

The spectral distribution of light was measured in air and underwater at a coral atoll. During twilight the underwater spectrum narrows and shifts to shorter wavelengths, as a result of increased absorption of yellow-orange light by the atmosphere. This effect at morning and evening twilight is unique among the general spectral events of each day.

Of critical importance to fish survival are the twilight periods, during which predation becomes maximal. Visually, these are awkward times of slow transition between the photopic and scotopic systems. These facts are reflected in complex behavioral sequences that accompany the exchange of diurnal and nocturnal faunas. In both predators and prey, rhodopsins have been selected that match the spectral distribution of twilight. The Purkinje shift is an evolutionary adaptation that maximizes visual sensitivity during the critical and visually difficult twilight periods.

CHANGES IN TROUT VISUAL PIGMENTS. D.M. Allen, Department of Zoology, Univ of Michigan, and W.N. McFarland, Ecology and Systematics, Cornell Univ.

The proportion of two visual pigments (rhodopsin and porphyropsin) in the retinae of some trouts changes in response to experimental or field photic conditions. The brown trout, Salmo trutta, has evolved a more red sensitive and less labile rhodopsin-porphyropsin mixture; its porphyropsin pigment always dominates regardless of photic conditions. Rainbow trout, Salmo gairdneri, and brook trout, Salvelinus fontinalis, have rhodopsin-porphyropsin mixtures that change in response to photic conditions - inceasing rhodopsin in darkness and decreasing rhodopsin in light. Wild cutthroat trout, Salmo clarki, living in shaded areas, had more rhodopsin during winter and spring than fish living in non-shaded areas of the same stream. The potential for demonstrating these responses may depend on several factors, but light intensity, photoperiod, season and temperature seem to be primary. This evidence confirms earlier evidence that at least two types of responses - one in which light favors rhodopsin and darkness porphyropsin - and one in which the opposite is true (trouts) co-exist amongst the freshwater fishes. In species of both types, rhodopsin increases in summer and decreases in winter. The opposite condition has not been reported.

TAM-D6

THE EFFECT OF TEMPERATURE ON RHODOPSIN-PORPHYROPSIN RATIOS IN PAIRED PIGMENT FISHES. William N. McFarland and Donald M. Allen, Division of Biology, Cornell University, Ithaca, N.Y. and Dept. of Zoology, University of Michigan, Ann Arbor, Mich.

The trout, Salmo gairdneri, and the common shiner, Notropis cornutus, were exposed for a period of one-month to continuous temperatures of 4.5° and 14°C. Groups of 16 fish were maintained in each temperature and exposed to either continuous light, a 12L/12D photoperiod, or, to continuous darkness. After sampling and analyzing for the percent porphyropsin in initial controls in each species, 8 fish were sampled after two and after four weeks of exposure to each treatment. For both the trouts and shiners, fish exposed to the higher temperature (14°C) had a smaller percentage of porphyropsin in their retinae than fish exposed to the lower temperature (4.5°C). The effect of higher temperature in reducing porphyropsin percentage was independent of the effect of light or darkness on porphyropsin percentage in rainbow trout and the common shiner. Normally, light has opposite effects, as does darkness, on these species, light inducing an increase in porphyropsin in the trout and a decrease in the shiner. The mechanism of temperature action remains obscure, but the net effect is to set the rhodopsin-porphyropsin balance under given light conditions at a lower level. The possible involvement of seasonal temperature changes in changing rhodopsin-porphyropsin percentage is discussed.

STUDIES ON THE MOLECULAR ORGANIZATION OF ROD OUTER SEGMENT MEMBRANES. Edward Dratz, Rodger Raubach, Peter Nemes*, Janine Perlman*, Kendall Madden* and Stephen Schwartz*, Division of Natural Sciences, Univ. of California, Santa Cruz 95060.

Several membrane impermeable reagents react with rhodopsin in the disk membranes. A membrane permeable imidoester, ethyl acetimidate, labels all the amino groups of rhodopsin and the lipids in the intact membrane. The impermeable analog, sodium isethionyl acetimidate, reacts with four of rhodopsin's amino groups in the intact membrane.

We have found that a large amount of polysaccaride is normally bound to the disk membrane in the extradiscal space of the rod. In our previously reported preparation fluorescein isothiocyanate (FITC) would not react with rhodopsin in the membranes[1]. Howevever, if the polysaccaride is removed from the membrane preparation by water washing four FITC molecules react per rhodopsin.

Results will be presented on Concanavalin A and wheat germ agglutinin binding to the membranes and on other membrane impermeable reagents.

Rhodopsin is exposed to the aqueous medium, probably on the extradiscal side of the membrane. An analysis of the low angle X-ray data on the membranes will be compared with the chemical data with regard to the structure of the membrane.

1. Dratz, E.A., Gaw, J.E., Schwartz, S. and Ching, W. Nature New Biol. 237, 99 (1972).

TAM-D8

LIGHT EVOKED RESPONSE FROM THE EPIPHYSIS OF CHICKS. D. I. Hamasaki, Bascom Palmer Eye Institute, Univ. Miami, Fla.

Stainless steel electrodes were directed into the epiphysis of anesthetized chicks. Illumination of the lateral eyes elicited a response which had a latency of approximately 40 msec. The threshold for eliciting a response from the epiphysis was comparable to the threshold for evoking a response from the cortex. Direct illumination of the epiphysis did not elicit a response. The position of the electrodes was confirmed in all cases by marking techniques.

TAM-D9

AN EXPLICIT MODEL FOR THE ELECTRICAL RESPONSE OF THE ROD. Vic Elias*and Edward A. Dratz. Dept. of Physics, Univ. of Mass., Amherst and Div. of Nat. Sci., Univ. of Calif., Santa Cruz.

We have explicitly calculated the time course and relative magnitude of the rod outer segment plasma membrane sodium resistance in response to a light flash on the basis of the following model. The rod disks contain transmitter molecules which are released by light and which diffuse to sodium pores in the plasma membrane. Binding of the transmitter to the sodium pore closes it and a pump in the disk membrane restores the extradiskal transmitter concentration to a low value. We have related sodium resistance to measured voltage response based on published circuit models of the rod cell. The shape and persistance of the response to flashes in rat and *Necturus* indicates that the rate of return of transmitter to the disks is linear with concentration of transmitter. The topology of the outer segment interior requires a reduced effective transmitter diffusion constant and this reduction explains the rise kinetics of voltage response to dim flashes in *Necturus*. The interaction of transmitter ions with individual plasma membrane pores is discussed in terms of first order binding. Other types of binding are related to different forms for saturation of voltage response.

TAM-D10

ION MOVEMENTS IN VERTEBRATE PHOTORECEPTORS IN THE DARK AND UPON LIGHT EXPOSURE. W.T. Mason, Y.F. Lee*, R.S. Fager and E.W. Abrahamson
Dept. of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

This paper reports the direct experimental finding that calcium ions are released by outer segment disc membranes of photoreceptors upon light exposure. A technique was developed whereby the plasma membrane of the rod was removed by enzyme treatment; the disc membranes were then allowed to accumulate radioactive calcium ion. The mechanism of ion accumulation appears to be by means of a ouabain-sensitive pump in the dark in addition to a simple diffusion component. Disc membranes were bleached for varying periods of time and it was found that Ca^{++} was released from the discs in direct response to the amount of photopigment bleached. The kinetics of this release and uptake have been studied similarly in whole retina, crude and highly purified outer segment preparations of the frog and cow.

Similar experiments were performed on a somewhat different system. Pure rods were ultrasonically disrupted and allowed to reseal, thereby yielding a spherical vesicle of 700 Å diameter. By this technique the internal components of the vesicle preparation can be controlled. Using variations on this technique, the kinetics of the light dependent calcium ion release and uptake were studied and found to be identical to the intact disc. This system has further allowed us to study the compartmental localization of the pump components and well as to accurately measure quantitatively the ion fluxes across the disc membrane.

These results and our previous finding concerning translocation of rhodopsin in the disc membrane upon light exposure have allowed us to formulate a model of the visual process of the coupled absorption/excitation phenomenon in the vertebrate photoreceptor.

ATTACK OF VERTEBRATE AND INVERTEBRATE VISUAL PIGMENTS BY SCHIFF BASE ATTACKING REAGENTS.
Roger S. Fager, Michael Kandel,* Stuart Goldman and E.W. Abrahamson, Dept. of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

For both vertebrate and invertebrate rhodopsin in the dark-adapted state, the retinal chromophore is believed to be buried in a hydrophobic pocket, inaccessible to small hydrophilic attacking reagents. We have recently shown that the hydrophobic analogues for these reagents, i.e., cyanoborohydride for borohydride and ethanloamine for hydroxylamine, do attack the chromophore.

Cone pigments, on the other hand, normally function at high light intensities. For this reason, the bleached retinal chromophore must be regenerated more rapidly and the chromophore binding site must be more accessible from the molecule's exterior. Wald, Brown and Smith showed chemical attack of the cone pigment iodopsin by hydroxylamine and rapid regeneration by 11-cis retinal. Similarly, we have shown attack by sodium borohydride and denaturation by a variety of detergents under conditions where rhodopsin is unaffected.

TAM-D12

PHYSICOCHEMICAL PROPERTIES OF VERTEBRATE AND INVERTEBRATE PHOTORECEPTOR MEMBRANES.
W.T. Mason, R.S. Fager and E.W. Abrahamson, Dept. of Chemistry Case Western Reserve Univ., Cleveland, Ohio 44106

Many studies of the bovine photoreceptor membrane exist, but up to this time little attention has been given the frog or squid photreceptor membranes. This paper reports the lipid and fatty acid composition of purified rod and rhabdome outer segment membranes from the frog and squid, respectively. The squid membranes were found to contain high cholesterol and large amounts of saturated, short chain fatty acids, whereas the frog rod was found to contain little cholesterol and large amounts of polyunsaturated fatty acids. We have also studied the glycolipid content of these photoreceptor membranes and found them to vary widely, the bovine and frog containing less than 25% and the squid nearly 50% of the total lipid as glycolipid. Further results will be reported as the differential phospholipid extractibility of squid rhabdomes in light and dark. These results indicate that phosphatidyl choline is a tightly bound component.

The data reflecting the different lipid and fatty acid compositions of these membranes is further reflected in the deformability properties of the membranes, i.e., one is able to obtain protein/lipid fibers in the squid membrane, whereas upon deformability of vertebrate photoreceptors, no such fibers are obtained.

This report also contains results of differential scanning calorimetry on photoreceptor membranes. All membranes show reversible endothermic transitions which center around -30°C, although the transition temp. does vary somewhat for each membrane. Extracted lipids show a similar transition, though lower in temperature and higher in heat content.

These results are consistent with a view of the squid rhabdome as a rigid membrane structure and the vertebrate membranes as highly fluid.

THE MOLECULAR DESCRIPTION OF BIOLOGICAL CHROMOPHORES. Aaron Lewis, School of Applied and Engineering Physics, Cornell Univ., Ithaca, N.Y.

Photobiology is based on the fact that living systems contain biological chromophores, i.e. molecules that absorb solar radiation, and that this absorbed light energy in some way "transduced" into chemical, electrical or mechanical energy which characterize photobiological processes. We assume that these transduction processes can be understood in molecular terms and our purpose is to examine "primary processes" in biological chromophores in these terms. The photochemist understands the primary process to mean the direct consequence of light absorption by the chromophore, i.e. the initial photochemical or photophysical act which the excited molecule undergoes as distinct from thermal or dark processes which follow this act in the sequence of events producing particular photobiological phenomena, e.g. photosynthesis, vision phototaxis, etc. To gain an understanding of these primary processes in molecular terms we must refer naturally to the principles already established by spectroscopists and photochemists for such systems *in vitro*. Now electronically excited states, in addition to having much higher energies, generally have electronic charge densities and even geometrical shapes that differ from the ground state so that a molecule in a given excited electronic state must be viewed as a separate molecular species with a unique set of chemical and physical properties. Electronic states of molecules are most often described quantum mechanically in terms of molecular orbital theory. Such molecular orbitals have a convenient pictorial as well as mathematical description which is a reasonable approximation of the spatial wave functions of the individual electrons. In this lecture we shall develop, in essentially this pictorial form, a description of electronic states of biological chromophores in terms of molecular orbital theory and show how such states are populated by radiative and radiationless processes as well as the types of photochemical primary processes that can arise from them.

H. H. Jaffe and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley & Sons, Inc., 1962.

R. Dandel, R. Lefebvre and C. Moser, "Quantum Chemistry, Methods and Applications," Interscience Publishers, 1959.

J. Calvert and J. Pitts, "Photochemistry," John Wiley & Sons, 1966.

K. Smith and P. Hanawalt, "Molecular Photobiology," Academic Press, 1969.

E. W. Abrahamson and S. M. Japar, "Principles of the Interaction of Light and Matter," Handbook of Sensory Physiology VII/1, Photochemistry of Vision, Ed., H. J. A. Dartnall, Springer-Verlag, 1971.

THE ROLE OF LIGHT IN HUMAN ENVIRONMENT: Thomas R. C. Sisson*, Temple University School of Medicine, Philadelphia, Penna.

The development of varied and powerful sources of artificial light from electricity have led to sophisticated knowledge of illumination and its measurement -- usually in terms of its perception by the eye. But visible light, as much as U-V or infra-red radiation has the ability to exert measurable biologic effects. Medical uses of the visible spectrum have been virtually ignored by physicians for the past 90 years, since the "Blue Light Mania" in the last half of the 19th Century -- they have been, in fact, almost solely the preserve of quacks and cultists, aside from the practice of heliotherapy at one time. However, there is a new appreciation of these uses by medical scientists, stimulated in great part by the advent of phototherapy of the jaundiced newborn.

Directly beneficial effects of light produced by artificial sources include the photo-repair of U-V damage, the treatment of hyperbilirubinemia of the newly born infant by blue light, the destruction of the virus of herpes simplex when stained by certain photosensitizing vital dyes, the diagnosis of some hereditary disease _in utero_ by the activation of fluorescent dyes in fetal cells, the activation of immunofluorescence of amniotic fluid cells to diagnose infection in the fetus, and recently, the destruction of certain cancer cells by visible irradiation after their incorporation of photosensitizing agents. In the field of psychology, light has come to play a profoundly influential role in affecting the psyche, although the mechanisms involved are poorly understood.

Not all the effects of visible light are beneficial, however. Marked effects on the retina have been demonstrated under circumstances that were thought innocuous. Permanent damage to the visual receptors of nocturnal rodents by green fluorescent light has been shown to be accelerated when the body temperature is simultaneously raised. Blue as well as white fluorescent light has caused blindness in the newborn piglet after less than 12 hours of irradiation. Also, both blue and white fluorescent light increase damage to the retinas of piglets cuased by oxygen in high concentration.

Certain enzymes and other substances in blood and tissue will absorb light _in vivo_ and thus undergo photochemical decomposition. Among these are G-6-PD and GSSG, and the essential vitamin riboflavin. Their photodegradation in red cells leads to hemolysis. Although porphyria is an uncommon condition, the individuals with this disease are exquisitely sensitive to visible as well a U-V radiation since they have large accumulations of porphyrins which are highly phototoxic. The increasing use of artificial light sources containing near U-V along with visible spectral emissions offers a distinct hazard to such people.

We know that photosensitizing drugs given to pregnant women cross the placenta to the fetus readily. If, after delivery, the infant is exposed to

light of high intensity as with phototherapy, the chance of photosensitization is increased.

Biologic rhythms, not only of behavior, sleep, and unconscious bodily functions, but also rhythms of metabolic and biochemical processes, the reproduction of hormones, enzymes, amino acids and so on are regulated by the cycling of light and dark. If the natural cycle is altered by artificial illumination these rhythms will be disturbed. Elegant studies of the effects of constant dark or light environment, and light of differing spectral distributions have shown effects on organ size, growth patterns, and sexual maturation in some animal species, possibly including man.

The psychological effects of light, particularly of colored light, are well known but not well understood. These effects may bear a causal relationship to purely biologic processes in the brain induced by light which in turn will affect psychic behavior. Light intensity as well as wavelength specificity may alter productivity and mood. In the infant, sensory overload by prolonged exposure to highly intense illumination may produce undesirable effects on development. Indeed, the manipulation of the lighting environment of adults as well as infants can have consequences of which we may be quite unaware.

The penetrance of visible light rays into tissues deeper than the skin has not been adequately measured. The penetrance of U-V has been measured with some success, so that we do know much about the photochemical actions within these superficial cells, but the potency of visible light below the skin bears closer study. Our investigations, for instance, have demonstrated a significant transmission of blue light through the entire abdominal wall of the living rat. The photochemical action of light other than U-V upon deep tissues is an area of study that needs much further work. Some studies have shown an increase in blood flow in limbs, and an increase of insensible water loss of physiologically significant degree.

It is obvious that we must consider the types and sources of artificial light, intensities, spectral characteristics, and the chemical, physiological and psychological effects of the lighting environment in man -- not from the standpoint of illumination but specific photo-biologic consequences of its use.

BIOSYNTHESIS OF RHODOPSIN

Paul J. O'Brien, Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, Maryland 20014

Vertebrate photoreceptor cells are highly differentiated structures with equally specialized functions. They consist of two distinct compartments or segments. The inner segment contains the organelles responsible for most of the genetic and metabolic functions of the cell: nucleus, endoplasmic reticulum, Golgi complex, and mitochondria. The inner segment terminates in synaptic endings proximally, and connects with the outer segment distally through a narrow cilium. The outer segment is completely filled with a stack of lamellar membranous discs which are continuous with the plasma membrane in the cone but separate in the rod (1). These membranes are composed mainly of phospholipid and the visual pigments.

Rod outer segments are continuously renewed by the addition of new discs at the base of the outer segment (2). Autoradiographic studies show that protein is synthesized in the region of the inner segment containing the endoplasmic reticulum. Newly synthesized protein migrates first to the Golgi complex then proceeds through the connecting cilium to become incorporated into the basal discs of the outer segment. To compensate for this growth at the base of the outer segment, groups of discs are periodically shed from the apex of the outer segment, engulfed by the adjacent pigment epithelium cells and digested (3). Parallel autoradiographic and biochemical studies (4) have shown that the major protein labeled during this process is rhodopsin. Once incorporated, the rhodopsin remains in the membrane without exchange or turnover until the discs are finally shed.

In order to study this process in greater detail we developed an _in vitro_ system that sustains the incorporation of radioactive leucine into protein using intact, isolated bovine retinas (5). Microsomes are rapidly labeled and rod outer segments become labeled after a lag period. One of the outer segment components thus labeled is rhodopsin which can be isolated and extensively purified by chromatography. The identity of the labeled component as rhodopsin can be confirmed by exposure to light which bleaches the rhodopsin to opsin. After such bleaching, the opsin migrates on an Agarose column differently from rhodopsin (6), yet the label still remains with the protein, opsin.

The time course of rhodopsin labeling also shows a definite lag as anticipated from _in vivo_ studies. Likewise, in pulse-chase experiments, the _specific_ activity of rhodopsin after one hour of incubation can be increased seven-fold during a 3 hour chase. Rhodopsin synthesized in the inner segment during the first hour of exposure to radioactive leucine migrates to the outer segment during the 3 hour chase. Thus the _in vitro_ system appears capable of sustaining the fundamental _processes_ of rod outer segment renewal. In fact, biochemical and autoradiographic studies of frog retinas incubated in this system confirmed these results and established conclusively that outer segment renewal was taking place (7). A band of radioactivity was formed at the base of the outer segment exactly comparable to that seen _in vivo_ (4).

Rhodopsin is a glycoprotein with an oligosaccharide unit composed of three mannose and three N-acetylglucosamine residues (8). The *in vitro* bovine retina system sustains the incorporation of labeled glucosamine into rhodopsin. The product shows the expected light sensitivity and the radioactive sugar can be released by hydrolysis and shown to migrate with glucosamine on paper chromatography. The time course of labeling is similar to that seen with radioactive leucine. However, in double label experiments glucosamine appears in rhodopsin prior to leucine. No such differential labeling occurs in microsomes or mitochondria. The first glucosamine residue is probably added to the newly-synthesized polypeptide on the rough endoplasmic reticulum shortly after the leucine is incorporated. However, there is a transient accumulation of these polypeptides in the Golgi complex (4). This is the site of glycosylation for many glycoproteins, possibly including rhodopsin. The observed pattern of glucosamine label preceding leucine would correspond to the glycosylation of preformed polypeptides shortly before their migration to the outer segment. The longer lag in labeling rhodopsin with leucine would reflect the additional time required for synthesis and accumulation of polypeptide in the Golgi complex.

With either labeled substrate an opsin-like protein is always formed. It occurs as a very highly labeled component of the outer segment with little or no detectable absorbance at 278 nm. Whether precursor or finished product it strongly suggests that the polypeptide of rhodopsin can be transported to the outer segment prior to the addition of the chromphore, retinal.

The *in vitro* system is a convenient vehicle for the detailed study of rhodopsin biosynthesis and outer segment renewal.

1. Young, R.W. (1969), in The Retina: Morphology, Function and Clinical Characteristics (Straatsma, B.R., Hall, M.O., Allen, R.A., and Crescitelli, F., Eds.) p. 177, Univerity of California Press, Los Angeles.
2. Young, R.W. and Droz, B. (1968), *J. Cell Biol.* *39*, 169.
3. Young, R.W. and Bok, D. (1969), *J. Cell Biol.* *42*, 392.
4. Hall, M.O., Bok, D., and Bacharach, A.D.E. (1969), *J. Mol. Biol.* *45*, 397.
5. O'Brien, P.J., Muellenberg, C.G., and Bungenberg de Jong, J.J. (1972), *Biochemistry* *11*, 64.
6. Heller, J. (1968), *Biochemistry* 7, 2914.
7. Basinger, S.F. and Hall, M.O. (1973), *Biochemistry* 12, in press.
8. Heller, J. (1970), *Biochemistry* 9, 864.

PHOTOBIOLOGY OF VISUAL PIGMENTS. C.D.B. Bridges, Department of Ophthalmology, N.Y.U. Medical Center, New York, N.Y.

Visual pigments result from the combination of a variety of apoproteins (opsins) with the 11-*cis* isomers of retinal (forming *rhodopsins*) or 3-dehydroretinal (forming *porphyropsins*). The visual pigments we extract from dark-adapted animals are generally believed to be derived from the rods. With the possible exception of certain birds, cone pigments have not been brought into solution, although their presence in the cone outer segments has been demonstrated microspectrophotometrically (9).

The absorption properties of visual pigments are considered to be broadly related to the spectral quality of ambient light. Fishes from blue marine waters, particularly bathypelagic forms, have pigments that are displaced towards the blue when compared with those from fishes of reddish inland freshwaters (4,8,10). The absorption maxima of rhodopsins, as a group, lie nearer the blue end of the spectrum, between 440 and about 530 nm. Porphyropsins have λmax between 510 and about 540 nm. Hence rhodopsins prevail in marine fishes. Purely freshwater fishes fall into several categories: about half have porphyropsin only, most of the remainder have mixtures of rhodopsin and porphyropsin, while a few have rhodopsin alone. The rhodopsins of freshwater fishes have λmax at 500-520 nm.

The migratory salmons, lampreys and eels generally have mixtures of rhodopsin and porphyropsin (2,7,11), the latter predominating in upstream migrants, the former while the fish is still in the sea. This would appear to be a simple story, the fish synthesising the pigment with absorption properties most closely matched to the light of its environment. There are exceptions, however, exemplified by the Pacific Coast lamprey which has only rhodopsin (7). Moreover, it is becoming apparent that genetic factors are at work, often leading to selection of a visual pigment that is not manifestly adapted to the color of the aquatic surroundings. Thus a recent study has shown that various isolated populations of originally migratory smelt landlocked for 10,000 years since the end of the Wisconsin glacial epoch may differ markedly in their choice of prosthetic group (6). Some have pure porphyropsin and others almost pure rhodopsin, even though the porphyropsin precursor 3-dehydroretinol is present in the liver. The pure porphyropsin trait has persisted in one population for a period of 50 years since they were transplanted over a distance of 500 miles into a lake in the vicinity of that harboring a rhodopsin population.

In freshwater fishes the balance between rhodopsin and porphyropsin depends on a variety of circumstances. In some species thyroxine exercises a potent influence in favor of porphyropsin (1); a similar effect has been shown in migratory salmon and trout (3). One important factor is the level of environmental illumination. A number of species react to light by converting most of their porphyropsin into rhodopsin after a week or so (5,8). The effect is reversible: on placing them in the dark, the proportion of porphyropsin increases. In one case it proved possible to influence the pigments in one eye independently of the other by use of an opaque monocular cap (5). This demonstrated that the response occurred at the retinal level and was probably not mediated by hormones released into the general circulation. Recently, the

situation has become more complex, and experiments on one species have shown that photoperiod and color of illumination may be important under some circumstances (1).

Like the fishes, many amphibians utilise rhodopsin and porphyropsin at some stage in their lives (7,11). Tadpoles of *Rana*, *Hyla* and *Xenopus* have mainly porphyropsin, apparently in response to the requirements of seeing while immersed in freshwater habitats. This view is strengthened by the observation that there is a transition to characteristically terrestrial rhodopsin at metamorphosis in *Rana* (11,12) and *Hyla* (7) but not in *Xenopus* which remains aquatic throughout its life. On the other hand, tadpoles of *Bufo* and *Necturus* have rhodopsin, like the adults. As in the smelt, one is led to wonder whether the presence of rhodopsin or porphyropsin is determined by purely adaptive considerations or whether other, non-visual factors are implicated.

Recently it has been shown that Ranid tadpoles must be kept in the light in order to maintain a high proportion of porphyropsin (5). In the dark, the system gradually drifts towards rhodopsin, hitherto considered the typical adult pigment. The process is reversible and has been repeated over several light-dark cycles. By keeping tadpoles in special incubators illuminated at different wavelengths it is possible to determine the intensities required to sustain selected "criterion" levels of porphyropsin. The sensitivity obtained in this way differs conclusively from the photopic curve, where there is a 2.5 log unit discrepancy at 690 nm, suggesting that if the response originates in the eye it is mediated by light absorbed in the rods.

At present, the mechanism of the photic effect is little-understood. The pigment epithelium in tadpoles and fish often has stores of retinol and 3-dehydroretinol esters that reflect to some extent the changing composition of retinal visual pigments (5), so it is likely that this tissue has an important regulatory function.

References

(1) Allen, D.M. (1971). Vision Res. 11, 1077-1112.
(2) Beatty, D.D. (1966). Canad. J. Zool. 44, 429-455.
(3) Beatty, D.D. (1969). Vision Res. 9, 855-864.
(4) Bridges, C.D.B. (1965). Cold Spring Harb. Symp. Quant. Biol. 30, 317-334.
(5) Bridges, C.D.B. (1972). In Handbook of Sensory Physiology, vol. VII/1, pp.417-480. Springer, Berlin
(6) Bridges, C.D.B. & Delisle, C. unpublished.
(7) Crescitelli, F. (1972). Handbook of Sensory Physiology, vol. VII/1, pp.245-363. Springer, Berlin.
(8) Dartnall, H.J.A. (1962). In The Eye, vol.2, pp. 367-426. Academic Press, New York & London
(9) Liebman, P.A. (1972). Handbook of Sensory Physiology, vol. VII/1, pp. 481-528. Springer, Berlin.
(10) Munz, F.W. (1965). In Colour Vision, Ciba Foundation Symposium, pp. 27 - 45.
(11) Wald, G. (1960). Circulation 21, 916-938.
(12) Wilt, F.H. (1959). Develop. Biol. 1, 199-233.

VISUAL CYCLE IN VERTEBRATES

Sjoerd L. Bonting
Department of Biochemistry, Univ. of Nijmegen, Nijmegen, The Netherlands

The most widely studied vertebrate photoreceptor cells are the rods of cattle and frog. The outer segment of these cells contains 1000-2000 neatly-stacked sacs or discs. In the sac membranes the visual pigment rhodopsin with its characteristic absorption spectrum (λ_{max} = 500 nm) is embedded. Illumination of rhodopsin causes a series of chemical changes beginning with the isomerization of the chromophoric group 11-cis retinaldehyde to all-trans retinaldehyde and ending in vivo with the release of retinol, the reduced form of the chromophore. Spectrophotometrically several intermediates have been distinguished, so that the photolytic sequence can be written as follows: rhodopsin ⟶ prelumirhodopsin (543 nm) ⟶ lumirhodopsin (497 nm) ⟶ metarhodopsin I (478 nm) ⟶ metarhodopsin II (380 nm) ⟶ [metarhodopsin III (470 nm)] ⟶ retinol + opsin.

The initial steps are very fast, so that metarhodopsin II is formed in about 1 msec at physiological temperature.

Our studies, all carried out on cattle photoreceptor membranes, are aimed at the chemical characterization of the visual pigment and its photolytic products. Isolation of rod outer segments is carried out by mild homogenization of the retina, followed by sucrose gradient centrifugation. Any bleached rhodopsin is regenerated by treatment with 11-cis retinaldehyde, yielding an aqueous suspension of photoreceptor membranes with maximal and constant rhodopsin content without the use of detergents (De Grip et al, Vision Res., 12, 1597, 1972). Such aqueous suspensions, used in most of our experiments, are less subject to structural and chemical deterioration than detergent solubilized preparations.

Definite proof of the identity of the chromophore as 11-cis retinaldehyde has been obtained by cautious extraction of the chromophore in organic solvent and its identification through thin layer chromatography and the spectral change upon iodine-catalyzed photoisomerization (Rotmans et al, Vision Res., 12, 337, 1972). Molecular weight (38,900) and molar absorbance (40,300) of cattle rhodopsin have been determined (Daemen et al, BBA, 271, 419, 1972). This study showed that rhodopsin represents 87% of the total membrane protein.

The binding site of retinaldehyde in rhodopsin as well as in metarhodopsin II has been established as the ε-amino group of a lysine residue in opsin. Previously phospholipid amino groups as a binding site had been excluded by means of enzymatic delipidation (Borggreven et al, Arch. Biochem. Biophys., 145, 290, 1971; 151, 1, 1972; Daemen et al, Arch. Biochem. Biophys., 145, 300, 1971). Blocking of all free amino groups in photoreceptor membranes by amidination with methylacetimidate without removal of the chromophore and subsequent determination and identification of the non-amidinated amino groups with DANSyl reagent established the binding site of the chromophore (De Grip et al, BBA, 303, 189, 1973).

Since there are 16 lysine residues present per chromophoric group and a protonated aldimine bond readily reacts with free amino groups, the possibility of transiminization of the chromophore during the metarhodopsin I ⟶ II transition had to be studied. This was done by adding $NaBH_4$ (fixes the aldimine bond by reduction) during or after illumination, then probing the chromophore binding site by treatment with 11-cis retinaldehyde, and observing the formation of any light-sensitive pigment (Rotmans et al, to be published). The results indicate that the chromophore does not migrate during the metarhodopsin I ⟶ II transition, but does so subsequently during metarhodopsin II decay. Surprisingly, it is found that in the absence of reduction to retinol (no NADPH present) 60% of the chro-

mophore remains bound, mostly to protein amino groups and a few percent to phospholipid amino groups.

The photoreceptor membrane contains a retinoldehydrogenase, able to reduce free and aldimine-linked retinaldehyde to retinol (De Pont et al, Arch. Biochem. Biophys., 140, 275, 1970). Studies based on $NaBH_4$ reduction as well as on amidination show that this enzyme has an amino group in its active site, which is able to receive the migrating chromophore. In the presence of the coenzyme NADPH this leads to reduction of the chromophore (Rotmans et al, to be published).

Modification of sulfhydryl groups in photoreceptor membranes showed that out of 6 SH-groups present per rhodopsin molecule only 2 are reactive in darkness. In contrast to earlier reports for detergent-solubilized pigment, no additional SH-groups become accessible upon illumination. Modification of the 2 reactive SH-groups has no effect on spectral integrity and regeneration capacity of the pigment (De Grip et al, to be published).

The search for an isomerase in the photoreceptor membrane, which is able to convert all-trans retinaldehyde to 11-cis retinaldehyde, has not yet been successful. An artefactual isomerization to 9-cis retinaldehyde and formation of isorhodopsin has been found in the presence of bacteria, the bacteria forming an isomerizing cofactor and the membrane possibly acting as an isomerase (Rotmans et al, BBA, 267, 583, 1972).

Combination of information from spectrophotometric and chemical studies is gradually yielding a fairly detailed picture of the molecular events in the visual cycle.

PRACTICAL APPLICATION OF LIGHT AND COLOR TO HUMAN ENVIRONMENTS. Faber Birren, Color Consultant, Stamford, Ct.

The author has worked extensively in the specification of illumination and color for offices, industrial plants, schools, hospitals, and for the facilities of the U. S. Army and Navy. He has applied the pure research of others to everyday environmental problems. Out of this practical experience he has reached several conclusions which have proved resultful in his work. That in habitats occupied over long periods of time, the artificial light source should (a) emit some ultraviolet radiation and (b) that it should be balanced in terms of spectral emission from visible red through orange, yellow, green, blue into violet. That high brightness should not exist in the field of view where critical seeing tasks may be encountered in order to avoid severe muscular and fatiguing constriction of the pupil of the eye. That monotony of illumination level, brightness and color should be set aside for an environment in which moderate sensory change is planned and put into operation to eliminate the emotional hazards of sensory deprivation. That tendencies toward physiological activation with bright and warm colors, and relaxation with softer and cooler colors can be given functional meaning depending on room purpose and occupant need. That any esthetic approaches to color should be supported by tangible evidence reached through competent research into the visual, physiological and psychological responses of the human organism.

TPM-D2

A FLUX DAY--OBSERVATIONS ON FACTORS INFLUENCING THE LIGHT ENVIRONMENT OF INFANTS. Jerold Lucey, Thomas Wolk, James Bottoggi and Richard Klein. Depts. of Ped. and Botany, Univ. of Vt., Med. Ctr, Hosp. of Vt. Burlington

Little is known about the normal light environment of the newborn infant. Nothing is known about the optimal light environment for an infant. Concern has been expressed that phototherapy might expose infants to risks from excessive radiant energy exposure. Observations in our nursery using a spectroradiometer indicate that a normal infant in an incubator near a window exposed to 6 hours of summer sunlight receives approximately 4700 microwatts/cm^2 at 450 mu in 24 hours (a flux day). An infant receiving 24 hours of conventional phototherapy receives 3800 microwatts/cm^2. A number of factors (skin surface area, thickness, and fetal age) profoundly affect the actual light dosage an infant receives. Transmission of light through the skull of 2 premature infants was studied at postmortem and found to be about 10% at 450 mu. These and other important environmental factors affecting phototherapy will be discussed. The hypothesis that a component of physiologic jaundice is due to "light deprivation" will be presented.

Study supported by NIH Grant PHS R01 05561, United Cerebral Palsy Grant and Easter Seal Foundation Grant.

INFLUENCE OF LIGHT INTENSITY AND PHOTOPERIOD ON THE REPRODUCTIVE SYSTEM OF HAMSTERS. Roger A. Hoffman. Colgate Univ., Hamilton, N.Y. 13346

Standard lighting in animal colonies and in most experimental conditions usually ranges from perhaps 50 to 200 ft. candles in intensity. Yet rats, hamsters and mice are nocturnal, exposed under original conditions to intensities characteristic of moonlight or dusk and dawn. To determine if the responses of normal hamsters to short photoperiods of low intensity (LI) are the same as those to high intensity (HI), animals were exposed to one hr. of light per day at two different intensities. At six weeks, testicular and accessory gland weights were 1237 mg and 368 mg respectively in animals in HI while they were 2245 mg and 523 mg respectively in LI. A second experiment with 4 hrs. of light per day showed no differences at six weeks, modest changes at eight weeks and major differences at ten weeks (701 and 350 mg vs 2562 and 718 mg). The evidence suggests a time/intensity function by light and that the pineal activity may be regulated or controlled by light intensity as well as by photoperiod. (Supported by NSF Grant #GB-18350 and by the McGrorty Fndn.)

TPM-D4

LIGHT INTENSITY AND SPECTRAL REQUIREMENTS FOR THE PHOTOSEXUAL RESPONSE IN QUAIL. T. Oishi* and J. K. Lauber, Department of Zoology, Univ. of Alberta, Edmonton, Alberta, Canada.

Mature male Japanese quail (*Coturnix coturnix japonica*) reared under continuous light have gonads of maximal size, which then regress when birds are transferred to short photoperiod. The photoreceptor for this photosexual response is located in the head region, but is extra-retinal, extra-pineal and extra-Harderian gland, although either the eyes or the pineal may serve as auxiliary photoreceptor or as light guide to the deep (hypothalamic ?) receptor. The light intensity threshold is between 15.7 and 1.57 $\mu w/cm^2$ (white, incandescent), for both intact and blinded birds, higher intensities eliciting no further response. In intact birds, both red (λ max 625 nm) and green (500 nm) light promoted gonadal maintenance at low intensity (4.0 μw for red, 9.6 μw for green), while only red light was effective at very low intensity (1/10 of above energy levels). Blue light (450 nm) was not stimulatory at either low or very low intensity. In blinded birds maintained in several spectral environments at low intensity (as defined above), only red light was effective, the gonads being the same size as those of intact quail at very low intensity. The key variable in the photic environment for quail appears to be wavelength rather than quantum level. Orange-red radioluminescent paint (15 mg, λ max 600 nm), placed beneath the skull immediately over the pineal body, elicited a marked gonadal response, while smaller amounts of orange-red paint (5 mg or 0.5 mg), or a higher amount of orange-red paint after pinealectomy, or green radioluminescent paint (15 mg, λ max 520 nm) were ineffective. (Supported by grant A-3446, National Research Council of Canada.)

RESPONSE OF DOGS TO COMMERCIALLY AVAILABLE LIGHT SOURCES AS A SOLE SOURCE OF ILLUMINATION. A. L. Rogers, J. W. Templeton* and J. Cottingham*, Departments of Animal Care and Surgery, University of Oregon Medical School and Portland General Electric Company, Portland, Oregon.

Eighteen 12 week old Labenji female puppies were placed under standard ized foot candles of incandescent, mercury arc vapour, warm white fluorescent and broad spectrum fluorescent bulbs. The engineering, husbandry and veterinary monitoring of these dogs for the first 15 months is discussed.

TPM-D6

COMPARISON BETWEEN PHOTOREACTIVITIES OF CARCINOGENIC BENZPYRENE AND SKIN-SENSITIZING COUMARINS. P. S. Song, T. A. Moore,* and W. W. Mantulin, Department of Chemistry, Texas Tech University, Lubbock, Texas 79409.

Significant spectroscopic difference was found between the $^3(\pi,\pi^*)$ state of coumarins and the ^3L_a state of benzpyrene. No analogy of the localized triplet state associated with the coumarin chromophore was revealed in the case of the potent carcinogen, 3,4-benzpyrene. Instead, the ^3L_a state of 3,4-benzpyrene is characterized by more delocalization than that of non-carcinogenic 1,2-benzpyrene. Therefore, predominant photoreaction between 3,4-benzpyrene and DNA bases do not seem to involve cycloaddition in contrast to the coumarin-pyrimidine system. Reactivity indices have been calculated, and results are consistent with recent experimental findings. In addition, spectroscopic properties of 1,2- and 3,4-benzpyrenes have been comparatively described in terms of relatively high resolution spectra, polarization measurements, and MO calculations.

A MODEL FOR THE LUMINESCENCE OF LYSOZYMES AND LACTALBUMINS.

J. W. Longworth and V. L. Koenig,* Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tn. 37830

Human leukemia (HLL) and hen egg-white lysozyme (HEL) are believed to share a common conformation with bovine a-lactalbumin (BAL). It is possible to consider the detailed environment of the individual tryptophan residues of these three proteins using the atomic structure for HEL and the three primary sequences. For example BAL shares 4 W residues in common with the 6 of HEL. In HEL, W62 and W108 fluoresce and only W108 in HLL; there are two fluorescence components of HEL and one for HLL, judged by spectra and lifetime. In HEL W111 is quenched by K116 and W28 transfers to W111. W63 interacts with disulfide 64-80 and becomes the source of phosphorescence, probably through triplet sensitization from W62 and W108; it also acts as a quencher for W108 through transfer. W123 is quenched by K33. HEL lacks W62 and W123, hence only W108 fluoresces, hence the blue short-lived fluorescence. The additional W34 is quenched by K33. BAL lacks W111, so W28 contributes to fluorescence as does W123 since K is replaced by F33. Hence the greater intensity and long-lived phosphorescence. Fluorescence anisotropy spectra of all three proteins show no depolarization, consistent with either no transfers between the fluorescent tryptophan residues, or a parallel orientation (e.g., W62 and W108 of HEL). This result is in agreement with the molecular structure. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.) (V.L.K. is a Special Fellow of DHEW.)

THE BEHAVIOR OF ACRIDINE ORANGE-CHONDROITIN SULFATE-A COMPLEXES

J. M. Menter and S. S. West, University of Alabama in Birmingham, Birmingham, Alabama, and P. Gentilcore, R. S. Fager, G. P. P. Kuntz and E. W. Abrahamson, Case Western Reserve University, Cleveland, Ohio.

A systematic study of the absorption, fluorescence, ORD and CD spectroscopic properties of acridine orange-chondroitin sulfate-A (AO-CSA) complexes in solution has been undertaken. At relatively low concentrations, shifts in absorption and fluorescence spectra occur under complex formation which are similar to those occuring in AO alone at high concentrations. These shifts have been attributed to aggregation of AO to form dimers and higher aggregates.(6) Fluorescence excitation spectra of mixtures of AO and CSA have indicated the presence of more than one emitting species. In addition, the dependence of the ORD-CD properties, as well as the solubility behavior of these solutions on CSA/AO ratio indicate the possible existence of at least two distinct AO-CSA complexes. These spectroscopic effects are sensitive to changes in ionic strength. Some degree of photolability seems to exist for these complexes, thus caution is urged in the interpretation of spectroscopic behavior in such systems.

TRYPTOPHAN FLUORESCENCE AND FMN BINDING IN *AZOTOBACTER* FLAVODOXIN.

L. Andrews*, M. MacKnight, J. Ryan* and G. Tollin, Department of Chemistry, University of Arizona, Tucson, Arizona.

Previous work [J. A. D'Anna, Jr. and G. Tollin, *Biochem.* 10, 57 (1971)] has shown that the binding of FMN to *Azotobacter* apoflavodoxin results in the quenching of 90% of the tryptophan fluorescence. In an effort to elucidate the mechanism of this quenching, we have measured the lifetimes (with a pulsed laser) and relative intensities of apoprotein and holoprotein fluorescence, using both native and N-bromosuccinimide (NBS) oxidized materials. The tryptophan fluorescence of the apoprotein decays exponentially with a lifetime of 4.1 nsec; the holoprotein fluorescence decay is clearly non-exponential and has components with lifetimes close to 2 nsec. This lifetime decrease is not sufficient to account for the decrease in quantum yield and thus some quenching must occur at the ground state level by a contact mechanism. NBS (at pH 6 or 7) is found to oxidize two (out of four) tryptophan side chains in the apoprotein and 1.3-1.5 tryptophan side chains in the holoprotein. Both of these oxidations are accompanied by complete loss of FMN binding, small but definite changes in far UV CD spectra and complete elimination of the near UV CD spectrum. In both cases, 85-90% of the tryptophan fluorescence is lost. However, the fluorescence decay of these modified proteins is approximately exponential and the lifetimes are about 4 nsec. These results are interpreted in terms of the presence of a tryptophan side chain in the coenzyme binding site, which is oxidizable by NBS in both apo and holoproteins, and which is responsible for approximately 90% of the fluorescence of the protein and the near UV CD spectrum. The binding of FMN quenches this tryptophan by a contact mechanism and partially quenches the remaining tryptophans by energy transfer.