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BIOMEDICAL EFFECTS OF EXPOSURE TO ELECTROMAGNETIC RADIATION

PART I — ULTRAVIOLET

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FOREWORD

This study consists of three sections each reporting the results of the research conducted by Dr. Archibald R. Buchanan, Dr. Harold C. Heim, and Dr. Donald W. Stilson of the Physics, Engineering, Chemistry Corporation, 1001 Mapleton Avenue, Boulder, Colorado. The work was performed under the direction of Dr. Donald G. Burkhard, Research Director of the Corporation.

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ABSTRACT

Literature concerning the biomedical effects of ultraviolet radiation is reviewed. Ultraviolet absorption results in mitotic alterations and abnormal cell divisions, regressive changes in the somatic structures of some lower animals, and skin and eye tumors in mammals. Damage to the eye from high intensity ultraviolet is probably limited to the cornea and, to a slight extent, the lens.

The effects elicited by ultraviolet irradiation of certain proteins, nucleotides, enzymes, hormones, and amino acids are reviewed.

Literature pertinent to the visibility and hue of ultraviolet, the effects of ultraviolet wavelengths on scotopic and photopic visual sensitivity and on the "reactivity" of the organism is surveyed.

PUBLICATION REVIEW

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SECTION I

REVIEW OF LITERATURE PERTAINING TO THE BIOMEDICAL EFFECTS OF EXPOSURE TO ELECTROMAGNETIC RADIATION

Dr. A. R. Buchanan

Blum (1), in a review of the physiological effects of sunlight on man, stated the following: "Man now having added a third dimension to the 'habitable' earth, one must consider the physiological effects of sunlight at altitudes as high as airplanes can operate. The increase in total solar radiation in going from the earth's surface to the outside of the atmosphere is not a very important factor, amounting to only about 35%. The effects of the cold surrounding air and greater radiations to the heavens, make cold rather than heat the important problem in high altitude flying. On the other hand the sunburn-producing ultraviolet radiation varies with altitude in a quite different manner and to a different extent; the ozone of the atmosphere, which is the component most important in cutting out this radiation, is stratified in its distribution, occurring in greatest concentration in the stratosphere. A number of measurements of the intensity of ultraviolet radiation at various altitudes indicate a considerable increase in the sunburn-producing portion, beginning rather close to the earth; but the most complete measurements yet obtained, taken by O'Brien and his collaborators in connection with the stratosphere flight of the 'Explorer II', indicates no appreciable ozone below about 15 kilometers, a height above the present limit of airplane operation. Whether or not there is an important increase in the incidence of sunburn-producing radiation at present flying altitudes, and whatever the possibility that the ceiling may be raised in the future, adequate protection should be readily available by the use of plastics which do not transmit these wavelengths, or of window glass, in transparent enclosure surfaces."

Sunlight is arbitrarily divided into ultraviolet, visible, and infrared spectral regions. Light, or visible radiation, is usually defined as that radiation perceived by the human eye and is often said to lie between 4000 Å and 7000 Å, the approximate limits of photopic vision; radiation of shorter wavelengths is referred to as ultraviolet, and that of longer wavelengths as infrared. Actually, the rods in the retina of the human eye perceive radiation of wavelengths as short as 3000 Å but the sensitivity is so slight as to be negligible. Wavelengths that

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elicit photobiological effects are determined by the spectral absorption of some photochemically active substance in the living system and such absorption may occur anywhere in the ultraviolet, visible, or very near infrared, depending upon the chemical constitution of the absorbing substance.

The direct effects of sunlight are of two kinds: first, specific effects initiated by a photochemical reaction; and second, nonspecific or radiant heat effects which merely result from the local rise in temperature(1). The former results in the activation of molecules by the capture of quanta of radiation. Such capture constitutes the primary act in a photochemical reaction. The type of chemical reaction is determined by the kind of molecule present in the affected system as well as by the activated molecule itself. Such an effect is characterized by a specific action spectrum; it is produced only by certain wavelengths that are specifically absorbed by the light absorber, i.e., the compound whose molecules are activated as the primary event in the underlying photochemical reaction. Radiant heat effects also result from the capture of quanta of radiant energy by molecules but such capture is not followed by chemical reaction in this case. Instead, the energy of the absorbed quanta is distributed among the molecules of the system in such a way as to increase the average kinetic energy and, hence, the temperature of the system.

Ultraviolet radiation produces injurious effects in living systems, indicating that it acts upon some components of all cells. This concept is strengthened by the fact that, with very few exceptions, such effects have their long wavelength limits at about 3200 Å and have similar action spectra. All these action spectra show remarkable similarity to the absorption spectra of typical unconjugated protein or of nucleic acid. The universal importance of these two substances, their presence in quantity in all cells, and the fact that both are photolabile, leads to the conclusion that ultraviolet radiation exerts its injurious action by altering either or both. Proteins may be principally concerned in some instances and nucleic acid in others, as there is some evidence that in cells with considerable cytoplasm to absorb the ultraviolet radiation, protein is the principal substance attacked. Comparison of the erythema spectrum with the absorption spectra of a typical protein and of nucleic acid reveals that in all three there is a long wavelength limit near 3200 Å, and that each has a discrete maximum; but the erythema maximum at 2970 Å does not agree with either the protein absorption maximum of 2800 Å or the nucleic acid absorption maximum of 2600 Å. Since the erythema spectrum is measured at skin surface, it is not a correct index of the photochemical reactions underlying erythema because this reaction takes place in the living layers of the epidermis, beneath the superficial dead corneum which acts as a semiopaque screen by absorbing the active wavelengths. Careful consideration

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of all the evidence suggests that protein is the light absorber in the sunburn mechanism and it is credited with that role by most investigators. Whether protein or nucleic acid or both act as light absorbers makes no difference insofar as subsequent steps in the process are concerned, once the postulate is accepted that ultraviolet radiation causes injury to epidermal cells. The relationship between the sunburn and the carcinogenic mechanisms is suggested by the fact that both have the same long wavelengths limit at about 3200 Å. It seems reasonable to relate the carcinogenic mechanism to the same fundamental injurious action on the cells that is associated with sunburn and is characteristic of ultraviolet radiation of wavelengths shorter than this limit. An alternative hypothesis has been offered to the effect that the ultraviolet radiation brings about somatic mutations by direct action on the nucleic acid of the chromosomes. Supporting this hypothesis is the fact that ultraviolet radiation is effective in inducing mutations in unicellular organisms and in the sex cells of Drosophila, the action spectra of the former indicating that nucleic acid is the light absorber. It has been suggested that ultraviolet radiation acts on the steroids of the skin to produce a chemical carcinogen but recent evidence indicates that this is very unlikely.

As knowledge of the few clearly established cutaneous responses to sunlight increases, there seems correspondingly less reason to credit the numerous claims for additional effects (1). Most evidence supports the view that wavelengths longer than 3200 Å have no specific photochemical effect on normal skin other than the pigment-darkening effect, which extends to about 4200 Å. There have been reports that ultraviolet wavelengths longer than 3200 Å may bring about erythema and there is also some evidence that such wavelengths affect living systems in general. Lethal effects on bacteria and other lower forms brought about by the longer wavelengths of ultraviolet have been the subject of studies by Hollaender (2) and his group, whose findings indicate an essentially different character for these effects than for those produced by wavelengths shorter than 3200 Å. Blum has suggested that such processes may represent photodynamic action for which point of view there is some evidence in earlier literature. The possibility that such effects are common to all living systems must be entertained but it seems improbable that they will prove to constitute an important cutaneous response to sunlight.

Carbon arc radiation which has been employed in several studies on circulatory effects, contains wavelengths other than those that cause sunburn, being particularly rich in the longer ultraviolet in the region of 3900 Å. The possibility of some specific effects of these wavelengths, which are also present in sunlight, must be entertained but for the present there is no direct evidence that they play a part in circulatory changes. The total intensity of the radiation used in some of these investigations has been approximately equal to that of maximum

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sunlight and hence the effects of increased heat load are not excluded.

With respect to the formed elements of the blood, exposure to ultraviolet radiation seems to produce a slight increase in red cells, white cells, and platelet counts but there is disagreement among various reports, due probably to the lack of uniformity in the doses and procedures employed.

The ultraviolet microscope has made possible many important contributions, particularly to physiological genetics (2). Its value in the problem of the effects of ultraviolet lies in the fact that it indicates quantitatively where the radiation is absorbed in the cell and it helps in recognizing the changes which take place during radiation in the distribution of protein and nucleic acid. The irradiation of horse serum and human serum and certain protein fractions of the latter results in increased viscosity, a decrease in colloid osmotic pressure and homogeneous electrophoretic mobility; such experiments indicate that the major effect of 2537 Å irradiation on proteins is the unfolding and subsequent splitting of the protein molecule.

Viruses, as far as their chemical composition has been determined, in most cases have proved to be nucleoproteins; therefore, it would be expected that their inactivation spectra would resemble the absorption spectra of typical nucleoproteins. Hollaender and Oliphant (3) reported a high sensitivity of influenza virus at 2650 Å with a decreased sensitivity to shorter and longer wavelengths. Similar inactivation curves have been reported for vaccine virus and bacteriophage.

Comparative studies of the effects of x-rays and ultraviolet radiation on neurospora have suggested that ultraviolet effects are localized, whereas x-ray effects are more diversified. The latter includes chromosome breaks and rearrangements. The rate of mutation production from ultraviolet radiation is linear up to certain energy values. With further increase of energy the mutation rate becomes more or less irregular. In contrast to this, the mutation rates produced by increasing energies in the x-ray region follow a straight line relationship over most of the range.

Giese (4) studied the effects of monochromatic ultraviolet radiation between 2483 and 3650 Å on well-fed and starved paramecia. He found that the action spectrum for retardation of division is similar to the absorption spectrum of a typical nucleic acid. The action spectrum for the retardation of ciliary movement resembled closely the absorption of a typical protein. This seemed to indicate that the first effect is probably on the nucleus and the second on the cytoplasm.

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The mechanics of the effects of ultraviolet on mitosis is of considerable interest because such information is very useful in the further understanding of radiation effects. Tradescantia pollen grains are good material for such studies because ultraviolet penetrates the pollen tube readily. Swanson (5) described the effects of ultraviolet radiation on the pollen grains (2537, 2976, and 3022 Å). Chromatid breaks induced by 2537 Å are proportional to dosage. Of particular interest is the observation that pretreatment of pollen tube chromosomes with radiation of 2537 Å inhibits all the visible breaks usually produced by x-radiation but treatment with ultraviolet after x-radiation has no effect. It was suggested that ultraviolet produces changes in the chromosome matrix which result in greater resistance to x-rays.

Several interesting physiological problems have arisen in connection with the wide use of 2537 Å radiation in air-borne disinfection. One such problem concerns the maximum amount of 2537 Å radiation to which men can be safely exposed for a given length of time. The Council of Physical Medicine of the American Medical Association has set a 0.5 microwatt per sq. cm. for 7 hours and a 0.1 microwatt per sq. cm. for 24 hours exposure as the highest permissible limit. These values were based on the experience of workers in the field who have noticed no detrimental effects upon equivalent exposure.

Arc lamps differ from the sun in providing rays shorter than 2900 Å, but owing to the very slight penetrability of these rays they have very small effect, if any, with exception of the rays which lie in the region 2500 Å. Rays shorter than 2500 Å do not penetrate the horny layer of the skin but are very powerful in their lethal action on single cells such as a film of red corpuscles, or the surface layer of a culture of bacteria. A very thin layer of protein absorbs the shorter rays. Thus bacteria or red cells centrifuged with water in a quartz flask are easily destroyed by exposure to a mercury vapor lamp (6) but when the same are mixed with serum and centrifuged, they are protected to a very great extent by the very thin protein film of the serum which covers the inside of the rotating quartz flask. Not more than about one-thousandth of the active short ultraviolet rays penetrate through the horny layer of the skin to reach the living cells beneath and scarcely any reach the most superficial blood capillaries in the dermis.

It is estimated that some 80,000 people died in Hiroshima and 45,000 in Nagasaki as the result of the two atomic bombs used in combat, and it has been further estimated that 20% of them died of irradiation (7). In addition to the gamma rays and neutrons given off at the time of the explosion, there were large amounts of infrared, visible light, and ultraviolet. It is reported that superficial effects from such radiations were by far the most widespread in Nagasaki, and were found 1½ miles

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from ground zero. Flash burns affected only exposed surfaces because shadows were sufficient to protect the skin, except in zones close to the explosion where burns occurred beneath the clothing. Those at great distances from the center felt no heat but noted burns several seconds after the explosion.

It has been widely believed that the sun's rays, by damaging the brain or spinal cord, could cause pyrexia, delirium, coma, and death. It has now been established that the biologically active ultraviolet portion of the sun's rays does not penetrate more than 1 or 2 mm. into the skin and, therefore, the possibility of direct injury to nerve centers may be excluded (8). It has been shown, however, that, after exposure to ultraviolet radiation, there may be a reduction in sweating rate which is partly caused by occlusion of the mouths of sweat gland ducts in the necrosed surface layer ofthe epidermis and, probably, partly by reduction of secretory pressure of sweat. It can be shown that, under arid conditions, a sweat deficit of 100 ml. will allow a rise of 1 degree C. in body temperature.

An ultraviolet microscope, having an achromatic objective, was used by Brumberg and Larionow (9) for photographing living tissue cultures. The latter were grown by the hanging-drop method in a quartz cover glass. The source of light was a high pressure quartz mercury lamp. All radiations except those of wavelengths 2540 to 2750 Å were prevented from reaching the object by means of filters. Focusing was performed under conditions of visible light which completely prevented the ultraviolet rays from reaching the cells before they were photographed. The time of exposure was 20 seconds. Photographs of living cells of mouse mammary carcinoma cultures as well as of mouse or chicken fibroblasts revealed that the nuclei of living cells failed entirely to absorb any ultraviolet rays within the region 2540 to 2750 Å. The nucleoli alone revealed moderate absorption. Cancer cell cytoplasm was likewise found to absorb ultraviolet rays only moderately. These investigators explained the disparity between their results and the earlier results of Caspersson on the basis of the use by Caspersson of a monochromatic objective which obliged him to take a few preliminary photographs in ultraviolet light in order to get the objects in focus. This resulted in damage and death of the cells. Apparently desoxyribonucleic acid is contained in the nuclei of living cells in a somewhat different state than in damaged or dead cells -- a state in which it does not absorb ultraviolet waves of lengths about 2600 Å. Absorption apparently develops in connection with the injury and death of the cells.

Ultraviolet radiation produces skin erythema, destruction of enzymes, activation of ergosterol, and denaturation of proteins. It has been found that denaturation and coagulation of isoelectric salt-free egg albumin involves three distinct processes, of which the first is light denaturation of the

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protein molecule, the second, a reaction between the light-denatured molecule and water, and the third, a flocculation of the light- and heat-denatured molecules to form a coagulum. The object of an investigation by Clark (10) was, first, to determine the temperature coefficient of the second step in protein coagulation, in the presence of salt and at different hydrogen ion concentrations and, second, to determine the temperature coefficient of erythema production in order to see if there was any evidence for a relationship between these two reactions. Her conclusions were as follows: Coagulation of proteins by ultraviolet radiation involves three different processes (as listed above). The first has a temperature coefficient of 1, the second, a temperature coefficient of more than 10. The temperature coefficient of the second step in the coagulation process is modified by the presence of salts and by changes in hydrogen ion concentration but, under the conditions tested, has been found to be of the order of magnitude of 8 or 10. The temperature coefficient of the initial change produced in skin tissue by ultraviolet light radiation was found to be approximately 1. The temperature coefficient of the latent period of erythema production was found to be 2.3 in human skin, at temperatures between 30 degrees and 40 degrees C. and 1.9 in frog skin at temperatures between 20 and 30 degrees C. The production of erythema, therefore, which is supposed to be due to the release of a vasodilator substance in damaged tissue, is probably not related to the coagulation of tissue proteins by ultraviolet light.

Giese (11) has dealt extensively with the subject of "protozoa in photobiological research." He noted that the photolethal effect of ultraviolet light was discovered in 1877 by Downes and Blunt when they found that sunlight paralyzed a culture of bacteria. Giese called attention to the fact that the ultraviolet region of the spectrum is a wide span bordering on the visible at the long end and overlapping with x-rays at the short end and, for convenience, divided into the long ultraviolet (4000 to 3000 Å), the short ultraviolet (3000 to 2000 Å) and the very short ultraviolet (2000 to 150 Å). The long ultraviolet is less effective on protozoa; in most studies short ultraviolet has been used; very short ultraviolet is so readily absorbed by air and water that few studies have been attempted.

Protozoa are stimulated when they swim into the pathway of ultraviolet light. Ciliates so stimulated perform the avoidance reaction and attempt to move out of the irritating field; an amoeba will withdraw its pseudopodia. If protozoans cannot escape the light, increased activity continues only briefly; then movements decrease and swimming becomes much slower. The contractile vacuole in irradiated fresh-water ciliates begins to fail at about the same time as the cilia and contacts only slowly. Once it has failed the vacuolar contents increase in quantity and the animal swells because of failure of extrusion

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of water which is continuously entering into a paramecium or other fresh-water ciliates. Alternately there is rupture of the entire animal. Variation of environmental conditions (pH, temperature, salt content) had little effect on the mobilization of the protozoa by ultraviolet within the limits tested. Starved paramecia and tetrahymena were much more readily immobilized, however.

Radiation of wavelengths 2650 Å has been found to be the most effective in killing paramecia (12). Giese and Leighton (13) found maximal effects after radiation with wavelengths of 2804 Å, although the differences between 2537, 2654, and 2804 Å were not great. Weinstein (12) showed that, within a four-fold change in intensity (at 2800 Å), the reciprocity law held for killing of paramecia; for each decrease in intensity a corresponding increase in time of exposure was required to produce the same effect.

Observed alterations in protozoa, after ultraviolet treatment, include segregation of mitochondria into small granular masses, degeneration and disintegration of the macronucleus, elongation of the micronucleus, and disappearance of the nuclear membrane. The viscosity of the cortical gel of an amoeba is decreased by ultraviolet treatment. Since removal of calcium has the same effect, it has been suggested that ultraviolet may act by removal of calcium. The endoplasmic sol of the amoeba is gelated by ultraviolet but this does not occur if calcium is first removed.

The following variants have been observed in protozoa subjected to ultraviolet treatment: (1) forms with more contractile vacuoles and with a finer oral basket; (2) tetraploids; (3) triploids; (4) transparencies; (5) fused organisms; (6) truncated organisms; (7) chains of organisms; (8) giants with dimensions of 44 to 54 micra as compared to controls of 18 to 30 micra. Ultraviolet also induced mutations in protozoa although it was difficult to find specific and easily recognized characters for mutational studies.

The retarding action of ultraviolet upon division of protozoa depends upon the dosage. A small dosage produces a lag or delay before division begins but larger doses cause both a lag and a decrease in the rate of division. The effect is much greater when the protozoa are starved to some degree before exposure.

To produce an effect on a chemical or biological system light must be absorbed. It has been found that the macronucleus of paramecium absorbs very strongly at 2749 Å and somewhat less strongly at 2313 Å. The difference in absorption between nucleus and cytoplasm was greater at 2749 Å than at 2313 Å. At wavelength 3525 Å the absorption by nucleus and cytoplasm was similar as it was also in the visible range. The difference in absorption between nucleus and cytoplasm decreases with exposure to

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ultraviolet. Small granules which may be mitochondria also strongly absorbed ultraviolet. For retardation of division of protozoa, absorption by nucleic acid is of primary importance, since the relative efficiency of different wavelengths correlates well with the absorption by these compounds. For immobilization, effects on ciliary movements, and sensitization of protozoa to heat, the action spectrum suggests absorption by non-conjugated proteins, such as albumin. Whether ultraviolet acts directly upon nucleic acid and unconjugated proteins, which absorb the radiations falling upon the protozoan, or whether these compounds merely absorb the radiant energy and sensitize the formation of a diffusible poison, possibly a peroxide, is impossible to say (11).

Kline and Rusch (14) called attention to the fact that the 3125 Å band accounts for approximately 0.5% of the total radiant energy of daylight fluorescent lamps. They exposed albino rats to the light from such fluorescent lamps, at a distance of 4 inches, for a period of 1 year. They calculated that the amount of this wavelength received by the mice was 28×10^7 ergs per sq. cm. Although this is slightly more than the minimum of 26.4×10^7 ergs per sq. cm. reported to be carcinogenic when a mercury burner is the source of radiant energy, no tumors or other pathologic changes resulted from this type of irradiation. These investigators took care to note that comparisons between the daylight fluorescent lamps and the mercury lamp were not valid because the calculations involved in the latter included all the wavelengths from 2900 to 3341 Å. Also, the radiations from a mercury lamp are regularly given over a short period and are considerably more intense than those received from fluorescent lamps.

A "flying spot" ultraviolet microscope has been utilized by Montgomery and Bonner (15) to determine what happens when a cell is deliberately damaged with ultraviolet light. They applied a graded damaging dose to the whole cell either by stepping up the strength of the current or by increasing the scanning speed which increases the frequency of attack by the flying spot on each point of the cell. They found that a moderately punishing dose causes the cell's cytoplasm to gel, as if it were suddenly frozen into immobility. The cell stops drinking at its surface and the tiny round bodies in the cytoplasm stop moving. When this dose catches the cell in the act of dividing, more striking effects are sometimes seen. Not only does the division stop abruptly but many big bubbles break out on the surface. The response of cells to heavier doses of ultraviolet radiation is dramatic; the cell suddenly becomes completely opaque (black) and contracts into a small round ball. Each cell reacts as an individual; that is to say that no two cells, even in a group of a single type, show exactly the same vulnerability to ultraviolet irradiation. Exposure to the same damaging dose causes the cells to "give up the ghost" one by one, each after a different time,

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and a few survive even after 30 minutes of exposure.

The effect of ultraviolet radiation upon mitochondria of the yeast, saccharomyces, has been studied by Sarachek and Townsend (16). They reported disruption of mitochondria, becoming microscopically detectable prior to onset of cytoplasmic precipitation. The effect was first observed with ultraviolet doses sufficient to reduce irradiated yeast suspensions to approximately 1% survival; it became more pronounced as dosage was increased. It was found, in general, that there was an inverse relation between the size of the mitochondria and the metabolic activity of the cell. Large mitochondria may be considered to consist of an enzyme protein core essentially isolated from the cytoplasm of the cell by a lipoid cortex. As cellular activity declines, mitochondria increase in size. Following irradiation of yeast cells containing an abundance of large mitochondria, slight swelling of the mitochondria occurred after 2 minutes and complete disruption and coalescence after 10 minutes of exposure. Although there was considerable cellular shrinkage and the nuclear vacuole was usually obscured, there was no apparent precipitation of the cytoplasm. A different result was observed when cells containing small mitochondria were irradiated. Presumably these organisms differ from those containing large mitochondria in possessing lesser amount of lipid in the external cortex and less extensive aggregation of enzyme at the mitochondrial core.

It appeared that the changes in mitochondria described by Sarachek and Townsend (16) were due to direct ultraviolet absorption by the mitochondria and the cytoplasmic matrix. Disruption of large mitochondria can be attributed to high absorption of radiant energy of wavelengths 2537 Å by the sterols and unsaturated fatty acids of the lipid surface. Such absorption may effect decarboxylation of fatty acids. If loss of lipid polar groups occurs, a weakening of attraction between the surface lipid and the proteinaceous mitochondrion core might be expected, resulting in mutual attraction and coalescence of lipid substances. It is not possible, however, to ascribe a fundamental relation to the disorganization of mitochondria and ultraviolet-effected cellular inactivation since the changes reported are brought about only under specific conditions and at relatively high doses.

Parallel or "consensual" reactions in the two arms of 14 individuals following irradiation of one one arm with a combination of ultraviolet and infrared light were observed by Schulz and Amelung (17). The results of skin temperature determinations on the nonirradiated extremity showed a marked increase in temperature which could only be explained by an increased flow of blood. These authors assumed, on the basis of their findings, that the combination of ultraviolet and infrared irradiation was responsible for the production or mobilization of a vaso-active

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substance in one part of the body which resulted in a generalized effect in other areas of the body. The increase in caliber of the blood vessels in areas not directly affected by the irradiation was explained, at least in part, on the basis of prevailing theories concerning ultraviolet effects. They elected not to speculate as to whether the dilatation was caused by a so-called H-substance or by a sulfhydryl body. They stated that the active hyperemia involved not only the sub-papillary plexus but also the deeper blood vessels of the dermis and they assumed that the deeper vessels participated in the transport of a "vasodilatory" substance produced by the ultraviolet irradiation.

The sensitivity to lysis by high pressures of ultraviolet-irradiated Blepharisma undulans has been studied (18). In each experiment the minimum pressure required to induce complete lysis in 50% of the irradiated organisms was determined. This was designated as the critical lysis pressure. The nonirradiated controls displayed a minimum sensitivity with the critical lysis pressure being 10,000 pounds per sq. in. Among the irradiated specimens greatest sensitivity was evident by those organisms irradiated at 2300 Å; slightly less sensitivity was manifest at 2800 Å and still less at 2650 Å; the critical lysis pressures (after 6000 ergs per sq. mm. at the stated wavelength) were 5000, 6500, and 7000 pounds per sq. in., respectively. Other wavelengths had little or no sensitizing effect and at the effective wavelengths the sensitizing effect was directly related to the dosage. Tentatively, these relative sensitivities to pressure are interpreted on the basis of the fairly specific ultraviolet effects upon proteins (at 2800 Å) and nucleic acids (at 2650 Å) and on the basis of more generalized effects upon the cell cortex (at 2300 Å). It seems likely that the phenomenon of lysis by high pressure and/or ultraviolet irradiation may involve the well-known solational effect of pressure upon protoplasmic gel structures. In most, if not all animal cells, the cortical cytoplasm immediately subjacent to the plasma membrane and pellicle, displays an organized gel structure. It might be expected, therefore, that complete solation of this cortical cytoplasm would lead to molecular rearrangements which could very drastically alter the structure and permeability characteristics of the cell. Such an expectation seems particularly valid for highly organized cell types, such as blepharisma, which tend to maintain a farily complex and elongate cell form. It is generally agreed that the formation of a protoplasmic gel involves the unfolding of globular (protein) units into elongate fibrils which then become interlinked, forming a three-dimensional colloidal network. It appears that one primary effect of pressure is to weaken the intermolecular linkages of the system and a similar effect may be postulated for the ultraviolet treatment. This implies that high pressure and ultraviolet irradiation have additive effects because both agents produce a weakening of the intermolecular bonds of the gel system. It also seems possible that ultraviolet denaturation

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of a protein which normally participates in the formation of a gel structure might be involved. This would result in formation of a weaker gel, more susceptible to pressure solation. Such a view is supported by the recent report of Giese (11) that sublethal dosages of ultraviolet increase the susceptibility of paramecium to the killing effect of heat treatments, and that the action spectrum for this effect is that of a protein component.

An interesting in vitro experiment to determine the effect of ultraviolet irradiation on hyaluronic acid has been reported by Hvidberg, Kvorning, Schmidt and Schou (19). Hyaluronic acid is an essential constituent of connective tissue ground substance; it is a highly viscous product whose viscosity can be reduced by various other materials. Of these the specific hyaluronidase is the most important. The viscosity is a function of the degree of polymerization; therefore ultrasonic waves, ionizing rays and ultraviolet light can each reduce the viscosity of hyaluronic acid. Potassium hyaluronate prepared from umbilical cords was used for the experiment. A mercury-quartz high pressure burner was the source of radiation. The irradiation took place through a quartz plate constituting the bottom of the cuvette which contained the solution and whose height, which determined the light pathway through the solution, was 6 mm. On irradiating hyaluronic acid under the experimental conditions described, the viscosity was found to decrease with increase in time of exposure. By prolonged irradiation the viscosity could be reduced to the same value as that of the solvent. This corresponds to the effect obtained by adding excess hyaluronidase. It was found that when rays of wavelengths under 3000 Å were filtered off, the viscosity was not reduced. These investigators suggest that the main viscosity-reducing activity is exercised by the radiation emitted at a wavelength of about 2550 Å whose share in the emission of the burner used is of the order of about 5%. Decrease in viscosity stopped simultaneously with discontinuation of ultraviolet irradiation. It was felt of interest to find out whether exposure of a solution of chondroitin sulfate to the same source of radiation might reduce its viscosity. This substance is another important mucopolysaccharide in connective tissue. Solutions of the compound were exposed to prolonged irradiations. The viscosity was found to remain unchanged. Irradiation of dextran solution likewise had no influence on its viscosity. The results left no doubt that ultraviolet irradiation can depolymerize hyaluronic acid to such an extent as to reduce its viscosity when this experiment is carried out in vitro. The authors concluded as follows: "There is thus hardly any doubt that depolymerization of the hyaluronic acid only is the cause of the appreciably increased spreading reaction after exposure of connective tissue ground substance to ultraviolet light." Hvidberg et al have also conducted in vivo experiments on the effect of ultraviolet irradiation of connective tissue (20). Ultraviolet irradiation on the inside of the skins of dead mice increased the spreading reaction of an intradermally

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injected dye. This was presumed to be due to depolymerization of hyaluronic acid in the connective tissue ground substance of the skin. The spreading reaction in living rabbits remained unchanged after ultraviolet irradiation for up to 30 seconds. In living rabbits ultraviolet irradiation for 120 seconds caused the spreading reaction to increase after an interval of 24 hours whereas the spread was unchanged shortly after the irradiation had been stopped. Water and hexosamine concentrations in the skin of living rats remained unchanged after ultraviolet irradiation for up to 90 seconds. It was concluded that ultraviolet irradiation has a marked effect on the connective tissue ground substance, probably by depolymerizing the hyaluronic acid. If the epithelium is intact this will protect the underlying tissues against direct action. If, however, the dose of ultraviolet light is sufficiently large a secondary increase in spread will occur after a certain latency, presumably due to a development of an inflammatory reaction to irradiation.

Blum, Grady and Kirby-Smith (21, 22, 23) studied the effects of mercury arc radiation on the weight of male albino mice incidental to studies on the induction of cancer. Such radiation, including wavelengths shorter than 3200 Å, was applied at regular intervals in varying doses and intensities. A decreased rate of gain in body weight resulted. The degree of skin damage paralleled the inhibition of weight gain. There were no changes in internal organs suggestive of major systemic effects. These investigators believed that the slower gain in body weight was probably accounted for by decreased food intake and resulted from superficial damage.

The antirachitic effect of ultraviolet radiation has been studied by Demina (24). Experiments carried out in white mice revealed that ultraviolet radiation corresponding to one-eighth of the erythema dose represented, when administered daily, an antirachitic effect equal to that of one international unit of vitamin D_2. If the radiation was increased to one-fourth of the erythema dose the antirachitic effect was reinforced whereas an increase in the dose of vitamin D_2 to two international units resulted in mild hypervitaminosis. From studies carried out in children Demina concluded that daily ultraviolet irradiation in doses equal to one-eighth to one-sixth of the erythema dose, or administered together with a dose of 5000 international units of vitamin D_2, has a more marked antirachitic effect than the administration of 5000 to 10,000 international units of vitamin D_2 alone.

Gusarova (25) obtained marked improvement of their condition in 97 patients with recurrent types of pyodermatitis. Good results were evident even in patients unsuccessfully treated in the past with antibiotics and other methods. It should be noted that Gusarova's ultraviolet irradiation of these patients was combined with the administration of staphylococcal antitoxin.

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Irradiation for 5 to 20 minutes by ultraviolet light of wavelength 2597 Å of 1 to 10% brain suspensions infected with Russian tick-borne or Japanese encephalitis virus, resulted in complete inactivation of the virus (26). The same suspensions irradiated by wavelengths of 2970 to 3020 Å retained their infectiveness, even after 30 minutes of irradiation. In complement fixation tests, virus antigens inactivated by short wavelengths irradiation reacted positively with specific serum.

Ultraviolet Light Absorbers

Any abnormal lesions brought about by action of light must be initiated by a photochemical reaction (27). This reaction in man or other mammals must take place in the superficial region of the skin or in the eye because of low penetration of light into the body. The first law of photochemistry is that light must be absorbed in order to bring about photochemical reactions and this means that for every type of photopathological lesion, there must be some specific substance that absorbs light. Substances that are likely to be light absorbers in living tissues are more or less complex organic compounds having characteristically rather broad absorption spectra. Wavelengths which produce a given pathologic lesion must lie within the range of absorption of the particular absorbing substance. It should be possible to distinguish pathologic effects on the basis of the wavelengths that elicit them and this should constitute one of the most basic differentiations that could be made. Unfortunately, it is difficult to differentiate on this basis because accurate measurements may not be possible in instances where the absorption spectra of the light absorbers overlap.

Dürken and Graul (28) compared the absorptions of ultraviolet and x-rays. They observed that ultraviolet light, in contrast to roentgen light, exerts its effect primarily upon the cytoplasm of the cells and particularly upon the cell membrane. Roentgen rays have their most marked effect upon the nuclear elements of the cell, according to their observations.

Bradfield and Errera (29) studied ultraviolet absorption in living cells mounted in appropriate Ringer solutions. They found that paramecia showed both nuclear and cytoplasmic absorption from the moment they swam into the ultraviolet beam. There was increased absorption in the nuclei of frog and fowl erythrocytes which they considered to be due to the increasing density of the surroudning cytoplasm. In smears of sarcoma cells strong nuclear and cytoplasmic absorption showed little change with continuous radiation at 2650 Å. Myeloblasts, erythroblasts and other stem cells in bone marrow as well as lymphocytes and thymocytes showed a fall in nuclear absorption of about 15% when

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irradiated at 2650 Å. The fall in nuclear absorption in the stem cells of bone marrow was thought to be due to swelling of the nucleus, a change in the state of the nuclear material, partial depolymerization and loss of nucleoprotein, and changes in membrane permeability facilitating the loss of nuclear material.

Bradfield and Errera (29) summarized their observations as follows: Living cells absorbed ultraviolet irradiation around 2600 Å as soon as they could be observed but continued irradiation of the intensity required for measurement caused changes in several cases. The fall in absorption produced in some cells was arrested by switching off the light and was prevented, even during continuous irradiation, by the presence of sulfhydryl inactivators. It should be noted that many investigations have revealed an abundance of sulfhydryl groups in cells just before and during the prophase of mitosis, when extensive changes in nucleic acid distribution are taking place.

Montgomery, Bonner and Roberts (30) studied nuclear absorption in living HeLa cells by "ultra-violet flying spot television microscopy." They described the undamaged nucleus as showing little or no absorption at 2680 Å and noted that this was in marked contrast to the observations of Caspersson whose use of ultraviolet light for visualization of cellular structure was supposed to have resulted in irradiation damage. Montgomery et al continued that lack of nuclear absorption by visible or ultraviolet light in normal living cells is one criterion of the absence of significant irradiation damage. As irradiation damage occurs the nuclear absorption increases until the nucleus becomes homogeneously opaque. The apparent increasing density of the nucleus in contrast to its normal transmissiveness may represent an alteration in the physical state of the DNA of the chromosomes.

Blum, Robinson and Loos (31), in investigating the loci of action of ultraviolet in the egg and sperm of the sea urchin, took advantage of the fact that the eggs can be separated by centrifugation into nucleate and enucleate halves. The whole egg may be exposed to ultraviolet, either before or after fertilization with normal sperm. The sperm could, at will, also be exposed to ultraviolet radiation before being used to fertilize the egg or the halves of eggs. Delay in cleavage was observed in all cases except those where the enucleate half of the egg was exposed to ultraviolet radiation before fertilization with normal sperm. If the sperm nucleus is introduced into the enucleate half of the egg before exposure or if the sperm itself is exposed to ultraviolet radiation, delay of cleavage results. The conclusion seemed obvious that the locus of action of the radiation was the nucleus or something closely associated with it. After initial delay by ultraviolet radiation, there was a gradual return toward normal cleavage rate. If the sperm considered to be comparable to bacteriophage and the egg to be

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comparable to E. coli the parallelism with the reports of Kelner (32, 33) becomes evident. Sperm, like bacteriophage, does not show recovery from the effect of ultraviolet radiation. Only when the irradiated sperm or bacteriophage is associated with egg cytoplasm or with E. coli, respectively does recovery take place. Since bacteriophage is virtually pure nucleoprotein it seems almost certain this substance is the absorber of the ultraviolet radiation. It is also reasonable to consider that nucleoprotein of the nucleus is again the absorber of ultraviolet radiation in the primary photochemical act initiating cleavage delay. It is important, however, that these experiments do not preclude action of ultraviolet radiation on parts of the cell other than the nucleus but only indicate that such action does not affect the rate of cleavage of the egg. Bacteriophage multiplies only in association with the host cell which is presumably essential for synthesis of nucleoprotein. Similarly, the sperm nucleus is associated with cell division and the synthesis of nucleoprotein only after it is brought into the presence of the egg cytoplasm. In attempting to explain this parallelism, it was postulated that ultraviolet radiation alters the nucleoprotein, bringing about some minor changes in configuration that can be reversed by the synthetic processes carried out by systems generally present in the cytoplasm but absent in the case of sperm. Blum et al (31) say "It is tempting to associate the repair after exposure to ultraviolet radiation with the synthesis of nucleoprotein; and since the latter no doubt involves endergonic processes, to regard the repair, too, as endergonic."

Changes in the nucleoli of grasshopper neuroblasts, induced by different wavelengths of ultraviolet radiation, were studied by Carlson and McMaster (34). If the nucleoli were irradiated and examined between telophase and their disappearance at late prophase, each seemed to break up into a group of about 10 nucleolar fragments which generally separated and formed refractile spherioles. These gradually fused to form one large sphere and disappeared at late prophase. Spheration was induced in the nucleolus of the grasshopper neuroblast by wavelengths 2250, 2399, 2537, 2650, 2804, 3022, and 3130 Å. Wavelengths 2650 and 2804 Å were most effective in inducing nucleolar spheration. Above 2804 and below 2650 Å the effectiveness of the ultraviolet gradually decreased. At 3650 no effect was attained even after prolonged exposure to radiation of high intensity. The time interval between termination of ultraviolet treatment and maximum spheration was independent of wavelengths and dose. Interphase nucleoli were the most sensitive, followed by very early prophase, early prophase, middle prophase, and late prophase in order of decreasing sensitivity. Spheration appeared to result from direct action of the radiation on the nucleolus or on the material immediately surrounding it. The effects produced on the nucleolus by ultraviolet, heat, and x-rays resembled each other only superficially. The fact that the wavelengths of 2650 and 2804 Å were more effective in producing

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spheration than wavelengths either above or below these levels was of interest because of the close parallelism between the extent to which the higher wavelengths are absorbed by nucleic acid and proteins and by the extent of which the wavelengths below 2650 become progressively less effective. In the latter wavelength range absorption by nucleic acid at first diminishes and then increases at 2250 Å. Absorption by protein of this wavelength is so high that most of the energy is apparently filtered out by the overlying protoplasm and does not reach the nucleolus. Since the action spectrum of nucleolar spheration shows a maximum effectiveness of the 2650 and 2804 Å lines, it would be logical to conclude that nucleolar spheration can be produced by ultraviolet-induced alterations of either the nucleic acid or protein of the nucleolus.

Errera and Vanderhaeghe (35) investigated the effects of ultraviolet rays on acetabularia Mediterranea. They found the cytoplasm of this organism to be more sensitive to ultraviolet irradiation than the nucleus, as demonstrated by decreased survival and regeneration of anucleate fragments as compared with nucleate ones. Irradiation of the cytoplasm only of the whole cell had the same effect as irradiation of the entire cell. When only the rhizoid (which contains the nucleus) is irradiated, there is less injury than when the whole cell or only the cytoplasm is irradiated. Mazia and Hirshfield (36) found that the survival of anucleate fragments of amoebae could be reduced by exposure to 2540 Å radiation. The anucleate fragments were more affected by such radiation than were the nucleate fragments or whole amoebae. It appeared to these investigators, therefore, that the survival of the anucleate fragments was intimately associated with nucleic acid metabolism. Zimmerman, Landau, and Marsland (37) found that 2800 Å was the most effective wavelength in reducing gel strength in both fragments and in whole amoebae. Because of the correspondence of this wavelength with the protein absorption band, it was concluded that structural protein is intimately associated with gel maintenance. Iverson (38) found that ultraviolet injury of the cytoplasm could be reversed by replacement of an irradiated nucleus with an unirradiated nucleus but that unirradiated cytoplasm was unable to reverse the ultraviolet injury of the nucleus. Unirradiated cytoplasm was even injured by the presence of an irradiated nucleus. An amoeba with an unirradiated nucleus in irradiated cytoplasm was inhibited in the incorporation of phenylalanine more than a cell with an irradiated nucleus in unirradiated cytoplasm or an ultraviolet treated amoeba without nuclear transfer.

The absorption spectra of visual purple and indicator yellow were studied by Lythgoe (39) and the maximum absorption of visual purple was found to be at 5020 Å. Lythgoe explained variations in his maximum absorption wavelength and those obtained by previous investigators on the basis that their

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solutions were probably more acid than pH 9.0. Absorption curves for indicator yellow, which is an intermediate substance formed during the course of bleaching of visual purple, have been determined to be between 3950 and 6500 Å over a range of hydrogen ion concentration from pH 3.3 to 10.8.

The combination of time lapse motion picture studies and ultraviolet irradiation of HeLa cells by Montgomery, Bonner and Roberts (40, 41) indicated that undamaged intermitotic HeLa cell nuclei, exclusive of the nucleoli, show little or no ultraviolet absorption at 2650 Å. They indicated further that this condition obtains, even after significant ultraviolet damage can be observed elsewhere in the cell, this damage consisting of gelling of the cytoplasm with cessation of Brownian motion of the lipid droplets and cessation of pinocytosis. Increase the intensity of the ultraviolet beam resulted in ability to complete mitosis, increased syneresis during mitosis and decreased cytoplasmic bubbling during mitosis. When the beam current was further increased, intermitotic cells showed a sudden generalized increase in absorption followed immediately by a marked contraction of the cell into a small round intensely absorbing mass.

Ultraviolet radiation is absorbed by various kinds of molecules in the cell and hence it is possible that a number of photochemical reactions may be promoted (42). There is, moreover, the possibility that one effect of radiation may be modified by another. This is more likely to occur with polychromatic radiation than with monochromatic. Where action spectra overlap, the use of monochromatic radiation does not in itself eliminate the possibility of mixed effects. The term "cytolysis" refers to a process which ends in the egg breaking up into small globules (the "egg" referred to by Blum, Cook and Loos is the Arbacia egg). The first observable changes were found to occur within minutes to a few hours after exposure to ultraviolet radiation, depending upon the dose. Characteristically the echinochrome pigment was accumulated in a limited region at the periphery of the cell. The break-up of the cell consisted of the formation of globules so that in viewing a field of cytolyzed cells there appeared numerous ruby-like, gleaming spheres accompanied by a much larger number of small, colorless ones. The cell nucleus was usually indistinguishable in this mass of cellular debris. Eggs subjected to high doses of unfiltered radiation may not break up into globules, but remain intact, and retain the approximate volume of normal eggs or even show moderate increases in some cases. There may be extensive changes in the interior of the cell including concentrations of pigment to form a ruby-like spot as in eggs about to cytolyze with the remainder of the eggs showing changes also similar to those that go on prior to cytolysis. This effect of ultraviolet irradiation has been termed "fixation." That there is more than one light absorber concerned in the photochemical reactions underlying these changes

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is clear from the respective ratios of effectiveness of filtered and unfiltered radiation. The lower ratio in the case of cytolysis is, however, difficult to interpret. Calculations of the relative numbers of quanta absorbed by a typical protein gives the ratio of about 1:10 for filtered as against unfiltered radiation. This ratio is higher than that for nucleic acid and altogether out of line with the ratio of effectiveness in producing cytolysis. It must be remembered that the intensity of filtered radiation is much lower than that of unfiltered, although we have no way of estimating accurately the relative intensities without knowing the spectrum of the light absorber. It seems likely that the rate of photochemical change may be important in determining whether cytolysis occurs and, hence, intensity may influence the ratio of effectiveness. This leaves us with no real evidence as to the nature of the light absorber for cytolysis. That this effect is based on cytoplasmic rather than on nuclear changes is suggested by experiments in which normal eggs were fertilized with sperm that had been exposed to ultraviolet radiation. These eggs did not cytolyze (at least not for many hours). If cytolysis were due to production of a cytolytic agent in nuclear material, one would expect it to occur in this case. Sonne (43) suggested that ultraviolet radiation may act upon the lipid faction of the erythrocyte surface to produce hemolysis and that the action spectrum reflects the absorption spectrum of that faction. One is tempted to carry this explanation over to the inactivation of the Arbacia egg, particularly since it is thought that activation involves changes in the lipids of the cell membrane.

Ultraviolet Radiations and Cell Division

Unfiltered radiation may bring about a series of changes in unfertilized eggs termed "activation." Furrows develop on the surface of the egg, often followed by more or less normal cleavage. Occasionally one finds such a cleavage in which the nuclei can be detected in the two divided cells. For the most part the cleavages are irregular and usually the eggs break up after going through one or a few cleavages. The proportion of activated eggs varies widely among samples from different females; always present with the activated eggs are cells which are distorted in shape and which do not display a tendency to cleave, together with a certain number of cytolyzed or fixed cells (42). In the course of activation the eggs sometimes develop a membrane which is usually assumed to be identical with the fertilization membrane that appears after the entrance of the sperm.

Partial-cell irradiation has been utilized to determine the normal function of various cell parts by selectively altering

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them and leaving the rest of the cell more or less intact in structure and function (44). Experiments were performed on cells in tissue culture. Some were cultures of mouse embryo and of HeLa but the majority were cultures of heart fragments from adult newts. In each experiment Zirkle et al (44) observed the dividing cells by medium dark phase contrast microscopy with photomicrographs being obtained 15 times per minute both before and after irradiation. If part of the chromosomal material of a cell is exposed to an ultraviolet microbeam, the chromosomal index of refraction is lower and approaches that of the surrounding cytoplasm so that the irradiated portion of the chromosome becomes pale as viewed in the phase contrast microscope. "Paling" has been observed in prophase, metaphase, anaphase and interphase and has not yet been investigated in telephase and reconstruction (44). In interphase the paling of the chromatin granules requires several times the dose that is effective on mitotic chromosomes. In prophase or interphase ultraviolet doses that pale the chromosomes or chromatin produced no visible effect on nucleoli. Wavelengths of 2250, 2400, 2500, 2600, 2700, 2800 and 3000 Å all produced paling. Wavelength 2250 Å is some 40 or 50 times as effective as wavelength 3000, and the other wavelengths are logarithmically about midway between these two and do not differ by more than a factor of 2 or 3 among themselves in effectiveness. It is possible, with an ultraviolet microbeam, to destroy the spindle of a given cell in such a fashion that cell division is not interfered with. The separation of the 2 groups of chromatin material is followed by cell constriction and by reconstruction of each chromosome group into a microscopically normal nucleus. The total number of chromosomes following such division is 24 but they are not always evenly distributed (12 and 12); combinations such as 11 and 13, 10 and 14, 9 and 15, etc. also occur. It is evident, therefore, that in this abnormal process involving no visible spindle, groups of whole chromosomes instead of half chromosomes are moving apart. This process has been termed "false anaphase." It is worthy of note that, to destroy the spindle and thus induce deranged metaphase and false anaphase, it was not necessary to include any part of the spindle in the irradiated volume. Exposure of cytoplasm well away from the spindle produced the same effects. Since, when the spindle was irradiated, intervening cytoplasm was exposed to the converging cone of the ultraviolet microbeam, spindle destruction is probably due, entirely or nearly so, to absorption of ultraviolet by some cytoplasmic component. "This component can be visualized as a precursor which, when altered by irradiation, becomes a spindle posion that presumably reaches the spindle by diffusion." To gain some hint as to the nature of the hypothetical percursor Zirkle et al (44) undertook comparative quantitative experiments with various ultraviolet wavelengths. Each of the wavelengths 2250, 2400, 2600, 2800 and 3000 Å destroyed the spindle when applied to cytoplasm. Wavelength 2250 was about 10 times as effective as the others, which did not differ among themselves by more than a factor of 2 or 3.

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There was some indication of a maximum in the vicinity of 2800 Å. False anaphase is obviously an excellent means of producing aneuploidy. Assuming random distribution of any pair of homologous chromosomes the probability that both will go into one daughter cell and neither to the other is 0.5. Thus the probabilities of very severe aneuploid distributions are quite considerable. The probability that no aneuploidy will result (i.e., each of the two daughter cells will receive a perfect chromosome set) is only 1 in 4096. Another phenomenon observed by Zirkle et al (44) is chromosome "stickiness." With either a proton or an ultraviolet microbeam stickiness is produced between a pair of chromatids or among chromosomes of a group. An interesting result of stickiness is the formation of "anaphase hinges." One side of a metaphase chromosome group may be suitably irradiated with the production of sticky bridges only in the irradiated chromosome. The nonirradiated chromosomes attempt a normal anaphase movement but, instead of proceeding individually and directly to the ends of the spindle, they swing as a group around the sticky ones as a hinge. The hinge action is apparently brought about by the combined action of normal anaphase forces and the tension in the sticky bridge. In terms of incident energy, 2250 Å is about 100 times as effective as 3000 Å in producing stickiness. Here again there is clear evidence of a maximum at or near 2800 Å and there probably is another at or near 2600 Å. This suggests that stickiness may be produced by alteration either of nucleic acid or of proteins containing aromatic amino acids.

Electron microscopic observations showed that most bacteria in a suspension of E. coli, receiving doses of ultraviolet light many times that sufficient to reduce plate counts of viable organisms by a factor of one million, can grow and multiply for some time after irradiation. Evidence of this residual metabolic potential diminishes with increased doses of radiation. Such bacteria are lysed by bacteriophage with yields of new bacteriophage that also diminish with increased irradiation. These experiments (45) demonstrate that bacteria which have received many times the dose of radiation sufficient to prevent their indefinite multiplication are not immediately killed by this exposure. Instead, such mortally damaged cells continue to grow and to undergo seemingly normal division even though they and all their daughter cells succumb after a few hours of incubation. For the periods of irradiation used, which did not allow more than 1 in about 100,000 of the irradiated organisms to proceed to normal colony formation, this period of bacterial survival appeared to be of the order of 2 hours. The metabolic activity of the damaged bacteria, as shown by cellular enlargement and division and as substantiated by phosphorus uptake studies, during this period of survival is diminished with increased exposure to radiation. There was little perceptible activity in bacteria which received about 100 times the minimal dose of radiation used in these experiments.

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The electron microscopic observation appeared to be compatible with the hypothesis that bacteriophage produced in cultures of E. coli "killed" with ultraviolet light, developed through interaction with these mortally injured but not yet "dead" cells. The damaged bacteria can be lysed in normal fashion by bacteriophage with the production of a large yield of new bacteriophage. There is, however, an absence of such bacteriophage from cells irradiated so heavily that they do not give evidence of further growth and multiplication.

The ability of ultraviolet to protect or to reactivate x-rayed dividing yeast cells (cells in division when irradiated) has been reported by Elkind and Sutton (46). The survival curve obtained from these experiments suggests that dividing cells are less sensitive to ultraviolet as well as to x-rays, than interdivisional cells. The mitigating action of ultraviolet is independent of the sequence of administration. There is quantitative agreement between the protection afforded by preultraviolet and the reactivation resulting from postultraviolet exposures. Mitigating ultraviolet exposure can be given during the x-ray exposures with results equivalent to those of the preceding. It has been shown also that ultraviolet protection is totally reversible by visible light. These results support the view that the sites of action of lethal irradiation are chromosomal deoxyribonucleic acid. In addition, this mitigating effect of ultraviolet compares with cytogenic observations in tradescantia pollen tubes and drosophila sperm where it has been observed that pre- or postultraviolet irradiation decreased the observable number of x-ray chromosome breaks. The results suggest considerable overlap between x-ray and ultraviolet "lethality sites" in dividing cells.

Hirshfield and Pecora (47) noted that abolition of nuclear or cell division without immediate cell destruction can be accomplished by critical dosages of x-rays, ultraviolet irradiation, or by such drugs as colchicine. Depending upon the degree of nuclear damage produced by such agents the effects resembled those produced by enucleation. With respect to ultraviolet irradiation, its effects could not be referred with certainty to either nuclear or cytoplasmic damage but rather appeared to involve both. The process of fission was shown to be more sensitive to the same wavelength that delayed regeneration.

Mouse sperm has been irradiated by ultraviolet light in vitro with dosages up to 156.6×10^5 ergs per sq. cm. on the surface of the sperm suspension (48, 49). The irradiated sperm were then artifically inseminated into eostrous females. Ultraviolet irradiation reduced the activity of sperm and probably caused delayed penetration. After short exposures considerable embryonic mortality occurred in presumably diploid embryos at implantation, and the size was very small. One of the few

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offspring was deformed. Slight increase in exposure time completely suppressed implantation. These defects could have been due to induced changes in the sperm chromosome. The spermatozoa which were probably heavily radiated and therefore may have contained inactivated chromatin presumably would fertilize proportionately fewer eggs than spermatozoa of the same sample which had received less irradiation and, therefore, were more active. Total inactivation of male chromatin may have been due to the high proportion of ultraviolet rays of wavelengths 2550 to 2650 Å. Ultraviolet irradiation may have had three progressive effects on the sperm chromatin: (1) induction of mutations and other genetic changes; (2) loss of individual chromosomes; and (3) complete inactivation by failure to participate in syngamy. It should be noted that retardation in development, characteristic for each dosage, was more consistent after x-ray; this may have been due to the lower penetrative power of ultraviolet rays which allowed spermatozoa in the lower levels of the suspension to escape the full effect of the irradiation.

With respect to roentgen rays, Henshaw (50) found that it took about 50,000 R of unfiltered x-radiation to prevent the eggs of Arbacia punctulata from fertilizing and undergoing cleavage. The onset of the first cleavage (measured from the time of insemination) was delayed by exposure of Arbacia eggs to much smaller doses of roentgen rays before fertilization. With an increase in dosage there was a corresponding increase in the amount of delay. The most important conclusion to be drawn from the investigations of Henshaw is, that as soon as any irradiation effect has been produced, a process of recovery begins which seems to be independent of the magnitude of the effect and governed by exponential law.

Effects of Ultraviolet Irradiation on Mitosis

The mitotic effects of ultraviolet radiation of 2537 Å on grasshopper neuroblasts were determined by treatment and observation of the living cell in artificial culture medium (51). Early prophase proved to be the stage of mitosis retarded the most by irradiation. Interphase, middle prophase, and late prophase showed less sensitivity while very late prophase, metaphase and anaphase were unaffected by doses as high as 36,000 ergs per sq. cm. The same dose given in 1, 15, and 1500 seconds retarded mitosis to the same extent. The mitotic effect was essentially the same for a given total dose whether it was given continuously or in two fractions separated by an interval of time 100 times the length of the total dose. Unlike x-radiation, ultraviolet radiation was not followed by a compensatory effect during recovery of the cells from the effects of treatment. Nucleoli were changed by treatment from

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their normal, irregular form to a spherical shape (34). This was frequently accompanied by an increase in number and a corresponding decrease in the size of the nucleolar bodies. The 2537 Å wavelength is close to the region of maximal absorption by nucleic acid (2600 Å). It is probably as a result of its high absorption by these compounds that this wavelength produces striking effects on living cells. The chromosomes of the living cell are part of a very delicately balanced and precisely adjusted living unit in which changes induced in a few molecules by relatively low doses of radiation may alter radically certain readily detectable functional and structural characteristics of the cell. The finding that mitosis is most easily affected by ultraviolet radiation in early prophase and by x-radiation in late prophase could hardly have been predicted on the basis of knowledge of the living cell and the properties of its physical components. Since it has been reported that nucleic acid content of the nucleus increases as the cell approaches the metaphase condition, it might have been expected that the sensitivity toward ultraviolet of 2537 Å would be more pronounced in this stage. On the other hand the slender, twisted chromosome threads of early prophase offer a much greater surface for absorption of ultraviolet than the shorter, thicker chromosomes of late prophase and this would not influence x-ray penetration. It was noted by Carlson and Hollaender (51) that, while this interpretation is based on the assumption that mitotic effect is exerted through chromosomes, it is not inconceivable that ultraviolet radiation may affect some other important mitotic factor regarding which there is no evidence. Although the nature of the mitosis-inhibiting primary and secondary effects induced in the grasshopper neuroblast by wavelength 2537 Å are obscure, it seems not unlikely that alterations in nucleic acids which exhibit a high absorption at this region of the radiation spectrum, may be involved (52). If these are the cell substances affected it seems more likely that the alterations are physical rather than chemical since it has been demonstrated that measurable physical alterations rather than primary chemical changes, i.e., the breaking of covalent linkages are induced by moderate amounts of 2537 Å radiation of sodium thymonucleate outside the cell. There is no evidence of reversibility of the physical changes thus produced in sodium thymonucleate in vitro which contrasts with the situation in the living cell which does show recovery. This is probably due to the presence within the living protoplasm of enzymatic substances that promote repair. X-radiation of the generative nucleus in tradescantia pollen grains reveals that most of the chromosomes are effectively split into sister chromatids (5). A considerable proportion of the chromatid aberrations involve deletions of both chromatids of a chromosome at identical loci, thus confirming the genetic data in maize with respect to endosperm deficiencies. Ultraviolet radiation of the generative nucleus in the pollen tube induces only simple chromatid deletions. The loss of only one of the two chromatids is in accord with the genetic observation that ultraviolet radiation produces

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primarily fractional endosperm deficiencies in maize. No configurations representing an interchange of chromatin between non-homologous chromosomes were found. The qualitative differences between the types of breaks induced by x-ray and ultraviolet radiation was tentatively explained by assuming that the sphere of influence of a single x-ray quantum is much greater in area than that of a single ultraviolet quantum. The vast difference in energy values and the difference in the physical behavior attendant to absorption of the respective quanta supply a possible physical and chemical basis for this variation in degree of effectiveness. The absence of translocation under ultraviolet treatment is not explained, however, by the above observation. The question arises as to whether or not chromosome breaks induced by ultraviolet radiation are capable of reuniting back into their original position or into new associations. It has been shown that x-ray-induced breaks can remain in an unstable condition and be capable of refusion for as long as an hour after the time of irradiation. Swanson suggests the possibility that the chemical action of ultraviolet rays leaves a satisfied bond at the broken end such that refusion is impossible. If this were true it might serve to explain the absence of gross chromosomal rearrangement with ultraviolet pigment under circumstances where comparable doses of x-ray, as judged from the frequency of endosperm deficiencies and lethals, produce an abundance of these types of aberrations.

The effects of radiation have been presumed to result from chemical change produced directly in biologically important molecules which absorb radiation. The demonstrated influences of oxygen concentration during irradiation, of postirradiation treatment, and the demonstration of the protection afforded by various chemicals show that a major share of the effects of ionizing radiations occur by indirect mechanisms (53). The effects of ultraviolet radiation similarly have been believed to result from chemical changes produced by the excitation of the molecules absorbing the radiation and the philosophy behind the action spectrum technique has been that a study of the relative effectiveness of different wavelengths would parallel the absorption spectrum of the biologically important molecule and hence furnish evidence concerning the cellular components affected. With respect to ionizing radiation, the consequence of a single ionization is chemical change in the ionized molecule. The lethal effects, therefore, appear to result from chemical change in a single molecule and the gene is the most logical candidate for the kind of molecules so vital to the cell that a change in a single one could be lethal to the cell. With ultraviolet the kinetic picture is not as clear but in many cases bonafide exponential as well as sigmoidal curves have been observed. The occurrence of exponential killing in some bacteria with ultraviolet suggests a unitary action and a similar argument likewise leads to the gene. This concept is supported by the commonly observed 2650 Å maximum in efficiency of ultraviolet

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killing and for mutation induction, which suggests absorption of ultraviolet quanta in nucleoprotein molecules as the initial step in both inactivation and induction of mutations.

Fluorescence is a property of certain substances that causes them to emit light when activated by ultraviolet light. In most instances the light emitted is differently colored and of different wavelengths than that of the substance. In minerals containing a fluorescent material it has been found possible to photograph in the dark, by means of ultraviolet light, the site of the fluorescent material. Biologists are acquainted with this phenomenon because certain dyes and oils change color in the sunlight, as for instance eosin and chlorophyll. The photosensitivity of living cells stained with vital dyes was discovered when motion pictures were made of living cells stained with neutral red. Cancer cells growing in tissue cultures containing eosin were found to be more photosensitive than normal cells in the same culture. Fluorescent substances -- chlorophyll, dibenzanthracene, methylcholanthrene, neutral red and eosin -- added to tissue cultures were not toxic so long as the cultures were kept in the dark (54). In the dark the cells containing the substances grew and multiplied, exhibiting about the same number of mitotic figures as the control cultures growing in media from fluorescent material. It was only when a strong light was passed through a culture containing a fluorescent substance that the cells were damaged. Cells in the prophase stage of division, growing in a medium containing neutral red or eosin, when exposed to light, failed to form a spindle. The scattered chromosomes became shrunken and stationary. As light activated the fluorescent substance the spindle in the cell in metaphase shortened and later disappeared; the mitochondria and other granules moved into the area previously occupied by the spindle and the chromosomes became agglutinated. The chromosomes failed to separate and remained clumped on the equatorial plate. The majority of cells in anaphase in cultures containing neutral red or eosin, when exposed to light, failed to complete their division; in a few instances the cells in late anaphase indented and were almost completely separated before the light caused a cessation of the division resulting in failure of the sister cells to separate. In many cells affected in the early anaphase stage chromosomes remained attached end-to-end as has been described in cultures having fluorescent material in their medium. It seemed apparent that cells were damaged by the change brought about in the medium during the activation of the fluorescent material present in the medium in which the cells were growing.

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Progress in understanding the photochemical action of ultraviolet on proteins and nucleic acids has been slow (55, 56). Several different chemical steps probably can take place simultaneously, however, and more than one of these may give rise to inactivation as discerned by some biochemical modification. The most fruitful approach to the problem in the immediate future may be the study of organic chemical changes accompanying exposure of enzymes and nucleic acid to ultraviolet, provided these changes are correlated with quanta absorbed per molecule altered and with change or loss in biochemical specificity. Destruction of nucleic acid involves rupture of the carbohydrate-phosphate chain and is accompanied by alteration of purine and pyrimidine residues. The nature of virus and phage inactivation is dependent on changes in the nucleic acid or protein moiety but the principal site of chemical action of ultraviolet has not been demonstrated clearly. Ionizing radiation can induce reactions in proteins by direct action, by indirect action, and by excitation. Excitation reactions are essentially photochemical reactions. Direct inactivation by ultraviolet and indirect action by ionizing radiation show similar kinetics and quantum yields are similar to ionic yields. Chymotrypsin loses its esterase and proteinase activities at equal rates with ultraviolet but esterase activity is lost more quickly with x-ray. With ultraviolet, inactivation is accompanied by denaturation. With both ultraviolet and x-rays enzymes are rendered thermolabile and undergo further postradiation inactivation.

The discovery of a method for synchronizing division in tetrahymena by heat treatment has provided interesting material for a study of the relationship between ultraviolet susceptibility and content of nucleic acid (57). Division of tetrahymena is delayed by irradiation, the delay increasing with dosage. Delay of the first division after irradiation is not marked but delay of the second and third divisions following irradiation is more noticeable. The effects of a given dosage of ultraviolet of wavelength 2650 Å retards division of tetrahymena more than a light dosage of wavelength 2260 Å. On the other hand, the latter radiation immobilized tetrahymena much more readily than the former. The greater resistance to ultraviolet radiation of tetrahymena at the end of heat treatment than in other physiological states is correlated with their greater content of DNA in that stage. Thus the ultraviolet sensitivity is inversely proportional to the amount of DNA present per cell, and shows a direct relationship with the amount of DNA which a cell must synthesize before division. Very short ultraviolet radiations (2260 Å) affect tetrahymena before and at the end of heat treatment to essentially the same degree. This suggests that radiations of this wavelength may produce damage elsewhere than

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by inhibiting nucleic acid synthesis. Since the very short ultraviolet radiations are superficially absorbed they may produce their effects in the cytoplasm as indicated by the rapid immobilization of the tetrahymena.

Alechinsky (58) conducted a series of investigations which indicated that nicotinamide in solution with histidine inhibited the appearance of a histamine substance as the result of the action of ultraviolet rays. His experiments demonstrated that histidine, irradiated with ultraviolet and injected into the vein of the penis of a rabbit, constantly provoked symptoms of histamine shock and that these symptoms were absent upon administration of the same volume of histidine-nicotinamide mixture irradiated in the same manner. He also irradiated one side of the face for 5 minutes through a quartz cuvette filled with a solution of nicotinamide. The other side of the face was irradiated in an identical manner through a quartz cuvette filled with distilled water. Under these conditions, erythema appeared on the side where irradiation was carried out through nicotinamide after a delay of 4 hours while erythema on the other side (irradiated through a cuvette filled with distilled water) appeared within 50 minutes. He reasoned that, on the side irradiated through the nicotinamide filter the histamine substance was inhibited by the formation of a collateral substance which provoked an erythema reaction more slowly. On the side without the nicotinamide filter the production of the histamine substance instead of a collateral substance resulted in a more precocious reaction. It has been believed that when human skin is irritated by mechanical or chemical stimuli, by heat or cold, or by ultraviolet light, the erythema which occurs is due to liberation of a so-called H-substance. Tests made with sunlight showed a very slow rate of formation of histamine--10^{-5} mgm. per sq. cm. of exposed surface per hour or less (59). While not inconceivable that a similar rate of formation of histamine in the skin might produce an evanescent erythema it was considered very improbable that sunlight, acting on the extremely minute quantities of free histidine presumably present in the skin, could produce histamine at an appreciable rate. Bourdillon et al (59) supported the theory that erythemas occurring after exposure to ultraviolet were due to the liberation of substances already present in the skin or to disintegration products of killed cells; they felt this to be especially likely since the greater part of the physiological reaction following exposure to ultraviolet radiation takes place "long after the stimulus is removed."

An interesting in vitro experiment on succinic and cytochrome oxidase activity of rat liver mitochondria after irradiation with ultraviolet light was carried out by Canzanelli, Sossen and Rapport (60). Suspensions of rat liver mitochondria were irradiated with ultraviolet light for varying periods of time and the succinoxidase and cytochrome oxidase activity were determined by their being placed in Warburg flasks immediately following

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irradiation. Both succinoxidase and cytochrome oxidase activity were reduced. The order of magnitude of the ultraviolet energy necessary to produce these changes was much less than that necessary to produce chemical changes in nucleic acid derivatives and approached the amount which has been shown to produce lethal, mutational and other biological effects. The radiation energy at the surface of the suspensions was calculated to be approximately 7000 ergs per sq. cm. per second. A number of experiments were done in which the effect of ultraviolet irradiation on the succinoxidase activity was studied without addition of cytochrome C. A comparison of these experiments with those carried out with cytochrome C showed that the cytochrome C remaining in the mitochondria after isolation is in part either destroyed or inactivated by irradiation; in all cases the percentage in oxygen consumption was greater in those experiments without added cytochrome C. In view of the apparent central role of ATP in the coupled oxidative phosphorylation system of mitochondria and their phosphorylative capacity, Beyer and Kennison (61) measured the release of mitochondrial nucleotides during ultraviolet irradiation. The results indicated a preferential release of nucleotides and inorganic phosphate from ultraviolet treated mitochondria.

Billen (62) observed a release of cellular constituents when x-irradiated (60,000 R) suspensions of E. coli B/r were incubated in a phosphate buffer-glucose solution at 37 degrees C. ATP accounted for less than 10% of the total 2600 Å-absorbing material released by such cells. The other constituents (showing maximum absorption at 2600 Å) were nucleic acid fragments varying from free bases to more complex nucleic acid fragments. No release of peptides above that found with unirradiated cells under similar conditions was observed. The release of nucleic acid fragments was inhibited in the absence of phosphate in the suspending fluid, in the absence of an exogenous metabolite, in the presence of arsenate, and at a low temperature of incubation. Ultraviolet light-exposed cells, suspended in a glucose-phosphate solution at 37 degrees C., released 2600 Å-absorbing material and this release was dependent upon the same conditions as those found for x-ray exposed cells.

It has been suggested that adenosine triphosphate (ATP) may be important in skin changes due to light. Ultraviolet irradiation causes breakdown of ATP in pure solutions and lowers its concentration in living cells. Findlay (63) irradiated a 1 millimole ATP solution by placing it in a quartz cuvette at the exit slit of an ultraviolet monochromator for 5 minutes at 2600 Å. Enzymatic breakdown of ATP was accomplished by using crude homogenates of guinea pig and human skin at 2 to 4% concentration, kept frozen until required. Each tube contained 0.4 ml. of homogenate and 10 to 15 micromoles of ATP and was allowed to react from 30 to 60 minutes at 37 degrees C. The reaction was run so that less than a quarter of the substrate was used and it was stopped with trichloroacetic acid. After

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centrifuging, a phosphate determination was made on the supernatant fluid. The results of these experiments indicated that irradiation of ATP solutions indicated no liberation of phosphates or change in the ultraviolet absorption of the solution compared with unirradiated duplicate material. The doses of ultraviolet were well above thos required to produce a minimal erythema. Findlay concluded that these experiments contradicted the suggestion that adenosine triphosphate is a substance directly concerned with light eruptions since it was shown that physiological doses of ultraviolet light cannot alter ATP itself and that ATP-ase activity of the skin is unaffected by relatively high concentrations of antimalarials (antimalarial drugs have been thought to antagonize some toxic effects of ATP and, in some of the "stress" disorders, they have been considered to act therapeutically in inhibiting the breakdown of ATP by ATP-ase).

Acetylcholinesterase is inactivated by ultraviolet radiation of 2537 Å (64). The enzyme, however, is resistant to ultraviolet radiation and doses of 6×10^4 ergs per sq. mm. were required to produce a clear inactivation under the experimental conditions. Three sulfhydryl reagents were tested for their influence upon the enzymatic activity of acetylcholinesterase and only one (P-choloromercuribenzoate) showed a slight inhibition. Hargreaves (64) believed that inactivation of the enzyme was due to the direct action of ultraviolet radiation rather than to an indirect oxidation of its sulfhydryl groups.

Aqueous extracts of human epidermis inhibit the oxidation of l-tyrosine and l-dihydroxyphenylalanine (DOPA), thus preventing the formation of melanin. It has been assumed that the inhibition is due to the presence of sulfhydryl compounds in the extracts. A similar relationship is demonstrated in vivo following ultraviolet irradiation of skin (65). An increase in melanin formation is preceded by a decrease in the sulfhydryl content of the skin. This suggests that pigment-producing stimuli act by eliminating the sulfhydryl inhibition, allowing the enzymatic oxidation of pigment precursors to occur. A clue as to the possible mechanism of this elimination is provided by another defense reaction of the skin to ultraviolet irradiation, namely, increased production of keratin, manifested as thickening of the horny layer in man and as excessive hair growth in animals. The production of keratin is characterized by the formation of disulfide bridges from sulfhydryl groups of native proteins. Possibly the same biochemical process, oxidation of sulfhydryl groups to disulfide, is responsible for both intensified keratinization and pigmentation.

It has been demonstrated that, on irradiation of cholesterol with ultraviolet light, a substance is formed which has an absorption maximum at about 2350 Å (66). The nature of the change in cholesterol on irradiation has not been fully elucidated. The facts are most readily understood if it is assumed

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that cholesterol forms an unstable ozonide, with subsequent production of H_2O_2 which is the immediate active agent on the photographic plate. In view of the presence of cholesterol in the skin and the undoubted production of skin tumors as a result of irradiation with ultraviolet and x-rays, further work on the chemical changes in cholesterol consequent on irradiation as well as a search for possible carcinogenic products is indicated.

Exposure of insulin to ultraviolet radiation destroyed it; this was indicated by the fact that the injection of the irradiated insulin into rabbits did not produce convulsions whereas the injection of the unexposed insulin did (67). Addition of insulin to irradiated paramecia sugar preparations (5 cc of paramecia in 100 cc of 0.1% sugar solutions) prevented decrease in sugar metabolism as observed in preparations to which no insulin was added. The decrease in sugar metabolism brought about by ultraviolet radiation of paramecia sugar preparations is attributed to destruction of the insulin in the paramecia. Estermann, Luse and McLaren (68) called attention to the fact that the ultraviolet absorption spectrum of insulin changes during irradiation; from this they concluded that some photochemical change occurred in the tyrosine residues and is responsible for the alteration in absorption. This hypothesis was considered to be consistent with the present view of the photochemistry of enzyme and hormone inactivation.

Eremeev (69) irradiated the livers of fasting mice with a "mitogenetic" source (1900 to 2700 Å) and found that this increased the "threshold exposures" necessary for detecting terminal amino groups which, in his opinion, proved that such amino groups were diminished in quantity. He accounted for this by assuming partial resynthesis of the protein substrate of the liver tissue by "mitogenetic" irradiation. "It thus follows that mitogenetic rays stimulate peptide synthesis in the livers of animals."

Friedman and Ceponis (70) produced mutants of the soft rot bacterium pseudomonas marginella by ultraviolet radiation. Radiation-induced mutants were then selected for loss of pathogenecity for lettuce and chickory. The avirulent mutants differed from the parent pathogens in their inability to synthesize pectolytic enzymes in culture or to ferment sodium pectate or sodium polygalacturonate as the sole carbon source in media. Experiments which suggest that protein synthesis is a prerequisite for the resumption of DNA synthesis in E. coli irradiated with ultraviolet light have been reported by Harold and Ziporin (71). Irradiation times were adjusted so as to result in an inhibition of DNA synthesis lasting 20 to 25 minutes. Growth and RNA synthesis were not affected. Irradiation reduced the viable count by 95 to 99%. The results of these experiments were believed to support the hypothesis that synthesis of a protein is one of the prerequisites for the

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resumption of DNA synthesis in E. coli irradiated with ultraviolet light. It appeared that the amount of protein synthesized prior to radiation determined the rate of subsequent DNA synthesis. It was also demonstrated that expression of mutations by ultraviolet light is a function of the rate of protein synthesis during the first hour after irradiation. "If expression of a mutation requires the duplication of DNA and if the latter process requires prior synthesis of a protein in the mutant as well as in the inactivated cells, the relationship between mutation and protein synthesis may become understandable in biochemical terms."

That many of the effects of radiation can be interpreted as inhibitions of the synthesis of specific micromolecular constituents of the organism has been emphasized by Kimball (72). He noted that there is direct evidence for inhibition of the synthesis of DNA and certain adaptive enzymes and that there is good evidence for believing that the effective primary lesions at low doses are mainly nuclear and that at least part of this nuclear damage is readily reversible. Inhibition of DNA synthesis has been suggested by a number of investigators as a major pathway for radiation damage to cells. Kelner (33) advocated this hypothesis for ultraviolet-irradiated bacteria and suggested that inactivation and mutation are secondary consequences of the effect on DNA synthesis. DNA synthesis was blocked while RNA and protein synthesis continued, suggesting that the resulting imbalance between nucleus and cytoplasm led to inactivation.

Photorecovery from Ultraviolet Irradiation

Dulbecco (73) first reported that exposure to visible light in the presence of bacterial cells enhances reactivation of coli-bacteriophage which has been inactivated by ultraviolet radiation. Kelner (32) reported that visible light enhanced recovery of streptomyces griseus conidia which had been treated with ultraviolet radiation. Kelner's experiments suggested that visible light has a factor which uniformly and reproducibly promotes the recovery of many of the cells rendered non-viable by ultraviolet irradiation. The magnitude of the effect was such that it seemed likely that a key factor in the lethal effect of ultraviolet light was being affected by the visible light. He speculated that much of the lethal effect of ultraviolet light is due to a light-labile alteration of some constituent in the cell and that exposure to visible light restored this altered constituent to its former state. He said "the powerful action of light on the resuscitation of the ultraviolet-treated cell leads us to hope that further study of this phenomenon may yield clues leading to the discovery of factors causing similar recovery from x-irradiation or irradiation from radioactive materials."

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The first enhancement by visible light of recovery from ultraviolet irradiation in animal cells was reported by Blum, Loos, Price and Robinson (74). They found that short doses of ultraviolet radiation (less than 3200 Å) caused delay in subsequent cleavages of eggs of the sea urchin. Gradual recovery occurred until after a few cleavages the normal cleavage rate was regained. The return to normal rate was greatly accelerated by light from the short end of the visible spectrum (4000 to 5000 Å). These investigators attempted to demonstrate photorecovery in human skin which had received threshold erythemal doses of ultraviolet radiation from an artificial source. The irradiated skin was afterward exposed to sunlight from which the erythema-producing wavelengths were removed by window glass. Experiments on seven persons indicated no effects of visible light on the threshold.

Little can be said about how visible light accelerates recovery from effects of ultraviolet radiation (75). Obviously the recovery process depends upon a photochemical reaction which is essentially different from that which produces the original changes in the cell. Different spectral ranges for the two effects indicate two different light absorbers. Two different kinds of molecules may be involved but it is possible that there are different absorbing structures in the same molecule and that the second may be an ultraviolet-induced product of the first. The possibility that photodynamic action (photosensitized oxidation) plays a role in the recovery process seems to have been eliminated by the finding that elimination of oxygen does not inhibit the process whereas photodynamic action is dependent upon oxygen (73, 76).

Extension of the known distribution of the phenomenon of photorecovery to the vertebrate level of the animal kingdom was first reported by Blum and Mathews (77). They exposed amphibian larvae 5 days per week to sublethal doses of intense ultraviolet radiation (2300 to 3130 Å). If the larvae were illuminated with visible light from fluorescent lamps after exposure to ultraviolet, they survived longer than if kept in the dark. The longest wavelengths causing recovery were about 5000 Å. The short wavelengths limit was not established but it was established that wavelengths in the near ultraviolet were effective. It was of particular interest that the wavelengths for photorecovery in amphibian larvae were restricted to the same spectral region as photoreactivation in fungi and bacteria and photorecovery of cleavage rate in sea urchin's eggs. All appear to represent the same fundamental process manifested by repair of the effects of ultraviolet radiation by a range of wavelengths extending from the very near ultraviolet to the blue.

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Most studies of photoreactivation have been concerned with reactivation of ultraviolet killed organisms. Wells and Giese (78) investigated visible light reversal of injury caused to eggs of the sea urchin by different monochromatic wavelengths of ultraviolet light. They also studied the effects of different dosages of ultraviolet radiation and they attempted photoreactivation before and after fertilization of the injured gametes. Finally, they studied the effects of visible light on sperm. They found that photoreactivation by visible light occurred following injury by any of the wavelengths tested (2450, 2537, 2654, 2804, 3025 and 3130 Å) but the phenomenon was less pronounced after injury by radiation of 2450 Å than by the other wavelengths. Wavelengths shorter than 4300 Å were most effective in photoreactivation. Zygotes formed from ultraviolet-induced eggs were more readily photoreactivated than unfertilized eggs. Visible light was injurious to sperm but sperm injured by ultraviolet radiation could be photoreactivated. They explained the reduction in photoreversibility following injury at 2450 Å as follows: The wavelength 2450 Å is strongly absorbed by surface proteins as indicated by raising of the membrane when eggs are injured by this wavelength. Several wavelengths tested are more selectively absorbed by nucleoprotein. It appears, therefore, that injury to nucleoproteins is most strongly photoreactivated. The reversible injury is not due to ozone formed by the ultraviolet light because wavelengths 2654 Å and longer do not produce appreciable amounts of ozone.

It has been suggested by Novick and Szilard (79) that the injurious effects of ultraviolet light may be due to the formation of some sort of poison. These investigators suggested since photoreactivation is never complete and is possible only for a time after ultraviolet irradiation, that the poison has two forms --one stable to visible light and one photolabile. As time passes after injury the photolabile form is transformed to the stable form. Novick and Szilard investigated the effects of varying dosages of ultraviolet light correlated with varying doses of light reactivation and found that there was a parallel effect upon the number of survivors (bacteria) which was dependent upon dose of ultraviolet and the amount of reactivation light administered, respectively. They also investigated ultraviolet-induced mutations in bacteriophage and concluded that there was consistency with the view that the effects of light reactivation on the appearance of mutants among the progeny of ultraviolet-irradiated bacteria is the same as its effect on the number of survivors and that this effect consists in the reduction of the effectiveness of the ultraviolet dose by a dose-independent factor. This led to their surmise that the killing of the bacteria and the production of the mutants might be due to the same chemical effect which was produced by ultraviolet irradiation.

Very large doses of visible light are required for photoreversal in colpidia (80); it appears, therefore, that

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the substance, or bond, absorbing the visible light and bringing about photoreversal is present in very small quantities or the reaction is very inefficient because of the small amount of energy available in quanta of visible light. Since photodesensitization is so slight in colpidia, it would appear likely that the compound concerned with photoreactivation appears in a form in which it can fruitfully absorb visible light only after absorption of ultraviolet. It has been suggested that one cellular compound absorbing light in the region of the spectrum effective in photoreactivation may be the enzyme D-glyceraldehyde-3-phosphate dehydrogenase, present with its reduced coenzyme. Absorption of the light by the absorbed coenzyme results in reduction of the enzyme. Good photoreversal by treatment with blue light even 4 hours after irradiation with ultraviolet indicates that the injurious substance has a long life and it does not exert its effect until sometime after its production.

Christensen and Giese (81) found that a photoreversing dose of monochromatic blue light of wavelength 4350 Å was more effective when delivered as continuous light at a low intensity or as intermittent light at a high intensity rather than as continuous light at high intensity; this was thought to indicate that a dark mechanism participates in photoreversal.

Rieck and Carlson (82) investigated photorecovery in the albino mouse, apparently the first example in a mammal. The highest dosage of ultraviolet used was approximately 1.6×10^8 ergs per sq. cm. at wavelengths of 3130 to 2000 Å. Exposures were for periods of about 35 to 40 minutes and each animal was exposed to radiation 5 days each week. Half of the animals were kept in darkness between exposures and the other half were exposed to visible illumination between radiations. Controls were kept under conditions of light or darkness with no deleterious effect in either situation. The death rate was higher and death occurred earlier when animals were kept in darkness between exposures to radiation than when kept in constant light between doses. The animals kept in darkness reached a point of continual survival sooner after the beginning of radiation, but at a lower percentage of survival, than the animals kept in continuous light. This was interpreted to indicate that the response of the skin--excessive thickening of the epidermis--occurs much more rapidly when a period of enhanced recovery with visible illumination does not interfere between exposures. This would render the skin effectively opaque to ultraviolet and would not allow penetration to the site of deleterious effects. Animals which remained in darkness between exposures to ultraviolet light exhibited much greater damage to the ears than those which were kept in light. The authors called attention to the fact that their results were interesting in view of the fact that Blum et al (75) were not successful in demonstrating photorecovery in human skin. They thought the high dosages and repetitive exposures utilized in their experiments were probably responsible for demonstrating photorecovery in the albino mouse.

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The effects of ultraviolet radiation on amphibian eggs have been studied by Schechtman (83). Frog eggs were irradiated with an instrument which delivered 95% of its energy at a wavelength of 2537 Å for periods varying from $\frac{1}{4}$ minute to 10 minutes. The eggs were observed at intervals during a period of 8 to 10 days following irradiation. No effect was observed in eggs exposed for less than 2 minutes, probably because of absorption of ultraviolet by the layer of egg jelly and by the chorionic membrane. Eggs exposed for 2 minutes or longer showed distinct difference between radiated and nonirradiated sides. Developmental processes of irradiated sides were inhibited. The appearance of the neural fold was retarded and frequently was absent on the radiated side. Gill filaments and adhesive glands developed more slowly on the radiated side. Many early gastrulae showed distinct curvature of the body with the concave side corresponding to the side previously irradiated. The following lines of light were used on another group of eggs: 3660, 3130, 3020, 2804, 2654, 2537, and 2350 Å. Of the various wavelengths, all except 3660 Å showed some inhibitory effect. Somewhat more pronounced abnormalities occurred in eggs exposed to 2537 Å but most showed no greater degrees of inhibition than can be found in eggs exposed to other effective wavelengths.

Dürken (84) studied the effects of ultraviolet radiation on older developmental stages of tritons. She concentrated on two developmental systems--the eye and the ear--and made the assumption that ultraviolet rays are nearly all absorbed in the ectoderm with the result that any abnormalities which appear in the developing ear are the result of a specific effect on ectodermal cells. Penetration studies in other tissues have indicated that this assumption may not be valid. The fact that mitotic activity was not greatly inhibited in the ectodermal portion of the ear which had been exposed to radiation led Dürken to the conclusion that the specific ultraviolet effect must be on a specific cytoplasmic entity. Ignorance of the phenomenon of photoreactivation by visible light after ultraviolet injury may make some of the conclusions of earlier investigators unreliable. It is now known that conditions of light subsequent to ultraviolet exposure must be carefully controlled. Rieck (85) undertook to determine the effects of single doses of ultraviolet irradiation on the forelimb of amblystoma at various stages of development. Of interest, in view of the report of Schulz and Amelung (17) on "consensuality of effects in the two limbs of human subjects when only one was irradiated," are the control studies carried out by Rieck which indicated that there was no detectable effect of irradiation of the right limb manifested in the left limb. Moreover the general vitality of the animal was unimpaired as the result of exposure of a small area of the right limb to ultraviolet irradiation. It also seemed evident

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the visible portion of the spectrum transmitted by the interposed glass plate had no noticeable effect on the contralateral limb. On the basis of these general observations, Rieck held that the unirradiated left limbs of the experimental animals could serve as adequate controls for the radiated right limb. In animals fixed immediately after irradiation the radiated area appeared blanched and the ectodermal cells were spherical. No damage to mesodermal cells was detectable. In animals fixed 20 minutes after exposure the primary result of the radiation was loss of ectoderm in the exposed region. Fixation at the end of 1 hour revealed that the mesodermal components no longer presented the normal compact appearance and some peripheral mesodermal cells evidently were being lost from the limb area. Four hours after exposure, enough cells had been cast off that the irradiated limb bud was far smaller than the control. Examination of the underlying mesodermal components showed a sharp shrinkage line; the portion distal to this line had a liquefied appearance whereas the proximal region appeared the same as the deep component of the normal limb area. At the end of 12 hours normal ectoderm had completely covered the damaged limb having probably migrated in from unaffected or slightly damaged regions. Underlying the ectoderm was a region about 100 micra in thickness which appeared completely chaotic. The size of the right limb bud was much smaller than the left or control bud. A distinct line which was the limit of visible damage could be seen easily. The nuclei medial to this line all appeared normal with occasional mitoses whereas on the distal side of the line much cellular debris and many pycnotic nuclei were present. At the end of 24 hours a remarkable degree of recovery had taken place with most of the cellular components of the irradiated limb appearing normal and with very few pycnotic nuclei in evidence. However, the experimental limb was much smaller than the control due to loss of cells and probably to inhibition of cellular division in the right limb after irradiation whereas the left limb had continued normal development. Although not true in the younger stages of development, the limbs of the older animals, kept in darkness, presented a strikingly different picture than those kept in the light. The period of retardation was greatly extended in those kept in darkness; there was some sign of growth in these limbs but they were generally retarded. All limbs exhibited some degree of recovery from ultraviolet damage but recovery was greatly enhanced by visible illumination. It appears that the effect of x-rays and of ultraviolet differ greatly in that the ability of x-rays to inhibit development decreases with progressive differentiation of the limb primordium while the effect of ultraviolet is quite the opposite--more susceptibility to ultraviolet with increasing differentiation.

Regenerative growth in the urodele forelimb following ultraviolet radiation was studied by Butler and Blum (86). They concluded that, whatever the basic effects of ultraviolet radiation on the urodele limb, subsequent regression and regeneration

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depended upon morphogenetic capacities characteristic of the species. They considered it possible that the radiation accomplishes, in essence, the same result as amputation of a limb which, in the urodele, is followed by transitory regression and later regeneration. It seemed likely that regression was an essential precursor to regeneration since irradiation of the whole elbow region was followed by regression and regeneration whereas irradiation of only a part of the elbow region was followed by regression only. Regression involves active destruction of cells; regeneration involves active proliferation of cells. "Hence the rate and extent of regression might be thought of for analytical purposes as an algebraic summation of these two opposing activities. The effect of visible light in inhibiting regression might be explained in part by an increase in proliferation relative to destruction of cells." In a more recent report Blum et al (87) stated that there is no significant difference in the growth of unirradiated limbs of urodele larvae in the dark as compared to those illuminated with visible light. They, therefore, considered the unexposed arm to be a valid datum for comparison with the length of an arm exposed to ultraviolet radiation, whether the animals were illuminated or kept in the dark after the exposure. Ultraviolet irradiation of the right forelimb of urodele larvae consisted of exposure to 6.2×10^7 ergs per sq. cm. of ultraviolet light of 3130 Å or shorter. It became apparent that the irradiated limbs were shortened relative to the control limbs in any event and that the limbs of the animals maintained in a dark chamber were shortened more than those of animals placed for 2 days in an illuminated chamber. It was found that positive growth, indicating active cell proliferation, goes on in the irradiated limb between 3 and 10 days and again between 20 and 50 days. Marked negative growth rate between 10 and 20 days must be dominated by active resportion of dead cells. The greater negative growth rate and greater shortening of the irradiated limbs of animals kept in the dark continually as compared to those illuminated for 2 days, indicates a greater resorption of tissues during the 7 to 10 day postirradiation period in the former. From this Blum et al (87) reasoned that the illumination for 2 days resulted in smaller number of cells that ultimately died and were resorbed. In all cases regression was greater with greater doses but photorecovery was always indicated by lesser effects on the limbs of the illuminated animals, in spite of greater doses.

Butler, Blum and Schmidt (88) undertook to determine whether changes produced by ultraviolet light have any effect on the degenerative capacity of an unirradiated region which is in close physiological association with an irradiated region through circulatory and nervous pathways. They were specifically concerned with the question whether irradiation of the proximal segment of the upper limb of the urodele larva would have an effect on the regenerative capacity or would alter the growth pattern of the unirradiated distal segment of the limb. The

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right upper arm of all animals was exposed to 6.2×10^7 ergs per sq. cm. of wavelengths 3330 Å and shorter. After irradiation half the animals were illuminated with visible light for 2 days and the other half were kept in darkness. After 2 days all the animals were kept in the dark. Amputation of both right and left forearms at the distal end of the radius and ulna was carried out in groups of 52 animals each. Half of the animals within each amputation group had been illuminated subsequent to ultraviolet irradiation while the other half had been kept continuously in the dark. There was no evident influence of illumination on the growth of unirradiated control limbs. Ultraviolet radiation brought about a decrease in the growth rate of the upper arm and illumination for only 2 days ameliorated the effect of ultraviolet irradiation. In the upper arm irradiation is followed by an immediate negative growth, indicating that regression (presumably involving the removal of material) is proceeding faster than cell proliferation. At about 20 days postirradiation, growth becomes positive for the "light animals" and the same occurs somewhat later for the "dark animals." In the shielded forearms of the irradiated right limbs, on the contrary, growth continued for about 10 days at the same positive rate as that shown by the controls. After this time the "light animals" showed a negative one. Amputation at the distal end of the forearm was followed by regeneration which showed no significant difference in its rate as compared with the totally unirradiated left limbs. It seems clear, therefore, that effects which ultraviolet exerts on regenerative growth are limited specifically to the region irradiated. The regenerative capacity of a region adjacent to an irradiated region remains unaffected, regardless of the close physiological association by way of nerves, blood vessels and other structures.

There is evidence of a strong correlation between presence of an apical epidermal cap at the amputation surface in regenerating limbs of both urodele and anuran amphibians. The apical cap is composed of epithelial cells which possess a high degree of mitotic activity. It is a transitory structure which is formed immediately after regenerating nerve fibers have penetrated the wound epithelium covering the amputation surface, and it disappears after a proliferating blastema has become established. The purpose of an investigation by Thornton (89) was to produce evidence that prevention of the formation of the apical cap by ultraviolet irradiation would prevent limb regeneration. His experiments did provide direct evidence that ultraviolet irradiation prevented the wound epithelium of the wound stump from developing an apical cap and that this inhibited regeneration. The internal mesodermal tissues of the limb were undamaged by exposure to the ultraviolet light (2537 Å, a wavelength of low penetrability into living tissues) and thus remained fully capable of providing cells for blastema formation. Daily exposure of one-half the amputation surface of the limb stump or double the threshold time, slowed but did not prevent accumulation of

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blastemal cells. Limb stumps whose amputation surfaces were exposed to ultraviolet light on alternate days only, but for double the threshold time, also regenerated. When exposure to ultraviolet light was begun after 5 days of wound healing, regeneration occurred. Five days after application the wound surface is covered by an epithelium which constitutes an apical cap with a beginning of blastemal cell accumulation. Daily exposure to ultraviolet light, begun at this phase of regeneration, results in damage to the outer layers of the apical cap but the remaining layers are protected from the radiation and apparently continue to influence accumulation of blastemal cells. Even after 3 to 4 weeks of daily threshold irradiation, mesodermal tissues of the limb stump are capable of undergoing regeneration when stimulated by an apical cap allowed to form after the limb is released from irradiation. It would seem, therefore, that the exposure to ultraviolet light prevents limb regeneration by effecting only the epidermal cells of the amputation surface.

Effects of Ultraviolet Irradiation on Nerve and Muscle

The effects of ultraviolet irradiation upon excitability and conductivity of the sciatic nerve of the frog were investigated by Audiat (90, 91). Both conductivity and excitability of the irradiated segment of the frog's sciatic nerve undergo alteration. If irradiation is prolonged until the irradiated segment of the nerve becomes inexcitable, there is also a lack of conductivity through this segment, as indicated by a lack of muscle response when the nerve is stimulated proximal to the level of irradiation. Stimulation of the nerve distal to the level of irradiation does produce contraction of muscle. One, therefore, cannot "explain the inexcitability or the lack of conductivity on the basis of a toxic product which diffuses throughout the nerve." The time necessary for reappearance of excitability, following ultraviolet irradiation to the point of inexcitability, depended on the duration of the irradiation. If the irradiation was not continued beyond the period of inexcitability, the return was very rapid. If, on the contrary, the radiation was prolonged after the beginning of the stage of inexcitability there was an increase in the time required for recovery which varied between 1 minute and 1½ hours. The reappearance of excitability and conductivity was simultaneous, indicating complete reversibility of both functions of the nerve. In his first experiments Audiat removed his nerve-muscle preparation from the frog; in a later experiment the skin was opened over the sciatic nerve and irradiated in situ. This resulted in a reversible paralysis of the leg involving those muscles innervated by fibers of the nerve given off below the irradiated segment. Those parts of the thigh innervated by fibers leaving above the irradiated region continued to produce muscular activity. Disappearance of the crossed reflex

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also indicated abolition of the sensory function of the sciatic nerve. Audiat (90, 91) considered his results to provide a satisfactory explanation for certain biologic effects of ultraviolet rays. He thought it likely that the hyperemia accompanying erythema as a result of ultraviolet irradiation of the skin was due to transient paralysis of terminal sympathetic fibers to the blood vessels of the skin. He explained hyperesthesia following analgesia, as produced after strong epidermal irradiation, on the same basis. Audiat, Auger and Fessard (92) applied stimuli on either side of an irradiated area of nerve and recorded action current oscillographically from the irradiated area and at half centimeter intervals distal and proximal to the irradiated area. They found that conduction was equally good in either direction and the heights of the action current led off were exactly the same regardless of whether stimulation was proximal or distal to the irradiated segment. There was progressive diminution in the height of the action current as they were recorded through the radiated segment, and the lowest level was that immediately beyond the radiated segment.

Ionic exchanges involving the laws of excitation, arresting potential and the production of action potentials constitute one aspect of the production and induction of excitation in peripheral nerves (93). A second aspect might be designated as free energy which, even in extremely minute amounts, is necessary for the maintenance of the resting potential and the repeated production of action potentials. Chemical reactions must furnish this energy and, therefore, finally are responsible for the maintenance of excitability. The observation that nerve is sensitive to shortwave ultraviolet radiation led to the hypothesis that absorbed photons produce chemical reactions during the normal sequence of pre-energy transfer. Booth et al (93) irradiated single myelinated nerve fibers at nodes of Ranvier and in internodal regions and found that irradiation of a node produced an increase of threshold. Only ultraviolet light below 3200 Å had a photochemical effect upon the nodes. Each wavelength had its specific activity. The photochemical activity curve between 3200 and 2480 Å was measured and premaxima were found at 2970, 2800, and 2650 Å. Fractional irradiation of nodes produces, for identical exposure times, the same effect as continuous irradiation. Irradiation of the internode and opposite effects -- an increase in threshold of the neighboring node. It seemed probable to these investigators that the substances in the node which were photochemically affected were related to the sodium shift during excitation. Thiamine, which absorbs between 2600 and 2650, may be one of these substances. Ultraviolet irradiation seemed to cause progressive destruction of the myelin sheath and to short-circuit the core with the outer medium; this view appeared to be confirmed by microphotographs showing irreversible loss of birefringence, bubble formation and core destruction. It has been shown that the anti-thiamine factor of fern has a similar effect on the threshold of a node as ultraviolet

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irradiation. It seems likely, therefore, that thiamine is one of the specific substances since it is well known that thiamine is destroyed by short-wave ultraviolet as well as by the action of anti-thiamine factors. The maximum at 2650 Å in the activity curve could well have a relation to the thiamine absorption which lies between 2650 and 2600 Å. Booth et al (93) speculated that irradiation produces photochemical disintegration of the carrier system which would prevent the inward current of sodium known to be essential for the excitatory process.

Ultraviolet irradiation of frog nerve at 2537 Å, according to Boyarsky (94), leads to a fall in action potential, a depolarization, and a prolongation of a relative refractory period. A slight fall in threshold was also observed. The results were explained as follows: Ultraviolet appears to affect nerve in much the same way as metabolic inhibitors such as cyanide and nitrogen which cause a fall in resting potential with little or no change in threshold. Ultraviolet differs from the effect of methylfluoroacetate in that the latter affects the threshold strikingly while leaving the resting potential unaltered. It appears probable that a major effect of ultraviolet on nerve is the result of the inhibition of one or several respiratory enzymes. Since the magnitude of the threshold change is slight, little direct effect on the membrane seemed to be involved. von Muralt and Stampfli (95) have stated also that the effects of ultraviolet (at wavelength 2800 Å) can be related with inactivation of enzymes, among which the inactivation of acetylcholinesterase and cholinacetylase deserves special attention. Marked increase of photochemical sensitivity of an excited node of Ranvier (especially at wavelength 2650 Å) suggests that chemical processes take place together with ionic shifts at the moment of nervous activity.

Giant squid axons have been irradiated with the unfiltered output of an ultraviolet lamp through a slit system permitting exposure of 4.7 to 5.2 millimeters of the axon (96). Recordings of thresholds and action potentials were made in control and experimental segments before, during, and after exposure. At the irradiated segment, threshold showed little or no decrease prior to the increase preceding block, while just outside this segment a definite decrease in threshold occurred, probably due to injury currents. Conduction was slowed and finally interrupted when the threshold increased about 80% and the action potential decreased approximately 60%. The initial decrease in threshold and the recovery from radiation block, which had been reported several times for frog nerve, were not observed in these experiments even though the total irradiation was reduced in successive experiments to a value which did not cause block. This suggested either that the rate of exposure was great enough to mask these changes or that, in this respect, invertebrate nerve differs from amphibian nerve. It should be noted, also, that the effects of ultraviolet on giant squid

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axons were not reversed by exposing the nerve to visible light.

Ultraviolet injury, as manifested by suppression of cell division or induction of mutation, is considered to be largely a nuclear phenomenon, presumably resulting from suppression of DNA synthesis which is limited to the nucleus (97). Photoreactivation appears to be the reversal of the suppression of DNA synthesis. Conversely, the effect of ultraviolet light on ciliary movement which is a strictly cytoplasmic phenomenon appears to be very little, if at all, subject to photoreversal. Consequently, it might be predicted that injurious effects of ultraviolet upon the nerve fiber which lacks a nucleus should show no photoreversal. Evidence presented by Pierce and Giese (97) for photoreversal of decreased action potential and sensitivity of ultraviolet-treated neurons of crab and frog indicates that photoreversal is not restricted to the nucleus or nuclear area since the fibers used were severed from the cell body containing the nucleus. Furthermore, photoreversal is not restricted to structures containing DNA since DNA is either lacking in nerve fibers or present in very small amounts. RNA is present in large amounts and plays a role in nerve metabolism. It is conceivable, therefore, that RNA production or utilization is retarded by ultraviolet and in turn is reversed by visible light. The action spectrum for ultraviolet effects on nerve with maxima at 2970, 2800, and 2650 Å, the middle peak being the highest, does not point unequivocally to any one protoplasmic constituent as the locus of ultraviolet injury, although thiamine was suggested by Pierce and Giese as one of these.

Punt and Schippers (98) investigated the influence of light on an acetylcholinesterase system, using the rectus abdominis muscle of the frog. The animals were kept in darkness and the preparations were made in dark red light. Contractures elicited by acetylcholine in the darkness were less than those made after exposure for a half-hour to a high pressure mercury burner. The statistical difference was significant. After treatment with eserine no significant difference could be found. It appears that the cholinesterase system is attacked by ultraviolet radiation. Punt and Schippers did not determine the most effective wavelength for this phenomenon. Punt, Nijhof-Rombach, and Schippers (99), in an extension of their investigations of muscle contracture elicited by ultraviolet irradiation, recorded contracture of the retractor-byssal muscles of the mussel after exposures to ultraviolet lasting from 1 to 10 minutes, the height of the contracture being proportional to the time of exposure. The rectus abdominis muscles of frogs were divided lengthwise. One-half was used in radiation experiments and the other half was treated in the same way but without exposure to ultraviolet light. In most experiments ultraviolet light caused contracture, lasting much longer than the duration of the irradiation. Since ultraviolet may inhibit the action of several enzymes and

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since cholinesterase is one of these enzymes, Punt et al (99) examined the influence of ultraviolet on acetylcholine contracture with the idea that, when cholinesterase was inhibited, the contractures evoked by application of acetylcholine must increase. The retractor-byssal and rectus abdominis muscles were exposed to irradiation with ultraviolet and, at the same time or afterwards, acetylcholine was applied by replacing the fluid bathing the muscles with a solution of acetylcholine in the same medium. In the experiments with retractor-byssal preparations the results were compared with a previous test with acetylcholine in the same concentration. In the rectus abdominis preparations the acetylcholine effect on the nonirradiated half of the muscle was used for comparison. Although the results were not as uniform as in earlier experiments, contracture could be noticed in most cases. In the retractor-byssal muscle it appeared that acetylcholine was added to the ultraviolet effect. In the rectus abdominis the increase of the contracture was not always obvious but the statistical evaluation proved that the stimulating effect of irradiation on acetylcholine contracture was significant. No significant difference could be found in acetylcholine contracture of irradiated and nonirradiated half muscles after previous bathing in eserine. The findings point to a possible influence of ultraviolet irradiation on the cholinesterase system. An interesting experiment indicating increased muscle tone as the result of systematic ultraviolet irradiation in humans was carried out by Seidl (100). It was stated that the power of skeletal muscle went up about 24% during 7 weeks in which systematic irradiation with ultraviolet light was carried out. Another indication of a systematic effect was a 10% decrease in pulse rate during this same 7-week period. The effect on the condition of the muscles was compared with normal variations throughout a normal year when the muscle strength reaches its maximum in September and is lowest in January. Another experiment was designed to determine the effect of muscle training on muscle strength during a preliminary period, followed by the effect of muscle training during a period of ultraviolet irradiation, followed by another period when muscle training was continued without ultraviolet. There were 5 weeks in each of these three periods and the muscle strength showed its greatest increase during the period of irradiation. In a somewhat similar vein, Sigmund (101) found that an erythema dose of ultraviolet irradiation delivered to the whole body (except head and neck) reduced the reaction time to optical stimulation. The reactions were muscular and compared with the observations that the reaction time to optical stimulation in normal people is fastest in June and lowest during the months of October, November, and December. Muscular reaction to optical stimulation in these two periods of the year required 160 milleseconds and 173 milleseconds, respectively. It was also found that the reduction in reaction time in irradiated people was at its lowest point 3 weeks after irradiation, with a gradual decrease to that level, followed by an increase to the original (unirradiated) level.

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Lawrence (102), although not the first to report that skin cancer appeared to be related to excessive exposure to sunlight, was responsible for a comprehensive and convincing series of observations. He found, in 20,000 patients during the years 1908 to 1927, 1,374 rodent ulcers, 877 keratoses, and 395 epitheliomas, all appearing in individuals who had lived for a considerable length of time in Australia. He concluded that the unusual prevalence of skin cancer in Australia was dependent upon relative low humidity and much sunshine. "That lessened humidity of the atmosphere can allow ultraviolet rays to have a greater irritating effect on the skin has been proved by many experiments which show that ultraviolet rays are actually impeded in accordance with the amount of moisture present in the atmosphere." In the same year (1928) Findlay (103), in order to determine experimentally whether ultraviolet light had a carcinogenic action on the skin, carried out experiments on mice. The spectrum of the mercury arc which he used covered a range from 2000 Å in the ultraviolet to 10,140 Å in the infrared but was "deficient in red and infrared rays." The albino mice were epilated with a solution of sodium sulfide on an area of the back between the shoulders and were then exposed to the light at a distance of 18 inches. He found that it was possible to produce papillomata and malignant epitheliomata of the skin by exposure of the mice to ultraviolet for a period of not less than 8 months. If the mice were tarred and exposed to ultraviolet light at the same time, the period necessary for the induction of cancer was shorter than when either tar or ultraviolet light was employed alone. A series of mice tarred for 1 month failed to develop cancer but when tarred and exposed to ultraviolet light for the same period, three mice developed malignant growths.

The carcinogenic actions of ultraviolet light on albino and black mice were compared by Rusch and Baumann (104). Of the albino mice irradiated for 30 to 60 minutes daily, 62 to 83% developed ear tumors in $3\frac{1}{2}$ to 9 months. Mice irradiated for long periods daily developed tumors more rapidly than those irradiated for short periods. Black mice developed a smaller percentage of tumors than albino mice and the time required was greater. Various gradations of papillomas, epitheliomas, and spindle-cell sarcomas were produced. Rusch, Baumann and Kline (105) investigated the effects of various substances on the development of ultraviolet tumors when applied directly to tissues in which neoplastic changes occur. Three types of material were used: oils, oxidizing agents, and carcinogenic compounds. Most of the oils applied accelerated the rate of tumor production with ultraviolet light. Mineral oil was the most active. Linseed oil retarded production of tumors probably due to the formation of a film of oxidized oil which decreased the penetration of the light. Cholesterol (an example of an oxidizing agent) markedly

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stimulated tumor development when applied in cottonseed oil but not when applied in benzene. The stimulating action of cholesterol on tumor development is probably not due to peroxides since neither hydrogen peroxide, benzoyl peroxide, nor irradiated cholesterol were carcinogenic when applied to the ears of non-irradiated animals. These investigators concluded that the rate of tumor production with ultraviolet light could be altered by the local application of certain substances to the tissues developing tumors.

The carcinogenic wavelengths of ultraviolet radiation definitely lie between 2537 and 3341 Å and probably lie between 2900 and 3341 Å (106). It was demonstrated that the 2537 Å band was relatively ineffective. The inactivity of the 2537 Å band is not the result of insufficient energy, for very large doses were given, nor does it appear that the ineffectiveness can be explained by inadequate penetration. The amount of radiant energy transmitted through the very thin corneum of the mouse is probably much greater than the amount for human skin (19% of the 2537 Å line and 34% for the wavelength 3000 Å) although the relative transmittancies for the two wavelengths may well be the same. The carcinogenic wavelengths appeared to be identical with those which produce erythema which reach their maximum effectiveness at about 3000 Å. While, in general, the length of the precancerous period varied inversely with the daily dose of radiant energy, the minimum time for the development of tumors appeared to be about $2\frac{1}{2}$ months. Rusch et al (106) found that it was not necessary to irradiate the animals throughout the precancerous period. Once initiated, carcinogenesis proceeded without further exposure to radiant energy, and in isolated cases several months elapsed between the end of irradiation and the appearance of tumors. Bain and Rusch (107) found, when mice were exposed to the entire spectrum of the mercury arc, that a pronounced erythema and thickening of the ears resulted, together with considerable irritation and tissue destruction. In addition to local effects a generalized toxicity appeared at the higher levels of irradiation and the mice became more susceptible to infection. When a filter, constructed for the isolation of the wavelength band 2800 to 3400 Å, was interposed between the mercury arc and the animal, the erythema, scratching, thickening of the ears, and tissue destruction were much less evident. The animals remained in good health and exhibited no toxic symptoms attributable to the irradiation. This indicated that the deleterious effects observed during irradiation with the entire mercury arc were probably due to the action of high total dosage and not to the carcinogenic portion of the spectrum per se. More than 75% of irradiated mice ultimately developed ear tumors and generally the irradiation was discontinued when this incidence was reached. In order to produce tumors with wavelength 2537 Å, it was found necessary to use exceedingly high dosages and to irradiate for long periods. It was stated by Bain and Rusch that, if the same amount of energy of wavelengths 2800 to 2400 Å

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had been employed, the tumor incidence would have been at least 50% and probably more. They suggested a screening effect of the outer layers of the skin as largely responsible for the weak carcinogenic effect of 2537 Å. It has been found that only 2% of wavelengths below 2800 Å is transmitted through the outer layers of the skin to the basal cells. Thus while shorter wavelengths are apparently intrinsically carcinogenic, it is not probable that wavelengths lying below 2800 Å contribute importantly in short-term experiments. "While the mode of action of ultraviolet light in the production of cancer is still obscure, the suggestion that some disturbance in the proteins or nucleic acids of the cells might be responsible, merits further consideration."

Other factors can be superimposed upon the effects of ultraviolet light as, for example, diet (108). Brain fraction, employed to retard the formation of tumors produced by tar, was found to retard the formation of ultraviolet tumors as well. Liver feeding appeared to increase the rate of tumor production without ultraviolet but when combined with irradiation it was found that mice on a diet containing 33% of liver developed tumors at a decreased rate. Increase in the production of ultraviolet tumors on high fat diets is probably due to a combination of local and systemic effects. Some mice had lost hair and were in reality receiving more light than those in other groups. However, within the high fat group there was no evidence that partially denuded mice developed tumors any faster than those which retained their hair. Fat-fed mice were all quite greasy and it is possible that a film of fat intensified the radiation effect. Mice on a 2% cholesterol diet developed tumors at exactly the same rate as those on stock diet notwithstanding the fact that these mice had responded to the cholesterol feeding by an increase in the fat and cholesterol content of the liver. Furthermore, on high fat diets, which accelerate tumor development, no increase in liver cholesterol was observed.

Bain, Rusch and Kline (109) investigated the effect of temperature upon ultraviolet carcinogenesis with wavelengths 2800 to 3400 Å. One group of irradiated mice was placed in a thermostatically controlled oven at 35 to 38 degrees C. (during irradiation); another group was placed in an insulated ice-cooled box at approximately 3 to 5 degrees C. while being irradiated; a control group was irradiated at room temperature. In the first two cases the mice were subjected to irradiation temperatures for 30 minutes before radiation was begun. In all cases the mice were irradiated 30 minutes per day, 6 days per week, with a medium pressure mercury lamp which isolated the wavelengths 2800 to 3400 Å. All groups received an intensity of approximately 4200 ergs per sq. cm. per second, resulting in a daily dose of about 7500×10^7 ergs per sq. cm. The production of tumors was more efficient at 35 to 38 degrees C. than at room temperature

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but there was little difference in the rate of carcinogenesis at 3 to 5 degrees C. or at room temperature. Results obtained at 35 to 38 degrees C. may not represent a direct effect on the carcinogenic process; the heat may have caused sweating of the mice and may have increased the transmission of ultraviolet radiation to the subdermal layer of the skin. The elevated temperature may have acted by promoting a better circulation in the skin and a consequent quickening in the metabolism of the affected cells. Since the mice were kept at the elevated temperature only during irradiation, this indirect effect did not seem so probable because of the short time of the increased blood supply. It is more likely that the increased efficiency represents a direct thermo-acceleration on the immediate reaction initiated by the ultraviolet radiation.

Blum (110) carefully reviewed all observations on sunlight and cancer of the skin and compared these with the production of tumors in animals by ultraviolet irradiation. He said, "In the case of tumor production only the general limits of the action spectrum are thus far known. These limits fall within the same spectral region as the action spectra of known photoprocesses in human skin, and hence a possible relationship to these processes must be considered. Three photoprocesses in normal skin are known to be brought about by wavelengths included in sunlight. The first is referred to as the sunburn mechanism and comprises a series of events, all of which result from damage to the stratum germinativum of the epidermis, principally the prickle cells. The first manifestation is erythema, representing dilatation of superficial vessels or vessels in the papillary layer, and this usually appears an hour or so after exposure. It is followed by pigmentation, i.e., the appearance of melanin pigment in the epidermis of the irradiated area. The second photoprocess, antirachitic action, seems to be a photochemical reaction by which 7-dehydrocholesterol or some closely related compound is changed into vitamin D or another antirachitic substance. The third process, only recently described with accuracy, is darkening of pigment already present in a colorless form. This process requires about one thousand times as much energy as the sunburn mechanism. Its action spectrum lies between 3000 Å and 4000 Å and, therefore, does not correspond with that for tumor production. It is doubtful that it has any role in this process. The first two processes, on the other hand, have approximately the same long wavelength limit as tumor production, i.e., 3200 Å, and must be examined, therefore, as to their possible involvement in that phenomenon." Blum goes on to note that the experiments of Rusch et al (106) indicate that the action spectrum for cancer production does not agree with the erythema spectrum since they could produce no tumors with radiation of wavelength 2537 Å although this is quite effective in producing erythema. In sunburn the primary change is in the epidermis whereas, for cancer production it may be necessary to affect deeper layers also. In this case the epidermis as a whole must be regarded as a filter with the action spectrum restricted virtually to only those wavelengths longer than 2800 Å. Such an explanation receives some support from the fact

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that sarcomas form a considerable part of the tumors produced by ultraviolet radiation in the laboratory. The epidermis of rodents is much thinner than that of man, at least before the former has been subjected to ultraviolet radiation. The erythema action spectrum for these animals has not been accurately determined although it is known to have the same long wavelength limit as in man. So far as the evidence from action spectrum goes, it is possible that cancer production is the result of photochemical changes similar to those causing the erythema of sunburn but occurring in cells at a deeper level. In a later review (111) of the relation between cancer and ultraviolet radiation, Blum tentatively accounted for the mechanism involved as follows: "Ultraviolet radiation causes acceleration of the rate of proliferation of the 'tumor' cells, each dose of the radiation causing an increase in the relative rate of proliferation proportional to the magnitude of the dose up to a certain limit set by physiologic factors limiting the absolute growth rate. The 'tumor' cells may not differ sharply from normal tissue cells, but represent those cells and their offspring whose growth rate is most effectively accelerated by ultraviolet radiation and which, therefore, outstrip their fellows and form a tumor." In his opinion this theory accounts for the sudden appearance of tumors after a period of apparent latency. He also noted that his idea of tumor cells being essentially normal cells, differing only in a quantitative sense, is consistent with the cytologic similarity between tumor and normal cells. The concept of accelerated growth does not seem out of harmony with some of the happenings in embryological development where cell proliferation speeds up locally for limited periods. Certain factors may affect the quantitative relationships. High doses of ultraviolet radiation cause lowered food intake and slower growth and such conditions are generally associated with increased tumor development time. Tumor development time increases somewhat with the age of mice which might again affect quantitative relationship. The finding that visible light promotes recovery from the effects of ultraviolet radiation could affect the data. Reproducibility of experiments at different times of the year speaks against this factor as having significant influence. "All in all it seems reasonable to assume all these factors to be trivial in their influence on the quantitative relationships."

Quantitative induction of tunors in mice with ultraviolet radiation was investigated by Blum, Kirby-Smith, and Grady (112). They described a carefully controlled method for exposure of mice to mercury arc radiation. One hundred percent incidence of tumors of the ears of male strain A mice can be induced under well-controlled conditions. At higher doses of radiation the time for appearance of tumor was little affected by the dosage; at lower dosage this time increased markedly with progressive decreases in dosage. They concluded that the production of tumors depends upon the quantity of radiant energy applied rather than upon the intensity of the radiation.

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Carcinogenesis may be limited by the saturation or exhaustion of some substance in the cell which is renewed in the interval between exposures (113). The exhaustion or saturation of this hypothetical substance should set a limit for the amount of carcinogenic action achieved in a single exposure. If the exposures were more frequent, more carcinogenic action could result from the same dosage. Tumor induction by ultraviolet radiation may be associated with injurious changes in the cells which are reversible in the sense that the cell may recover from them. If the injurious changes are too severe, the cell is killed; if they do not exceed a certain degree, the cell recovers. Thus, only those cells which receive neither too much nor too little injury develop the characteristics of cancer cells. This accounts for the minimum induction time since increasing the dosage above a certain level will only result in the destruction of more tissue cells without increasing the proportion of potential cancer cells. Different intervals between exposures permit different degrees of recovery and hence the minimum induction time shifts with the schedule of dosage. Hyperplasia of the epidermis and accompanying thickening of the corneum may affect induction time by altering the amount of radiant energy reaching the living cells. At higher doses tumors are always associated with a considerable degree of hyperplasia of both the epidermis and deeper tissues, present almost from the beginning of exposures. At lower doses only very slight hyperplasia may be observed even up to the time the tumor appears. It is questionable whether hyperplasia is an essential factor for tumor formation or whether the two processes are directly related. That ultraviolet radiation is a lethal agent which may actually accomplish its carcinogenic effect by partial damage to the cell is a possibility. At high dosages a considerable number of cells are destroyed and actual disappearance of part of the ear may result from such damage, followed by necrosis. Such destruction affects the induction time since it decreases the amount of tissue available for the development of tumors.

The carcinogenic effectiveness of ultraviolet radiation of wavelength 2537 Å has been the subject of considerable controversy. Blum and Lippincott (114) have discussed the subject in the light of their own investigations. Radiation of this wavelength is almost completely absorbed in the epidermis of the albino mouse and might be expected to produce a higher proportion of epidermal carcinomas than do longer wavelengths. The 2537 Å line is relatively ineffective in terms of surface dosage although it has approximately the same effectiveness when considered in terms of energy reaching the viable cells. The same relationship seems to apply as regards tumor induction although no accurate comparison can be made because of the early termination of this particular experiment. The first tumor induced by this wavelength was at 227 days which falls among the induction times of first tumor in five experiments in which the animals received the same dose of intermediate arc radiation. They concluded that the effectiveness of wavelength 2537 Å in tumor induction is roughly the same as

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that of wavelengths near 3000 Å when considered in terms of energy reaching viable cells, although the wavelength is much less effective when considered in terms of striking the surface of the skin. The fact that a number of epidermal lesions were found which might be expected to develop into carcinomas suggested that, other things being equal, this wavelength might induce a higher percent of carcinomas than do longer wavelengths. In conclusion, they said, "Considered in terms of surface dosage wavelength 2537 Å is much less effective than longer wavelengths of the carcinogenic spectrum in inducing tumors and in producing other tissue changes. This is best explained by the fact that wavelength 2537 Å is absorbed principally in the stratum corneum before it reaches the viable cells. It appears that photochemical changes taking place in the stratum corneum play no part in carcinogenesis and that the locus of carcinogenic action of ultraviolet radiation is the living cell itself. The penetration of ultraviolet light into the skin is of special interest when one considers the relationship of sunlight to human cutaneous cancer (115). Almost all cutaneous cancers in the human are epidermal in origin whereas those induced by ultraviolet radiation in albino mice arise chiefly in the dermis area. Wavelengths shorter than 3200 Å, which alone have carcinogenic action, pass through the epidermis of the albino mouse to a considerable extent. Transmission by the epidermis is less in mice which have been repeatedly irradiated than in normal mice but remains relatively high. The production of tumors of dermal origin in these animals is accounted for by a large fraction of the incident radiation which penetrates to the dermis. The transmission of these wavelengths by human epidermis is much lower than that by mouse epidermis and almost none reaches the dermis of well-tanned skin.

Clark, Luce-Clausen and Mider (116) decided to study mice subject to spontaneous mammary tumors under environmental conditions of darkness, artificial daylight, and ultraviolet radiation below the level necessary to produce skin cancer. It seemed possible that ultraviolet radiation, itself a carcinogenic agent when intense enough, might activate the tumor agent and increase the rate of tumor incidence. They obtained good evidence that C3H mice (subject to spontaneous mammary tumors), when exposed to ultraviolet rays, developed spontaneous mammary cancer more rapidly than did those reared in either light or dark environments in which they received no ultraviolet irradiation. They stated that the differences were not due solely to a more rapid rate of tumor production or to a decreased latent interval, but to a combination of both.

Griffin, Dolman, Böhlke, Bouvart, and Tatum (117) exposed Swiss mice to the total energy of quartz mercury vapor lamps and subsequently housed them in either the dark or in the white light of flourescent lamps, to determine if photoreactivation of ultraviolet carcinogenesis occurs. Less ultraviolet energy was required to produce tumors in the mice housed under white light than in

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those housed in the dark, which observation indicates an activation by white light of ultraviolet carcinogenesis. From all evidence it would appear doubtful that the very small ultraviolet emission from the fluorescent lights contributed to this higher tumor incidence. Kline and Rusch (14) have reported that albino mice placed under fluorescent light 4 to 6 hours per day, 6 days per week, for 1 year showed no signs of erythema or neoplastic changes in the skin. Mice exposed to the total ultraviolet energy and simultaneously exposed to visible white light appeared to be protected to a slight degree from the ultraviolet rays. More energy was required to produce tumors in 50% of these animals than was required to produce the same tumor incidence in mice exposed to ultraviolet light alone. These findings suggested that, if reactivation does occur, it is simultaneous with, or follows immediately, the ultraviolet exposure. There is no indication that exposure to white light after the exposure to ultraviolet has a reversing activity. Kelner and Taft (118) also studied the influence of photoreactivating light on the type and frequency of tumors induced by ultraviolet radiation. Tumors first appeared in their animals after 233 days of irradiation with 2537 Å, and after 189 days in the group irradiated with wavelengths 2800 to 3100 Å. When irradiation was stopped very few tumors had appeared in either group. It was only during the remaining period of observation that tumors appeared abundantly and regularly and most of their data are concerned with delayed, postirradiation tumors. The data show a reduction in frequency of tumors in visible light-treated animals. This reduction was more marked in the 2537 Å group which had a frequency of 0.41 in the dark and 0.19 in the light at 369 days; the reduction was less marked in the 2800 to 3100 Å group which had a frequency of 0.48 in the dark and 0.30 in the light at comparable stages in the experiment. Neither result is statistically significant. Kelner and Taft (118) commented upon the disparity between their results and those of Griffin et al (117) and conceded that the disparity might not be significant since the effect of reactivating light on the overall incidence of tumors in their 2800 to 3100 Å group was small at best.

Blum (119) reported on two types of experiments -- one in which ultraviolet irradiation was continued until the time of appearance of tumors and another where the irradiation was carried out with the same total dosage but was interrupted at a given time in the pretumoral period. The distribution in time of induction of tumors in these experiments opposed the hypothesis that a simple relation exists between dosage and latency. He found that the earlier in the pretumoral period that ultraviolet irradiation is interrupted, the longer the time from beginning the experiments until tumors appeared.

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The influence of ultraviolet light in the production of cancer of the skin has been referred to. It is of interest, however, that Hyde (120) stated that intense ultraviolet frequencies not only produce more or less "salutary pigmentations" but also produce changes in the vascular system and stimulation of both unstriped muscular fibers and of the "processes of metabolism." Hyde noted that the colored races suffer less than the white from cancer of the skin as well as from cancer of other organs. McCoy (121) believed both American Indians and Negroes to be immune to keratotic changes in the skin. He noted that the albino acquires no pigmentation under insolation, that he suffers intensely from dermatitis, and that he never acquires immunity to keratotic changes although he may persist in his efforts to minimize his exposure.

That the reaction of the skin to ultraviolet light is not strictly confined to the precise area of skin exposed to irradiation was noted by Lewis and Zotterman (122). They believed that the cellular structure of the skin is affected by the light only where the light falls upon it, that this skin shows a sharply defined reddening originally, that italone remains red in the stage of fading, that it alone becomes pigmented, and that it is the only skin which subsequently desquamates, but, while the reaction is at its height, the vascular reaction spreads widely into surrounding unexposed skin. The manner of this spreading and its extension along channels evidently lymphatic, constitute convincing evidence that substances having a vasodilator action are formed locally, are moved through the tissue spaces, and are picked up by lymphatic channels. The substance in question may not only dilate the minute vessels with which it comes into contact but may increase their permeability.

The sytemic effects of irradiation of the skin with ultraviolet light have also been noted by Macht, Anderson and Bell (123). "Advances in the phototherapy of ricketts, tuberculosis, and other pathologic conditions together with laboratory observations concerning the physiologic effects of light have established conclusively that ultraviolet rays produce profound changes in the blood, the metabolism, and other physiologic functions of the body." If one concedes that systemic effects do result from ultraviolet irradiation of the skin, one must also assume that the ultraviolet rays penetrate at least as far as the superficial blood circulation. Macht et al noted that earlier investigators had determined the depth of penetration for the shorter ultraviolet rays through the skin to be only 0.1 millimeters; they also noted that these investigators performed their experiments with dead skin and they, therefore, carried out an extensive investigation on the amount of penetration of various ultraviolet rays through living animal tissues and more particularly through

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the skin, with modern powerful quartz mercury vapor lamps. In their experiments on living skin it was found that ultraviolet waves of 3025 Å were repeatedly registered on a photographic plate through a 1 millimeter thickness of skin with an exposure of 1 minute. Occasionally, exposures of much shorter duration -- 5 and 10 seconds -- were sufficient to give this line in the spectrogram. The longer ultraviolet rays, still in the invisible region of the spectrum, could be registered by a few seconds exposure in all experiments. It was evident that the wavelength of 3025 Å and occasionally even shorter wavelengths, not only penetrated into a layer of skin 1 millimeter or more in thickness but actually, in part, passed through it. Experiments to determine what wavelengths would pass through the whole thickness of the rabbit's abdominal wall revealed that, after an exposure of 2 minutes to the Kromayer lamp at a distance of 5 centimeters, the line 3130 Å was definitely registered. Specimens of fresh human skin, obtained from the hospitals immediately after surgical operations, were much thicker than the skin of the rabbit or the cat and transmission of invisible rays was markedly reduced. Nevertheless, the wavelength 3650 Å (still below the visible length) was transmitted through a piece of white human skin 2.2 millimeters thick. It was found that the skin of the Negro, owing to pigmentation, absorbed practically all the shorter waves. They noted that, in the region between 2800 and 2537 Å, there exists an absorption band and the transmission is not as good. In recalling their statement that the positive and undeniable chemical results obtained by ultraviolet radiation in ricketts and other pathologic conditions cannot be explained except on the assumption that active ultraviolet rays penetrate deeply enough to affect at least the superficial capillary circulation, Macht et al (123) state that the present experiments have established the correctness of such an assumption. The difference found between white and negro skin with a spectrograph agrees with clinical experience; it has been learned that ultraviolet therapy in Negroes is much less satisfactory than in white persons.

After reviewing the essentially qualitative and occasionally contradictory results of previous investigators, Bachem and Reed (124) determined to measure the transmission of ultraviolet and a portion of visible light through human skin quantitatively. They included a number of substances which accompany or represent a part of the skin -- fat, fascia, horn, blood, and serum. They utilized photoelectric and photographic methods and found the latter more satisfactory. From all their observations they drew the following conclusions: (1) irradiation of more than 4000 Å should be used if pronounced penetration into the corium and into the subdermal layers if required; (2) the wavelength 4000 Å is particularly suited to be absorbed in the blood capillaries and to affect the blood stream; (3) a considerable percentage of the near ultraviolet at 2500 Å reaches the corium but the wavelengths 2700 to 2800 Å are practically all absorbed

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in the epidermis; (4) no radiation shorter than 2400 Å reaches the living parts of the epidermis, all being absorbed in the dead horny layer of the skin. In general it may be said that the results of Bachem and Kunz (125) were more nearly in agreement with those of Macht and his co-workers (123) than with those of earlier investigators (126, 127). The investigations of Miescher (128) convinced him that the degree of erythema resulting from ultraviolet radiation is proportional to the thickness of the epithelium. The protective effect of the stratum corneum appeared to depend as much upon dispersion and reflection as upon absorption. The result of exposure to ultraviolet radiation was, in every case, a thickening of the horny layer and, as a consequence thereof, a lowering of the light sensitivity of the skin through weakening of the effects of the rays striking living tissues. "The resistance against light is always a direct result of increased cornification."

Bachem and Reed (129, 130) set out to determine the amount of light of different wavelengths that penetrates through the horny layer, through the whole epidermis, and into different depths of the corium and the subcutaneous layers. They arrived at the following conclusions: (1) visible (and near infrared) rays are strongly absorbed by the blood of the corium and subcutaneous layers, with direct effects upon the blood to be expected. The heating effect must be mild on account of the small absorption in the epidermis and the strong convection by the blood stream; (2) the far infrared has very little penetrating power, most of it being absorbed in the epidermis. The heat produced is slowly conducted down to the deeper layers and up to the surface and convected away by the blood stream and air circulation; (3) the ultraviolet exhibits greater variations than the other parts of the spectrum; at 2800 Å the absorption in the corneum (and the granulosum) is very pronounced; the great antirachitic effect of this wavelength must occur in or above these layers. On either side, at 3000 and 2500 Å the penetration is greater, more radiation reaching the malpighian layer and the corium. These observations prompted some speculations as to the role of the skin in the systemic effects of irradiation. The fact that certain effects of a local application are systemic can be explained either by assuming that rays penetrate into the blood stream and produce changes that are reflected in the systemic reactions or that nerve endings in the skin are stimulated, producing reflex changes, or that some substance is liberated in the skin and taken up by the blood and lymph to be distributed to other organs or tissues. The skin as an organ serves several functions. Of special interest is the protection against excess radiation from sun. Since rays which do penetrate to the blood stream produce systemic effects that are at least unphysiologic, it is obvious that without the protective action of skin these effects would be much more severe. When skin is stimulated or irritated it gives rise to the "triple response": (1) local vasodilatation; (2) a flare or wheal; and (3) eventually, if

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the irritation is severe enough, local edema and finally blistering. The delayed erythema most commonly seen in sunburn is due to tissue changes which result from short ultraviolet rays. The temporary erythema is not strictly local, whereas the later erythema is confined sharply to the exposed area which is strong proof that it is dependent upon local tissue changes. It is conceivable that certain cells may be injured to such an extent that delayed metabolic changes occur which produce capillary dilatation. That this is what happens seems to be established by the demonstration that the site of degeneration from ultraviolet rays is the prickle cell layer and never the corneum or granulosum. This seems to be a typical irritation degeneration which results in capillary stasis and other phenomena of inflammation, finally increased permeability manifested by blistering. Changes in the capillary wall probably result secondarily from irritation by toxic products (H-substance) of prickle cell degeneration. Vasodilator substances result from any type of injury to cutaneous structures. Function of blister production is an interesting field of speculation and it is probably accidental, resulting from a great increase in permeability but it is also possible that it may serve a protective function since blister fluid absorbs heavily in the region of 2800 Å and would, therefore, protect against further injurious effects from the rays. With respect to the theory that systemic effects resulting from local cutaneous radiation are due to reflexes arising from stimulation of nerve end organs in the skin, it must be remembered that some of the responses, particularly the vasomotor reactions, result just as readily when denervated cutaneous areas are irradiated. That the various types of cutaneous end organs may be stimulated secondarily by tissue processes such as inflammation, is unquestionable. Local production of heat has been found to stimulate dermal receptors and so produce reflex effects. The systemic effects of cutaneous irradiation bear little resemblance to direct reflex stimulation but rather "resemble endocrine effects as manifested by the long latent period, slow maximal reaction and prolonged after effect. The question of production of changes in the blood by penetrating rays is open to question. Direct irradiation of circulating blood produces powerful vasotonic inhibition, mobilization of blood cells, decreased potential of red cells and various chemical changes that may be duplicated by cutaneous irradiation." Bachem and Reed (129, 130) concluded that at least a part of the systemic effects are due to blood changes with a production of substances that act as hormones. The blood changes must be due mainly to visible and near ultraviolet rays since experiments show that only these and the infrared rays penetrate far enough. It appears, therefore, that at least two of the three theoretical mechanisms play a part in the systemic effects of localized cutaneous irradiation. Primary pigment deposit occurs in the basal cells of the stratum malpighii. The greatest absorption in this layer occurs at 3000 and 2500 Å. Stimulation of pigment production, therefore, must be due mainly to these spectral regions, more particularly the former. Actinic pigmentation results only after erythema production. Any type of

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chronic irritation that results in inflammation will bring about some pigment deposition which indicates that pigmentation is an expression of a local response to irritation of a particular group of cutaneous cells, namely, the prickle cells which lie immediately above the basal cells in which the pigment is deposited. Since erythema seems to result from some degree of cytolysis and since it is a necessary preliminary to melanation, we may assume that the train of events is something as follows: Cytolysis of prickle cells liberates a substance such as dioxyphenylalanine which passes inward by lumph spaces until it comes in contact with the oxidase in the basal cells where it is precipitated as melanin by a molecular condensation. The generalized pigmentation of brunettes after irradiation of a limited cutaneous area is explained by the assumption that the skin bears very large amounts of the oxidase and that the precursors of melanin are so abundant as to permit a general distribution. This suggestion receives support from the observation that pigmentation sometimes follows the course of lymphatics in a nonirradiated area. That pigmentation is correlated with increased tolerance to radiation is unquestionable. Of the spectral regions which produce pigmentation most readily, a fairly large percentage penetrates beyond the pigment bearing cells. Whether these rays are injurious to the extent of requiring that the body be protected against them requires proof not now available. It is certain that pigment deposits will result in absorption of these rays in the malpighian layer. Such deposits would not account for the increased tolerance of prickle cells to subsequent irradiation since these latter cells are superficial to the pigment cells. The increased tolerance of the prickle cells must be due to some other mechanism, possibly decreased permeability or decreased fragility. When melanin is injected into the blood stream it gives a reaction, less transient, but otherwise similar to that of adrenalin. The possibility is suggested that organs other than the skin display a pharmacological reaction to melanin. It is possible that the increased tolerance to irradiation is not due alone to cutaneous melanation but also to systemic effects of this substance. Since the antirachitic effect is produced best by that part of the spectrum most heavily absorbed in the corneum, it follows that there must be chemical changes produced in that layer. It is possible that these changes may be a factor in the greater tolerance of prickle cells as produced by irradiation.

The permeability of human epidermis to ultraviolet radiation has also been investigated by Lucas (131). He concluded that the percentage of ultraviolet light of physiologically active wavelengths transmitted through the epidermis is higher than previously recorded by Hasselbalch (127); it was calculated to be about 1.3 to 30 times greater for wavelengths from 4040 Å to 2890 Å, respectively. The apparent absorption of ultraviolet light by epidermis, as determined by the usual methods of photometry, is not entirely due to true absorption but is in part due to scattering of the incident light by the epidermis which is not

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entirely due to true absorption but is in part due to scattering of the incident light by the epidermis which is not optically homogeneous. This scattering, while causing great diminution of the intensity of the light received by the recording spectrograph, causes much less interference in transmission of the light to the underlying tissues when skin is irradiated in vivo. For light of wavelengths 3000 Å and less the effect of scattering is dwarfed by true absorption by the epidermis. In shape, the absorption curves resemble those of many proteins and amino acids.

The possible relationship of cholesterol to skin cancer has been extensively studied by Roffo (132). He found that there was 3 to 6 times more cholesterol in the skin of the face than in that covering the abdomen. He thought that the large amount of cholesterol in the face resulted from exposure to sunlight and was able to confirm this idea. Experiments were carried out on two series of white rats, one of which was exposed to sunlight and the other to ultraviolet rays to test the validity of the hypothesis that the large amount of cholesterol in the face might result from exposure to sunlight. The results showed that cholesterol is fixed in the skin as a consequence of its heliotropism. There would seem to be some connection between the large amount of cholesterol in areas of the skin exposed to sunlight and to the high proportion of tumors found in such regions. The largest number of cancers occur on the nose which is most exposed. In the Negro where pigmentation of the skin causes it to resist the absorption of light, carcinoma of the skin is very rare. It is suggested that cholesterol prepares the soil for subsequent malignant growth by acting as an accumulator of light. An so we have a situation where the increased amounts of cholesterol in the skin are the result of light and whre in turn light, acting upon this cholesterol, is responsible for the production of tumors.

Mitchell (133) believed with Lewis and Zotterman (122) that the "diffusion flush" after localized ultraviolet irradiation of human skin provided direct evidence for the liberation of an active substance, the so called H-substance, responsible for vasodilatation. Upon histological examination, Mitchell found that the structural changes in ultraviolet erythema were usually limited almost entirely to the stratum mucosum. Based on this he came up with the simple hypothesis that the erythemal response is due to photochemical decomposition of some constituent of the cells of the stratum mucosum with liberation of active reaction products, which then diffuse to the region of the minute vessels of the subpapillary venous plexus of the corium and lead to vasodilatation. Two further assumptions were made as follows: (1) the stratum corneum behaves as an inert scattering and absorbing screen through which the incident radiation producing photolysis is transmitted with decreased intensity; (2) the pigment layer between the stratum mucosum and the corium behaves as a perfect absorbing screen preventing any transmission of ultraviolet radiation into the corium. Mitchell (131) calculated, within limits of experimental

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error, from absorption measurements of a typical protein and of the human stratum corneum, the form of the erythema curve. The calculated mean value of the wavelengths of maximum erythemal efficiency was 2975 Å (± 15), which is in close agreement with experimental observations. The known minimal erythema dose was consistent with the formation of photo-decomposition products in vitro in concentrations of a reasonable order of magnitude. It was suggested that the H-colloid may be a proteose formed by oxidative photolysis of proteins. The apparent resemblance between the effectiveness of the different wavelength regions for the production of erythema and of neoplasia is of interest. Carcinogenesis by ultraviolet irradiation is usually preceded by repeated severe erythemal reactions in normal kuman skin, in laboratory animals and in the abnormal skin of xerodermia pigmentosa, so that the relationship to erythemogenesis may signify that the neoplasia is related to chronic inflammatory and/or atrophic changes. Mitchell (133) suggested the possibility that carcinogenic agents may be liberated from proteins and from sterols.

Abramson (134) irradiated an area of the back measuring approximately 200 square inches, without any symptoms of histamine poisoning. The borders of the wheal produced by ultraviolet irradiation always coincided initially with the area irradiated. Spread of the wheal was slight but uniform and probably due to an increase in intradermal pressure by an excess of extravascular fluid. Nondevelopment of pseudopods is not in accord with the theory that a readily diffusible H-substance like histamine is liberated in the tissues subsequent to irradiation. When histamine was injected into the irradiated area, which had responded by whealing, a histamine wheal was readily superimposed. Similarly, a light wheal could be superimposed on a wheal formed by electrophoresis of a 1 to 10,000 solution of histamine. He concluded on the basis of these observations, that neither histamine nor a readily diffusible H-substance of low molecular weight was responsible for the skin response to ultraviolet irradiation. Blum, Baer, and Sulzberger (135) studied a case of urticaria solare in whom erythema was produced within 10 minutes or less by exposure to ultraviolet radiation of wavelengths less than 3700 Å. Injection of small quantities of serum from the patient into the skin of normal subjects rendered the immediate area of injection sensitive to ultraviolet radiation, exposure causing whealing. The observation on passive transfer of sensitivity indicated that wheals are produced on the areas of passive transfer of sensitivity indicated that wheals are produced on the areas of passive transfer by the same wavelength that elicits urticarial response in the patient studied. It was their opinion that proteins should be suspected in the role of light absorber since unconjugated proteins absorbed radiation in the same general spectral region and are probably the light absorbers in sunburn and other destructive effects of ultraviolet radiation. The possibility that the light absorber was an antibody was suggested. Blum, Barksdale, and Green (136) described a second case of urticaria solare who broke

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out with hives on the exposed parts of the body when subjected to sunlight. It was found on testing this patient that the response occurred only under those filters which transmitted radiation within the approximate wavelength limits 4000 Å to 5000 Å. He reported that just prior to the onset of his photosensitivity he was stung on the flank by a "sea nettle" with resultant transitory giant edema of the general region of the sting. These authors remarked that sudden onset at various ages has characterized all cases of uritcaria solare thus far reported but in only this case and one other has a sting been reported to have been associated with the onset.

The erythemal threshold for sunburn was studied by Blum and Terus (137). Measurements of thresholds under various conditions were described. It was found that the threshold of a given individual varied considerably from time to time. With respect to the factors of wetting of the skin, sweating, etc., which have generally been believed to make the skin more prone to sunburn than dry skin, Blum and Terus (137) found that application of water to the surface of the skin during exposure did not significantly affect the threshold. They noted that the thickness of the corneum is very different for different parts of the body and that it increases in thickness after exposure to ultraviolet radiation. In fact, the most important factor in the decrease of sensitivity to sunlight with successive exposures was considered to be the result of a decrease of the intensity of radiation reaching the malpighian layer due to corneal thickening. In another report, Blum and Terus (138) reported experiments to determine whether the erythema of sunburn could be inhibited by large doses of ultraviolet irradiation. The radiation used in these experiments consisted of the short wavelength end of the sunburn spectrum (2537 Å), longer wavelengths within the sunburn spectrum (chiefly 3130, 3020, and 3967 Å), and a mixture of short and long wavelengths. These types of radiation are referred to as short, long, and mixed ultraviolet. From their experiments they concluded that the course of developing erythema may be delayed by subsequent exposure to long ultraviolet or even caused to recede temporarily. The results were shown to depend upon the choice of appropriate doses, the time between the application of the first and second dose, and also upon the response of the subject. They were unable to demonstrate an inhibitory effect with short ultraviolet with any dose or intensity applied and they were not able to demonstrate inhibition by exposure to the intermediate pressure mercury arc passing through window glass which cuts off all the erythema producing wavelengths except a small fraction of the 3130 Å line, showing that the longer wavelengths of ultraviolet and visible light may be disregarded as factors in either erythema production or inhibition and that longer wavelengths of the erythema spectrum are the most effective in eliciting inhibition. In spite of clearly demonstrated optimum doses for erythema, there seemed no such optimum for total injury to the skin. The area of lowest dosage with long ultraviolet

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displayed the greatest erythema and showed only a mild desquamation of the corneum whereas areas which received doses higher than the optimum for erythema showed severe peeling of the epidermis with crusting. Total damage to the skin appeared to increase in proportion to the dose of ultraviolet radiation. They made three assumptions which they considered to be consistent with the experimental evidence: (1) there is essentially only one type of photochemical process involved, namely, alteration of protein components of living cells of skin which lead to injury of these cells; (2) the result of such damage to living cells of the epidermis is elaboration of a dilator substance which travels to the superficial minute vessels of the papillary layer, releasing them from the nervous or hormonal mechanism that keeps them in a state of normal constriction and resulting dilatation manifested grossly as erythema; (3) inhibition results from the same kind of primary photochemical reaction occurring in the papillary layer and having as its end result the prevention of release of constriction of the vessels of this layer by the dilator substance elaborated in the epidermis. Thus it was found that all wavelengths of the erythema spectrum could be expected to produce erythema because all are absorbed to some extent by the epidermis. On the other hand, only those wavelengths that penetrate the papillary layer cause inhibition of the vascular response in that layer. Accordingly, wavelength 2537 Å elicits only erythema since it is all absorbed in the epidermis, or virtually so, whereas long ultraviolet not only elicits erythema but causes inhibition as well because it is absorbed in both the malpighian and papillary layers. Mixed ultraviolet should be intermediate in relative effectiveness with regard to erythema and inhibition since it contains both deep and shallow penetration radiation.

Blum, Eicher and Terus (139) attempted an evaluation of protective measures against sunburn including the relative effects of the change in concentration and position of melanin pigment in the epidermis. Although difficult to assess, they concluded that melanin pigment is of less importance in protection against ultraviolet irradiation than thickening of the corneum. In this connection the observations of Rothman, Krysa and Smiljanic (140) are of interest. They found that aqueous extracts of human epidermis contained substances inhibiting melanin formation in the tyrosine-tyrosinase system and that this inhibition was interfered with by iodoacetamide. Their findings led to the hypothesis that in melanoblasts, both substrate and active enzyme are present with no reaction taking place between them because of the inhibitory action of sulfhydryl compounds. Pigmentogenic stimuli, such as sunshine, act by oxidizing or otherwise destroying these compounds, whereupon the enzyme freely acts on the substrate. In preliminary experiments the sulfhydryl content of the epidermal extract was found to be in the range of 0.0004 millimole of sulfhydryl per cc. The sulfhydryl content could be substantially depressed by ultraviolet irradiation of excised pieces of skin. Fitzpatrick, Becker, Lerner, and Montgomery (141)

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elaborated upon the mechanism by which ultraviolet energy activates the enzymatic reaction which leads to the production of melanin. At least two factors appeared to be involved. First, trace amounts of DOPA may be formed by direct photochemical oxidation of tryosine present in the tissues and may then catalyze the enzymatic oxidation of tyrosine to melanin. The conversion of tyrosine to DOPA by ultraviolet radiant energy in the absence of the enzyme has been demonstrated by Flesch and Rothman (65). Second, ultraviolet energy may decrease the concentration of normally occurring sulfhydryl groups in the epidermis as suggested by Rothman, Krysa and Smiljanic (140). Inhibition of the tyrosine-tyrosinase reaction in vitro by extracts of human epidermis is attributable to the presence of sulfhydryl groups. This inhibitory action was absent after irradiation of the epidermal extracts with ultraviolet radiant energy. Fitzpatrick et al (141) believed that sulfhydryl groups inhibit tyrosinase by combining with the copper which is required for enzyme activity. The inhibition of the human tyrosinase reaction by sodium diethyldithiocarbamate suggests that this enzyme, like tyrosinase in plants, insects, and in mouse melanomas, requires copper for its activity.

The superficial manifestations and the histopathology of "solar dermatitis" have been reported by Lamb, Shelmire, Cooper, Morgan and Keaty (142). Approximately 75% of patients with solar dermatitis presented plaque-like lesions, the commonest site of which were cheeks, sides of the neck below the mastoid regions, and the "V" of the neck. The plaque-like lesions ranged from 2 cm. in diameter to large plaques 4 to 5 cm. in diameter. The plaques were described as having a pinkish color in contrast to the tanned hyperpigmented surrounding skin. They were often excoriated and scaly. Many patients had vesicular lesions concurrently and in some there was follicular dilatation. In the so-called "contact eczematous solar dermatitis" the skin was erythematous, pruritic, thickened and excoriated. After prolonged exposure to the sun the affected sites became edematous and covered with fine vesicles, followed by oozing and crusting. Histopathologically, specimens from lesions of the plaque-like type showed atrophy of the epidermis with relative hyperkeratosis. Hair follicles were plugged and distended with keratin. Small areas of liquefaction degeneration of the basal layer were present in some cases. There was no conspicuous increase in pigment in the basal layer. Edema in the dermis was a constant observation. Dilatation of the blood vessels and lymph spaces was conspicuous. In some cases there were small focal areas of extravasation of red cells into the tissues surrounding blood vessels. In many there was moderate to dense perivascular cellular infiltrate made up largely of lymphocytes and epithelioid cells. Eosinophils were numerous and plasma cells were seen in some instances. The collagen and elastic tissue of the dermis showed basophilic degeneration of a degree greater than would be found in patients of the same age group, not afflicted with solar dermatitis.

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There were areas which, on gross inspection, appeared depigmented and melanin in the basal layer seemed to be rather spottily distributed. DOPA reaction testing on two lesions which were thought to be depigmented showed an essentially normal distribution of melanoblasts. Although the number of DOPA reaction tests done was too small for definite conclusions, the results suggested that, if there were any disturbance in pigment formation, it occurred in the conversion of the precursor to melanin and not through absence of melanoblasts.

Rottier and Mullink (143) found the erythemal reaction to the 3000 Å line to be much stronger on human skin from which the stratum corneum had been removed, than on normal skin; this was apparently due to the fact that a heavier dose of irradiation penetrated into the mucous layers of the skin when the screening effect of the horny layer was absent. Also, the absence of the scattering effect of the horny layer, which causes a broader area of the mucous layer to be irradiated in normal skin, should be taken into consideration. Conversely, the erythemal reaction after irradiation with light of wavelengths 2500 to 2600 Å was practically absent on skin without the horny layer. Since the absorption and scattering effects are much stronger at the shorter wavelengths, a much stronger reaction would have been expected after irradiation with these wavelengths than after irradiation with 3000 Å if the primary photochemical reaction is localized in the mucous layer, as is the case with the 3000 Å reaction. The absence of the erythemal reaction on the skin without horny layer led to the conclusion that the primary photochemical reaction to the 2500 to 2600 Å wavelengths is localized in the horny layer. In accordance with the known facts concerning formation of vitamin D by ultraviolet light from sterols in the upper layers of the skin, Rottier and Mullink suggested that photolysible substances in the stratum corneum are responsible for the erythemal reaction with ultraviolet light of wavelengths 2500 to 2600 Å. These substances might even be sterols.

A possible relationship between polymorphous light eruptions and subacute lupus erythematosus has been investigated by Cahn, Levy, Schaffer and Beerman (144). Nine patients suffering from polymorphous light eruptions received a single exposure of ultraviolet rays for 12 minutes at a distance of 75 cm. Two developed intense erythema and delayed papular eruptions in the test site which resembled the presenting eruption clinically. Morphologically and histologically the lesions were indistinguishable from lupus erythematosus. Although there is a wide quantitative variation in normal reactivity to sunburn radiation, polymorphous light eruptions are definitely abnormal responses in that they are qualitatively different from those of the normal sunburn reaction. Several reasons were advanced for failure to reproduce the eruption experimentally in 7 of the patients tested: (1) the patient's senstivity may have been lost or destroyed or the test site may not have been sensitive at the time of the

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test; (2) the light rays may not have been of sufficient intensity to elicit an adequate response; (3) the patient may have been sensitive to a particular wavelength not produced by the quartz-mercury arc lamp used in this investigation but produced by the sun which has a continuous spectral emission. Cahn et al (144) concluded that lupus erythematosus is not a clear-cut concept and that the reaction to light rays in these patients may represent stages in transition from subclinical to manifest clinical systemic lupus erythematosus. On the basis that there was a relationship between polymorphous light eruptions and lupus erythematosus, Cahn, Levy and Schaffer (145) investigated the effectiveness of chloroquine diphosphate (aralen) and quinacrine hydrochloride (atabrine) in the prevention of polymorphous light eruptions. This investigation was prompted by the observation that aralen and atabrine are effective in the treatment of lupus erythematosus. Sixteen individuals with a 2- to 19-year history of recurrent eruptions in the spring, summer and fall on sun-exposed regions of the body were treated with either aralen or atabrine and were cautioned to avoid exposure to sunlight for 3 weeks. They were then allowed to go into the bright midday sunlight without protection. The drugs were administered in spring and early summer when the eruptions were at their height. Following disappearance of the eruptions while taking medication, fourteen had no recurrences for the remainder of the summer months despite unrestricted sun exposure and avoidance of local sun-screening agents. The investigation indicates that aralen and atabrine will successfully prevent polymorphous eruptions in most cases.

The vascular response of the skin to ultraviolet light was investigated by Partington (146). He found that flares produced by pricking histamine into skin previously reddened by ultraviolet light were no different in size from flares produced in the same way on unirradiated skin. Also, lowering the histamine content of the skin by the injection of histamine liberators either before or just after irradiation did not alter the subsequent development of erythema. No evidence was found, through the use of histamine liberators, that skin irradiated with ultraviolet light 24 hours beforehand had a lowered histamine content. Pretreatment with antihistamines reduced the red reaction of the skin to histamine but not to that produced by ultraviolet light. No red reaction was produced in rabbit skin by histamine or the histamine liberators, but a red reaction similar to that seen in human skin followed irradiation with ultraviolet light. In a rabbit with trypan blue in its circulation, irradiation of the skin caused dye staining of the area irradiated and this was not prevented by antihistamines. Partington concluded that the vascular response of the skin to ultraviolet is not due to the release of histamine.

The influence of ultraviolet irradiation on the permeability of animal and human skin, utilizing an artificial radioactive isotope of iodine as an indicator, and an ionophoretic technique,

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has been investigated by Maizelis (147). Under the influence of repeated irradiation with ultraviolet rays, a significant decrease in permeability as indicated by the penetration of radioactive iodine into the skin, was observed. A less sharp decrease in permeability of the skin was observed on the opposite side of the body which was not subjected to irradiation. Experiments showed that the permeability of the skin of remote areas of the body was decreased insignificantly in comparison with the irradiated zone and the symmetric areas of the body. The permeability of the skin of healthy persons increased immediately after irradiation. Twenty-four to 48 hours after irradiation the permeability of the skin proved to be significantly lower than the original level in the erythematous zone. Maizelis explained the decreased permeability of the skin, observed during the period of erythema, as due to an increase in the protective function of the skin during the local inflammatory reaction produced by irradiation. Jarvinen (148) investigated the effect of cortisone on the reaction of skin to ultraviolet light. He observed a suppression of the reactivity of skin exposed to ultraviolet light during a period of administration of massive doses of cortisone. The minimum exposure required to produce an observable reaction in the skin was approximately double. Instead of the red erythematous reaction, the skin tended to react with an increase of brown pigment. Blister formation occurred much more rarely than normally. In one group of patients cortisone had been administered for at least 5 days with an average dose before exposure of 1038.6 milligrams and a minimum dose of 500 milligrams. In another group the administration of cortisone had been stopped on an average of 5.2 days previously, after administration for an average period of 18.8 days and an average total dose of 1969.3 milligrams. The view that the observed phenomena should be regarded as a suppression of the normal protective mechanisms is supported by observations made in connection with various infectious diseases. It has been found that massive doses of cortisone inhibit or prevent the typical symptoms of acute infectious diseases such as fever, the development of inflammatory tissue changes, rise in erythrocyte sedimentation rate, production of antibodies, etc., whereas the growth and propagation of organisms in bacterial infections proceed more rapidly than normally. Holti (149) also investigated the effect of cortisone upon skin reactions to ultraviolet irradiation and demonstrated that cortisone materially diminished and shortened the acute ultraviolet reaction. Vasodilatation as indicated by skin temperature was less elevated and for a shorter period than in a control area (irradiated prior to the ingestion of cortisone). No diffusion flush surrounded the area irradiated under cortisone at any stage of the acute reaction and edema was very slight and very transient in contrast to the control area. During the later part of the acute stage pigmentation was more conspicuous at the site exposed during cortisone administration but this appearance was due to less marked erythema in the area whereas the intense

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and prolonged erythema following the control exposure masked the early stages of pigmentation. Weeks or months after the experiment unbiased observers had no difficulty in picking out the skin irradiated while on cortisone because of its less marked pigmentation compared with that of the adjacent area irradiated while no cortisone had been taken. It was suggested that the reported changes were due to an altered viscosity of the tissue ground substance and not to an effect on the vascular endothelium. It has been stated that cortisone acts upon all components of connective tissue -- cells, fibrils, and ground substance. Nevertheless, since cortisone "neither combines with histamine, thereby inactivating it, nor influences histaminase activity, the weight of present evidence appears to favor a direct effect upon the vascular endothelium as explaining the diminished transudation during cortisone administration."

The minimum dose of ultraviolet irradiation needed to cause erythema of living skin has been thought to be independent of intensity. From this concept has come the "reciprocity law" which states that the minimal erythemal dose is equal to intensity times irradiation time which equals a constant. Claesson, Juhlin and Wettermark (150) investigated the validity of the reciprocity law with ultraviolet light of very high intensity. They reasoned that the effects of light with a high intensity might be of importance in studying phenomena caused by an atomic bomb since, at a distance of 1 kilometer from a 10 kiloton atomic explosion, the intensity of ultraviolet light has been estimated to be of the order of 100 watts per sq. cm. With special photolysis flashlamps, these investigators were able to vary intensity over a 10^7-fold range. They irradiated shaved areas of skin on the backs of albino mice and estimated the degree of tissue damage by studying the leakage into the irradiated areas of intravenously injected Evans blue. They determined an intensity level which gave maximal bluing and found that their was no bluing at half this dose. Furthermore, the bluing abated with further increase in the dose and was quite inappreciable after very high doses of irradiation. The factors determining the degree of bluing seemed to be an increase of permeability in the capillaries together with an increase in width of the capillaries and an increase in capillary pressure. The fact that an increase in irradiation dose beyond that producing maximum bluing gives weaker bluing, "might be explained by a vasoconstriction following larger doses." This would inhibit the leakage of the dye from the circulatory system. It was noted that it was only with respect to bluing and erythema that there was such an optimum dose. If one considered the microscopic changes in the skin, such as peeling, that occur after a few days there was an obvious increase with increasing doses. The results were considered to be similar to those obtained in studies on erythema in man 20 hours after irradiation with ultraviolet light (2960 to 3130 Å at low intensity). Erythema was demonstrated to be optimum at a certain dose that decreased when the dose was further increased. Blum

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and Terus (138) assumed the cause of this phenomenon to be that erythema is caused by damage to the epidermal cells which elaborate a dilator substance that penetrates to the superficial vessels in the papillary layer and frees them from their normal constriction. Inhibition was explained as a direct effect of the ultraviolet light on the papillary layer that resulted in its vessels not being affected by dilator substance from the epidermis. Claesson et al (150) concluded that the minimum dose of ultraviolet irradiation was independent of the intensity of the incident light. Consequently, the reciprocity law was shown to be valid at a wavelength of 3100 Å over a 10,000,000-fold variation in light intensity (0.00066 to 860 watts per sq. cm.). The minimum dose, 0.04 watts per sq. cm., is the same as that found in man at wavelengths of 2537 Å by Blum and Terus for the intensity range 0.00017 to 0.00001 watts per sq. cm.

Lipkin, Bailey and Hardy (151) found that the onset of erythema, resulting from ultraviolet irradiation, was associated with a fall in pain threshold. The maximum fall of pain threshold, the duration of this lowering, and the intensity of hyperalgesia and erythema increased with the ultraviolet radiation (intensity times time). It was shown that these experimental data were consistent with the hypothesis that, as a result of ultraviolet irradiation, there is inhibition of some tissue repair processes, thereby causing a pain threshold lowering from rought 45 degrees C. to 36 degrees C. In this state, even a slight elevation of skin temperature in a burned area evokes pain and may be sufficient to cause thermal inactivation of cellular proteins at a rate great enough to result in tissue damage.

Extracts of ammi majus have been used by the Egyptians as pigmenting agents for centuries. One of the active ingredients of this plant (8-methoxypsoralen) has been publicized as a "suntan pill." Becker (152) has investigated the combined effects of ultraviolet light and 8-methoxypsoralen in human skin. A blonde, blue-eyed, 33-year old white male exposed an area of his left thigh to ultraviolet irradiation with a mercury vapor lamp. Exposures were carried out on successive days with exposure times that were progressively increased from 1 minute to 15 minutes (15 minutes on the ninth to the fourteenth days inclusive). Afterward, a similar area of the right thigh was exposed in the same manner following the ingestion of 20 milligrams of 8-methoxypsoralen (2 hours before each exposure). Specimens of skin for microscopic examination were removed from the irradiated areas of both thighs on the seventh and fourteenth days of the irradiation series. After 14 days of irradiation the threshold erythema dose of ultraviolet light in the treated area of the left thigh was 25 minutes; 50 minutes of irradiation to the corresponding area of the right thigh produced no redness. Microscopic examination of the specimens of skin revealed the most prominent change in the horny layer which was thickened in both treated areas; the specimen from the right thigh also revealed

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the formation of a stratum lucidum. Becker states that a stratum lucidum is seen normally only in areas where the epidermis develops a thick horny layer, as on the palms and soles; physiologically it is never seen in the skin of the thigh. There was no melanin present in the stratum corneum of these specimens and it was therefore concluded that increased tolerance to ultraviolet light was due solely to the alteration in the horny layer. The combination of ultraviolet light and 8-methoxypsoralen resulted in more new pigment formation in the treated area of the right thigh than in the corresponding control area of the left thigh, but the quantity of newly formed pigment was not great. To Becker it appeared from these findings that, while 8-methoxypsoralen increases the pigmenting effect of ultraviolet light, the development of psoralen tan is due primarily to retention of pigment in the skin.

Rapid progress in knowledge concerning the physiology of pigmentation has indicated the complicated nature of pigmentary disorders and has pointed out the possibility of multiple mechanisms in the production of vitiligo (153). There appeared to be three different mechanisms that may play roles in the production of leukodermia. The first is failure of the intracellular enzymatic process of pigmentation; the supply of dihydroxyphenylalanine which is transformed into melanin may be at fault or the enzyme itself may be inactive due to relative lack of copper or, in the presence of active cooper containing enzymes, the enzymatic process could be inhibited by sulfhydryl groups. The second possibility in the production of leukodermia is failure of the melanoblast. All essential facotrs for enzymatic action may be available for the formation of melanin in vitro but this still may not be achieved under certain conditions in vivo. Enzymatic action can proceed in vitro without the presence of vitamins or hormones but in vivo intracellular melanin formation requires the presence of such substances. It is possible that melanogenesis could be profoundly affected by changes in the permeability of the cell membrane of the melanoblast, interfering with the chemical interchanges essential for successful intracellular melanin formation. The third possibility in the production of leukodermia is melanin destruction. The pathology of leukodermia has been thought of as essentially a failure of pigment formation. The possibility cannot be ignored that melanin may disintegrate into colorless substances. Certain foreign reducing agents, for example, hydroquinones, have been found to act as poisons to induce melanin disappearance in occupational leukodermia. Thus the mechanisms through which leukodermia may be acquired are so many that it is hardly possible to expect any single line of treatment to be effective in all cases. The observation that normal individuals coming into contact with ammi majus may show excessive local pigmentation favors the assumption that the drug as used in the treatment of vitiligo (leukodermia) acts by stimulating melanogenesis rather than by inhibiting melanin disintegration.

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Lerner, Denton and Fitzpatrick (154) gave 8-methoxypsoralen to three individuals with albinism and then followed their minimal erythema dose responses. Two of the albinos were treated for 3 months and the third for 2 months. All showed decreased sensitivity to ultraviolet light. All three believed that their skin was less photosensitive on exposure to sunlight following treatment with the drug. It should be noted that all of the albinos had associated photophobia and lateral nystagnus; administration of 8-methoxypsoralen had no effects on these phenomena. Fitzpatrick, Hopkins, Blickenstaff, and Swift (155) conducted experiments in 70 patients with vitiligo which indicated that 8-methoxypsoralen taken orally 2 to 3 hours before exposure increased intolerance to ultraviolet radiation. To extend these clinical observations, these investigators undertook a controlled field trial with 47 volunteer subjects, in the Arizona desert. Half of the subjects were given capsules containing 8-methoxypsoralen and the other half were given an identical appearing placebo capsule. Three hours after ingestion of the capsules the subjects were exposed in the prone position in direct noonday sunlight for 85 minutes. Direct visual readings showed significant differences between the placebo and the 8-methoxypsoralen treated individuals as follows: (1) significant differences did not appear until about 44 hours after exposure; (2) at 44 hours postexposure erythema and tenderness were significantly greater in the 8-methoxypsoralen subjects; (3) at 1 week after exposure the skin responses had disappeared in the placebo group except for tanning but there was significant erythema, edema, and tenderness in the 8-methoxypsoralen group. Thus the Arizona field trial provided evidence that 50 milligrams of 8-methoxypsoralen taken 3 hours before exposure augmented all cutaneous responses to solar radiation. In view of the fact that patients with vitiligo and many normal subjects had noted increased tolerance to sunlight, and in order to resolve these conflicting observations, the authors carried out a series of field trials in Oregon during the summer months. The results of these experiments indicated that, with a dose of 10 to 30 milligrams of 8-methoxypsoralen, exposure to solar radiation for not more than 1 hour will produce erythema followed by increased tanning without tenderness or exfoliation. In higher doses the erythema becomes progressively greater with increasing edema followed by exfoliation. Seventy-five milligrams of 8-methoxypsoralen, followed by exposure for 1 hour, produced marked tenderness and edema, followed by blistering. Fitzpatrick et al were forced to the conclusion that 8-methoxypsoralen does not have a primary protective function, but rather augments all cutaneous responses to solar irradiation. The "protection" reported by many subjects may be related to the markedly increased pigmentation which occurs after the use of 8-methoxypsoralen and repeated daily exposures to ultraviolet radiant energy. Fitzpatrick, Imbrie and Labby (156) carried out an investigation to determine whether 8-methoxypsoralen has an effect on liver function. Statistical analysis of their data indicated that it can be

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stated with 95% confidence that 73% of young healthy males receiving a daily dose of 30 milligrams of methoxypsoralen, with casual ultraviolet irradiation, for 3 months, will not develop a change in liver function as detected by the usual liver function tests. Six of 7 subjects who received methoxypsoralen during the spring noted an augmentation of their responses to sunlight, while none of the 6 subjects receiving a placebo noted any difference in erythema or pigmentation.

Intraperitoneal administration of 8-methoxypsoralen, followed 1 hour later by a 2-hour exposure to ultraviolet irradiation, produced an erythemal response that killed albino mice in 2 weeks (157). Eyes, ears, tail, face and feet were severely burned. The response was so great that it was necessary to reduce the exposure time to 10 minutes daily in order to keep the animals alive. Of great interest was the fact that a high tumor incidence was associated with this erythemal damage. Both fibrosarcomas and squamous carcinomas appeared in the animals. The importance of photosensitizing drugs of this type in experimental ultraviolet carcinogenesis becomes apparent when it is considered that the same or even far greater exposures to the same wavelengths do not produce effects in comparable animals not treated with the drug. Daily intraperitoneal administration of 8-methoxypsoralen just prior to irradiation with fluorescent light resulted in mild to moderate erythema in mice. After 8 months of exposure to fluorescent light, most animals had developed scar tissue on the edges of their ears and a few tumors were evident. It thus becomes apparent that the longer wavelength spectrum may initiate extensive alterations in the skin in association with the photosensitizing drugs. The slight ultraviolet emission from ordinary fluorescent lamps may be sufficient to activate such changes if the animal has been sensitized by compounds of this type. The authors were at a loss to explain the mechanism of action of the psoralen-type drugs. The drug absorbs strongly in the region of 2500 and 3000 Å which may account for certain of the observed effects. It would appear that the drug or metabolites thereof are capable of absorbing or receiving longer waves of the ultraviolet spectrum and converting this band into chemical energy within the outer layers of the skin or eyes. This is manifested by the extensive pigmentation and erythema that occur in normal individuals. Psoralen or its metabolites may acquire fluorescing properties following irradiation and emit ultraviolet light of wave bands that would initiate the changes noted above.

Ultraviolet Irradiation and the Eye

The basic relation which describes the response of the eye to radiation is its sensitivity to the various wavelengths of

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the spectrum (158). "The limits of this function define what is meant by light. Its form expresses the fundamental property of the retinal receptors and of the ocular structures through which light must penetrate to reach them." The human retina contains two groups of receptors -- rods which function in dim light, and cones which are the organs of vision in bright light and in color vision. In the passage from dim to bright light the spectral sensitivity of the eye shifts toward the red end of the spectrum; this is the Purkinje phenomenon. The intrinsic sensitivities of rods and cones are modified by the presence of colored intraocular structures. One of these is the lens which, in man, is yellow in color. The cornea and the ocular humors also absorb some light in the violet and ultraviolet. The retina itself contains a yellow pigment, concentrated in a diffuse central zone about the fovea -- the macula lutea. This pigmentation imposes special differences in spectral sensitivity upon the central as compared with the peripheral retina. The maximum sensitivity of the fovea, containing only cones, occurs at about 5620 Å. On either side of this wavelength the sensitivity declines, reaching an average about 0.0001 of its maximal value at 7500 Å and about 0.00040 at 3650 Å. Rod sensitivity is maximal at about 5050 Å; at 3650 Å it has fallen on the average to about 0.00020 and at 750 Å to about 0.0000025 of the maximum value. Below 5500 Å the dark-adapted periphery of the retina is 100 to more than 1000 times as sensitive as the fovea. Above this wavelength the peripheral and foveal functions draw together and, at about 6500 Å, they cross. In the farther red the fovea is more sensitive than the periphery. If the eye is light adapted and then placed in darkness, the threshold for time is due to the cones. During this interval the stimulus looks colored at all wavelengths. The threshold falls rapidly to a plateau, held constant from about the fourth to the eighth minute or longer. This is the threshold level of the completely dark-adapted cones. It is maintained long enough to permit its measurement with some precision. Later the dark-adaptation of the rods supervenes and the threshold falls again to a new and final level. The maximum sensitivity of the peripheral cone is found at about 5500 Å, about 120 Å below that of the fovea. Except for a narrow region between 4400 and 4900 Å, the sensitivity of the peripheral cones is lower than that of the fovea. At about 5500 Å both functions are parallel. Throughout this region the fovea is about $2\frac{1}{2}$ times more sensitive than the cone of a corresponding area of peripheral retina. Measurements of spectral sensitivity, rod and cone, centrally and peripherally, have been performed in subjects whose lenses have been removed in the operation for cataract. With the loss of the yellow lens the eye gains enormously in sensitivity in the violet and ultraviolet. The spectral sensitivity curve of the aphakic eye declines much less steeply at low wavelengths. At 3650 Å, rod or cone vision in the aphakic eye is still about one-third as sensitive as at the maximum. At this wavelength the aphakic rods are as sensitive as in yellow light and the cones are as sensitive as in the near red. The average sensitivity of the

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aphakic eye at 3650 Å is about 1000 times that of normal observers. Estimates of lens absorption show that it rises rapidly and regularly from about 5000 Å into the ultraviolet. At 4050 Å the average lens transmits about 15% and at 3650 Å about 0.1% of the radiation incident upon it.

Normal visible light is generally considered to extend from 7500 Å to 4000 Å but most spectroscopists are familiar with the fact that the 3650 Å line of the mercury spectrum is quite visible. It has been reported that this last line is visible after a period of accommodation and that its color sensation is identical with that of the 4047 Å line. Goodeve (159) found normal vision down to 3025 Å. Light of this wavelength also produced a violet color sensation similar to that of 4047 Å. Considerable difficulty was experienced in focusing the object unless it was within 4 inches of the eye. When in focus it appeared to be 9 to 12 inches away. This illusion was very definite and was "to be expected, owing to the presumably higher refractive index of the proteins of the eye lens for this wavelength." On removing the eye to a distance beyond 4 inches the object appeared to recede to a great distance and suddenly to become out of focus. At a distance of 6 feet the object appeared only as a large ring about 6 inches in diameter. These tests were carried out with a light-adapted eye and the object could be seen in the normal lighting of the room. The 3390, 3650 and 3906 Å mercury lines could also be seen and gave similar color sensations. The focusing problem was similar to that at 3125 Å but to a progressively lesser degree. With 3023 Å, no impression on the retina was observed -- only a pronounced fluorescence of the anterior part of the eye. Fluorescence was also obtained to a slight degree with the 3125 Å line but the two effects were quite separable, the fluorescence being merely an illumination with no sense of direction, similar to that produced by a bright light with a closed eyelid. Even with a dark-adapted eye it was impossible to be conscious of the position of the object when illuminated with light of 3023 Å. No vision or fluorescence was attained with either 2537 or 2625 Å lines. "The sharp cutoff of vision between 3125 and 3023 Å is probably due to a threshold of absorption of light by the proteins of the lens and such threshold is indicated by the work of T. Abe (160)."

The transmission of the ocular media has been studied by scores of investigators and the observations vary considerably. We may assume, however, that some human and other vertebrate eyes transmit wavelengths as low as 3100 Å (161, 162). Visual tests in the ultraviolet indicate that light of 3020 Å is perceived by very young subjects (6 to 10 years); with progressing age the threshold moves to longer wavelengths, middle-aged persons rising to 3100 or 3200 Å and, at higher ages, to 3600 Å and higher. Wolf (161, 162) exposed baby chicks to intense radiation (three 250 watt mercury vapor lamps mounted at eye level). Selection of specific spectral regions was accomplished by the insertion

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of appropriate filters. For exposure a standard period of 60 minutes was chosen. After irradiation individual chicks were tested for the onset of a nystagmus response and the tests were repeated at intervals until the threshold returned to its normal level. The first reading was taken at 60 minutes after cessation of exposure to allow for complete dark adaptation. Any deviation of the threshold from its normal level was considered to be due to the exposure to ultraviolet and not to incomplete adaptation. Control exposures to white light of equal intensity produced no effect on the threshold, after proper dark adaptation. After ultraviolet treatment the threshold was much higher, depending upon the extent of the spectral region employed. Wolf's (161, 162) conclusions were as follows: "If, based upon the similarity of the transmission of the ocular media, conclusions can be drawn from the findings on the eye of the chick, it is apparent, that under absence of exterior pathological conditions, recognizable by ophthalmoscopic inspection, the visual mechanism is impaired by ultraviolet light between 3000 and 3650 Å. Protective means for the eye should, therefore, be filters which absorb ultraviolet up to 3650 Å totally, or sufficiently to prevent injurious effects." Le Grand (163) has called attention to the practical problem of protection of vision against radiance which, by its nature or by its excessive intensity, is injurious or unpleasant. Corpuscular radiation might be responsible for disorders in the eyes after absorption occurs. The question is of importance for workers in the neighborhood of nuclear reactors and "it might also, in the future, be of interest to interplanetary travelers who would undergo cosmic radiation." Le Grand summarized his observations as follows: (1) natural light is harmless for the normal subject except in the case of strongly diffusive materials such as sand or snow which may be responsible for absorption of ultraviolet B (wavelengths shorter than 3150 Å); (2) fluorescent artificial lighting is harmless for normal subjects, if the subject avoids excessive proximity to the tube; (3) intense sources of ultraviolet B and C (2800 to 1800 Å) in industry require careful protection of the eyes; (4) some subjects present a special sensitivity to ultraviolet and must be protected from ultraviolet B even under the conditions which a normal subject can easily tolerate; (5) ultraviolet A (wavelengths longer than 3150 Å) seems to be harmless; it is absorbed by the crystalline lens and makes it fluorescent; the action of ultraviolet A, which has penetrated the crystalline lens, upon the retina is uncertain and seems unimportant; where night vision is required with its maximum effectiveness, it is beneficial to absorb ultraviolet A to avoid a veil of fluorescence; (6) finally, for the individual without a lens, it is preferable to absorb all the ultraviolet because, in the absence of a natural filter, chromatic aberration might be superimposed as a foggy ultraviolet image on the clear visible image of the retina and thereby diminish sharpness of contour."

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Ludvigh and Kinsey (164) have criticized the observations of Wolf (161, 162) on baby chicks and have carried out experiments on humans designed to refute his conclusions. Seven individuals 22 to 38 years of age, fixated upon a 1000 watt mercury arc whose radiations were filtered to remove most of the visible and almost all of the ultraviolet radiation shorter than 3200 Å, at a distance of 30 cm. for 5 minutes with the left eye while the right eye was covered. The foveal light-difference sensitivity and critical flicker frequency of both eyes of these individuals had been previously determined. The observers were tested 5 minutes and 1 hour after exposure and there was no statistically significant difference in the results between the two eyes of six observers or between the measurements of any one eye before and after irradiation. In the seventh individual the irradiation produced a statistically insignificant improvement in the light-difference sensitivity of the left eye. "The ultraviolet energy above 3200 Å, concentrated on the fovea in these experiments, was in excess of what could ordinarily be obtained in nature except by fixation on the sun. The discrepancy between the results of the previously reported experiments on chicks and these on human beings may be attributable to the use of the opticokinetic response in the dark-adapted eye in the tests on chicks and the light-difference threshold on human beings. The most likely cause for the discrepancy between results is the marked difference in absorption and general physiological characteristics between the eyes of baby chicks and those of adult human beings. It is concluded that ultraviolet radiations longer than 3200 Å, encountered in nature, are without deleterious effects on these two important functions of the normal eye." Wolf (165) responded to the criticisms of Ludvigh and Kinsey. He pointed out that, for the chick as well as the human eye, it has been shown that pre-exposure to radiation of the mercury vapor arc, emitting ultraviolet light above 2850 Å in addition to the visible range, raises the final dark-adapted threshold considerably above the normal level as compared with pre-exposures to the same source but with all ultraviolet filtered out. The adapting brightness is, in both cases, the same and hence it is assumed that the final threshold differences are due to the ultraviolet. Further experiments on humans and on chicks showed that, in both cases, the cone part of the duplex dark-adaptation curve is unaffected by pre-exposure to ultraviolet while the rod segments are clearly altered. For the human curve the onset of rod adaptation is delayed for about 2 minutes, the cone segment overshooting the normal beginning of rod adaptation and remaining above the previously established level until termination of the test. For the chick the slopes of the cone and rod segments are quite different from those of the human so that, due to the steepness of the cone segment, overshooting is not apparent. It was also found that reduction of the extent of the ultraviolet spectrum reduces the affect on final thresholds; light containing only wavelengths above 3650 Å has no effect. Ultraviolet alone, after largely eliminating visible

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light, acts in qualitatively the same manner as visible light to which ultraviolet has been added. The effect of ultraviolet upon the cones is probably prevented by their dense pigmentation while it acts upon the pigment-free rods. Therefore, while testing foveal intensity discrimination or flicker thresholds, after pre-exposure to ultraviolet, it is obvious that an effect upon visual threshold cannot be expected since one is dealing with an irresponsive pure cone population of sensory units with exclusion of the rods.

A shift of the dark-adaptation curve, produced by the presence of ultraviolet in the pre-exposure light can be demonstrated when a test light is used (166). With a blue test light (4880 Å), the ultraviolet effect is equally apparent. With green (5447 Å), however, only a very slight ultraviolet effect is found and with red (6690 Å) no ultraviolet effect is recognizable. Ultraviolet pre-exposure seems, therefore, to affect sensitivity mostly in the adjacent blue region of the spectrum and its effectiveness decreases rapidly as the red end of the spectrum is approached. Wolf and Zigler (166) admitted that, owing to the high absorption of the ocular media (particularly the lens, only small amounts of ultraviolet light reach the retina. They considered it surprising that a change in threshold sensitivity of considerable magnitude is found. They noted that, in addition to the direct action of ultraviolet on the retinal elements, an influence on threshold sensitivity caused by fluorescence of the ocular media during the pre-exposure should be considered. "It would, however, seem very unlikely that such an influence would persist beyond the light exposure period, unless some specific temporary changes in the transmissiveness of the ocular media had taken place or the retinal mechanism had been affected in some way."

Brandenburg (167) has indicated that exposure to light sources emitting ultraviolet radiations between 3000 and 4000 Å result in a decrease in sensitivity of at least 20% in dark-adaptation and visual discrimination when the ultraviolet light is allowed to reach the retina "unimpeded" as compared to the sensitivity when the ultraviolet is blocked by filters. "These ultraviolet effects may persist as long as 2 hours after exposure to unfiltered radiation from a television screen for 1 hour, fluorescent illumination, or the ultraviolet component of bright daylight. They may be a factor in the higher incidence of traffic accidents at dusk. Certain types of glass will prevent passage of ultraviolet without affecting the visible part of the spectrum. The use of such glass in front of various light sources or as spectacles might contribute to increased safety in twilight and night driving." Sexton, Malone and Farnsworth (168) have also reported that the ultraviolet energy emitted by fluorescent lamps, at sufficiently high brightnesses, is known to decrease dark-adaptation and to cause a decrement in visual acuity. Their report indicated that, so long as brightnesses in submarine

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compartments were kept below 20-foot lamberts, no reduction in visual efficiency of operating personnel was to be expected. They concluded that ultraviolet radiation is not produced in sufficient amounts by submarine fluorescent lights, held at or below recommended reflected brightnesses (10- to 20-foot lamberts), to yield measurable effects upon the eyes of the visual tasks employed in their tests. These tasks were of a moderate degree of difficulty and similar in visual requirements to the visual tasks performed aboard submarines.

Hecht, Hendley and Ross (169) studied the influence of exposure to intense sunlight on subsequent night vision. They concluded that exposures to ordinary sunlight produce temporary and cumulative effects on night vision. A single exposure of 2 or 3 hours delays the onset of rod dark-adaptation by 10 minutes or more and slows the process itself so that the normal night vision threshold is not reached for several hours. After repeated daily exposures to sunlight, the delay in reaching the normal threshold persists over night. The threshold, after complete dark-adaptation, rises higher each day for about 10 days and then remains at the high level. The elevated threshold corresponds to an average deterioration of about 50% in visual acuity, range of visibility, contrast discrimination, and in the frequency of picking up a target when it is barely visible. The effect shows considerable individual variation, but the average loss in night vision is nearly the same as is suffered when flying at 12,000 feet at night without oxygen. This chronic effect does not disappear even after 10 days of protection from sunlight. It was not the object of these investigators to inquire into the physiological mechanisms involved but they were inclined to believe that it is the visible part of the radiation that is effective. Ultraviolet forms only a small part of the radiation reaching the cornea and, while it can burn this part of the eye as in snow blindness, it does not reach the retina to any appreciable extent, because of the great absorption of the lens and of the eye media.

Kinsey, Cogan and Drinker (170) found that the energy necessary to produce keratitis in man by ultraviolet radiation is approximately two-thirds that required in rabbits. On exposure of human beings at a distance of 7 feet from a 300 amp. mercury arc, they found that ocular lesions were produced in 30 seconds. From their intensity measurements Coblentz (171) deduced that the ultraviolet energy required to produce keratitis was about 45,000 ergs per sq. cm. of equivalent 2537 Å radiation. Coblentz found, using homogeneous radiation of wavelength 2520 Å, that 600,000 ergs per sq. cm. was required to produce a threshold keratitic reaction, in comparison with about 365,000 ergs per sq. cm. in summer and 750,000 ergs per sq. cm. in winter to produce a minimum perceptible erythema with radiation from a cold quartz lamp emitting the same wavelength. In practice the erythema produced by this amount of radiation of wavelength 2537 Å

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is so evanescent that easily twice that amount of radiation is applied to get a definite reddening of the skin of average pigmentation with no ill effects. On the other hand, photophthalmia is a painful experience with no factor of safety from injury on overexposure. These data show that practically the same amount of energy of wavelength 2537 Å causes photophthalmia as is required to produce a minimum perceptible erythema of untanned skin. Hence, in irradiating the body with a cold quartz mercury lamp, unless eye-protective glasses are worn, there is no factor of safety between a dose giving minimum erythema and a photophthalmia consisting of conjunctivitis accompanied by stippling of the cornea. Bachem (172) referred to the statement of Coblentz (171) that keratitis and conjunctivitis may be produced by radiation of wavelengths shorter than 2800 Å and refuted the statement as follows: "According to the careful studies of Cogan and Kinsey (173) the maximal keratitic effect occurs above 2800 Å or at 2880 Å. The effect fades out toward the longer ultraviolet (above 3100 Å) and toward the short ultraviolet (around 2540 Å). Coblentz does not realize that all ultraviolet rays below 2930 Å are completely absorbed by the human cornea and cannot have any direct effect upon the lens (Bachem refers in this latter statement to another statement of Coblentz that wavelengths shorter than 2900 Å, with excessive exposures, may cause coagulation of albumin and cataract). An indirect effect of corneal injury upon the lens metabolism has not been observed and appears highly improbable. Coblentz discarded the possibility that ultraviolet rays of wavelengths 2930 to 4000 Å, passing through the cornea and absorbed and scattered through the lens, may produce cataract either directly through metabolic changes in the lens or indirectly through uveal injuries and subsequent interference with the nutrition of the lens. He also overlooked the fact that corneal and conjunctival responses are harmless inflammatory and pain reactions which protect the eyes against repeated ultraviolet exposures by closing the eyes." On the basis of his studies, Bachem concluded that, with a 3 to 5% reflection from the cornea, one must expect maximal biologic effects upon the latter below 3000 Å with the possibility of responses throughout the near ultraviolet between 3000 and 4000 Å. For the lens the situation is different; its true absorption is considerable in the near ultraviolet. Within the middle and far ultraviolet, however, the cornea protects the lens very effectively. Below 2930 Å this protection is complete. In situ or in vivo absorption of the lens is maximal around 3650 Å and it becomes practically zero in the visible and far ultraviolet. It appears that the near ultraviolet, including 2970 Å, has a better chance to produce lens injuries than visible light. As far as the human eye is concerned it is known that the lens absorption is greater than in the rabbit and that it increases with advancing age and cataractous conditions. The cornea of the guinea pig was found to be definitely more transparent than that of the rabbit. It was still perceptibly transparent for the wavelength 2930 Å. The lens of the guinea pig appeared to absorb more than the rabbit lens. These facts explain the high

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susceptibility of the guinea pig for experimental radiation cataract. Bachem's conclusions (172) were as follows: (1) ultraviolet rays ophthalmically most effective are the "actinic" rays near 3000 to 2880 Å for the cornea and 2970 Å for the lens; (2) ultraviolet rays having shorter wavelengths (far ultraviolet) are relatively harmless to the eye. They produce no lens injury but may cause corneal and conjunctival inflammation; (3) ultraviolet rays of longer wavelengths can cause cataract through the cumulative effect of repeated excessive dosage; this conclusion was based on the study of the true absorption of the eye media, biologic experiments on rabbits and the observations and discussions of "several ophthalmic authors."

Quantitative determinations of the absorption of ultraviolet radiations by different structures of the eye are of importance because of the various pathologic conditions of the eye ascribed to these radiations (174). In this investigation Kinsey obtained quantitative information by measuring photoelectrically the fraction of radiation transmitted. All measurements were made on the eyes of albino rabbits. The corneal epithelium was removed as a continuous sheet and placed between two thin quartz cover slips and the remainder of the cornea was placed between two polished plates of quartz. The aqueous and vitreous humors were placed in quartz microcells and the lens, with the capsule intact, was carefully removed and placed in a specially constructed lucite chamber. A chamber of design similar to that used for the lens measurement was made to hold the whole eye. A hole approximately 3 millimeters in diameter was cut through the coverings in the back of the eye and the light source was so oriented that the radiations traversed the eye in a postero-anterior direction. The limit of transmission for the whole eye is approximately 3330 Å, that for the lens, 3100 Å, and that for the aqueous and vitreous humors and cornea, separately, approximately 2800 Å. Measurements of the absorption of ultraviolet radiations by the corneal epithelium indicate that the chief absorbing element is nucleoprotein, its limit of transmission being less than 2300 Å. The correspondence of the absorption peak observed for corneal epithelium (2650 Å) with that for nucleoprotein suggests that this substance is chiefly responsible for its absorption of ultraviolet radiations. This interpretation is consistent with the highly cellular nature of the corneal epithelium. The relatively thick absorptive layer of the other ocular components obscure absorption peaks so that the nature of the absorbing constituents cannot be inferred from the curves derived. Presumably the radiations are absorbed primarily by mucoid and collagen in the cornea, by albumin and globulin in the aqueous and vitreous humors and by alpha and beta crystalbumin and albuminoid in the lens. On the assumption that the sensitivity of the lens epithelium to injury at any wavelength is the same as that of the corneal epithelium, the minimal amount of radiant energy to which the eye would have to be exposed before minimal damage would occur to the lens can be estimated. Kinsey's (174) calculations indicated the total effectivity for the cornea, expressed in arbitrary units, to be 4162 and that for the lens

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1369 with a ratio between the two of 3.02. He concluded, therefore, that the eye would have to be exposed to 3 times the dose necessary to produce minimal damage to the cornea before minimal injury to the lens would be encountered. It became evident that if a source of ultraviolet light emitted radiation shorter than 2900 Å, the ratio between the amount of energy needed to produce damage to the cornea and that required for the lens would tend to become infinite, since essentially no ultraviolet radiation would reach the lens. It became evident from the absorption curve that so little ultraviolet radiation in the abiotic range reaches the retina that damage from these rays would be extremely unlikely unless the sensitivity of the retina to ultraviolet radiation is much greater than that of other tissues. Experimental evidence in support of this conclusion came from the investigations of Verhoeff and Bell (175) who were unable to detect injury to the retina in monkeys and man after exposure to various doses of ultraviolet radiation. Ludvigh and McCarthy (176) studied the absorption of light between 4000 and 8000 Å by the media of eyes removed from human subjects. They found that only 8.6% of light of wavelength 4000 Å reached the posterior pole of the eye. There was a regular (linear) increase in light transmission from 4000 Å to 8200 Å at the latter of which levels the transmission by the ocular media amounted to 71.6%. They accounted for the low value at 4000 Å as follows: "These low values should not be surprising if one considers that the refractive media of the eyes of animals may absorb practically completely at 3800 Å."

Cellular reactions to ultraviolet radiation can be studied effectively in the cornea (177). Four characteristic reactions are noted: (1) inhibition of mitosis which is the most constant early change and may be seen after small doses of ultraviolet radiation with inhibition affecting preferentially the early prophase; (2) nuclear fragmentation which occurs with higher dosage and probably represents anomalous efforts in mitosis, eventually leading to destruction of the cell; (3) eosinophilic reaction which occurs both in the nucleus and cytoplasm with the denatured protein showing enhanced capacity to bind acid dyes; (4) loosening of the epithelial layer due to loss of cohesion with underlying structures which may result in sloughing off of the entire epithelium. According to Duke-Elder (177), a critical threshold of wavelengths and of intensity of radiation is necessary to excite the reaction to ultraviolet radiation. A certain amount of abiotic activity may be evident at 3650 or 3500 Å if conditions are favorable and exposure sufficiently intense; it is more readily seen at 3050 Å but it is found, for practical purposes, that only rays below 3000 may be considered abiotically active and these must be used in an intensity of about 2 million ergs per second per sq. cm. As has been noted by other investigators, the most active bands correspond to the maximal absorption of nucleoproteins (2650 Å) or of cytoplasmic albumin and globulin (2800 Å).

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Photophthalmia, an acute inflammatory reaction of the superficial parts of the eye to short-wave light, has been known and recognized from very early times and, in one of its commoner forms, is known as "snow blindness." It may be accepted that wavelengths from 3140 Å to 2500 Å are capable of setting up a photophthalmic reaction but the threshold intensity of energy required varies considerably with the wavelengths (177). It has been found that the most effective band is about 2880 Å. The threshold of energy necessary to excite the photophthalmic reaction in the rabbit when the eye is irradiated with the entire ultraviolet spectrum is of the order of 2 million ergs per second per sq. cm. The human eye is more sensitive than that of the rabbit and, in man, there are considerable variations -- blonde people being more readily affected than dark. The earliest sign of photophthalmia, after a latent period, is a granular appearance of the cornea. The granules are round and of the order of size of an epithelial cell, at first few in number and centrally distributed but ultimately forming a stippled homogeneous mosaic. The granules probably represent swollen epithelial cells. With greater exposure there may be vesicle formation, accompanied by hyperemia of the conjunctiva, and some discharge. After still greater exposure the central corneal epithelium is exfoliated with surrounding areas showing a heaped-up wall several layers in thickness in which mitosis is rarely seen. With heavy exposures there may be a marked central opacity with fairly sharply defined margins. A generalized diffuse cloudiness occurs only with radiation of such intensity that it penetrates the cornea in quantities sufficient to produce a reaction in the endothelium. The conjunctiva shows a considerable amount of inflammation with marked swelling and edema, associated with muco-purulent discharge and sometimes ecchymoses. Changes reach their height in 48 hours and thereafter gradually subside, the rate of recovery varying directly with the intensity of the radiation. In 3 to 5 days after a moderately severe exposure the epithelium has reformed and on clinical examination the eye appears normal. Solar photophthalmia (snow blindness) is the most widespread clinical manifestation of damage to the outer eye by ultraviolet light. It is not characteristic of the tropics since in many of these areas absorption by the heavy atmospheric concentration with moisture or dust keeps the ultraviolet content of sunlight at a low level. Rather the condition is seen in clear atmosphere of high altitudes where ultraviolet content of sunlight may rise from 1 or 2% at sea level to 5 or even 6%. Owing to irregular dispersion of the short waves, radiation from the sky contains from 2 to 4 times as much ultraviolet shorter than 3360 Å as direct sunlight. Since the liminal energy threshold is considerable, very short exposure is relatively harmless but it is to be remembered that short exposures are additive within a period of 24 hours and are therefore cumulatively dangerous.

Experimental lenticular opacities can be produced by ultraviolet rays only if the dosage is so massive as to induce

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gross pathological changes in the cornea and damage to the iris (177). Histologically, in the substance of the lens, degeneration of the superficial fibers can be seen associated with eosinophilic degeneration. In the capsular epithelium the changes are often more marked. There is complete absence of mitosis, eosinophilic degeneration, fragmentation of the nuclei, and eventual disintegration of the cells in the exposed papillary area while, just under the iris at the margin of this area, these cells may proliferate actively to form a heaped-up "epithelial wall" without evidence of great mitotic activity. Apart from histological changes, chemical alterations occur in the lens irradiated with ultraviolet light. The isolated lens thus radiated rapidly turns opaque and its proteins are denatured so that their subsequent coagulation by other influences such as an excess of calcium salts, is rendered easier. In view of the difficulty of experimental production of cataract with ultraviolet radiation, it is understandable that any direct relationship between the occurrence of senile cataract and the normal incidence of sunlight is questionable. A certain amount of evidence, none of it conclusive, can be brought forward to associate senile cataract with those long ultraviolet rays in sunlight which can penetrate to, and are absorbed by, the lens.

Since the radiation reaching the posterior segment of the normal eye begins to be cut off sharply at about 4000 Å and can persist in very small quantities only to the region of 3200 Å, it would not be expected that any abiotic effects would be observed in the retina. Even in the eye without a lens, the sensitivity of which is almost 1000 times greater than normal in the near ultraviolet at 3650 Å, the transmission of long ultraviolet ceases at 3130 Å and, since the upper limit of abiotic activity is about 3050 Å, it would seem to follow that an abiotic lesion could not occur even in the absence of the lens. It is interesting that, after irradiation of eyes of animals with filtered ultraviolet, histologic changes can be observed over a wide area of the retina in the posterior pole, consisting essentially of chromotolysis and acidophil tendency in the ganglion cells and the inner nuclear layer (177). From the clinical point of view some functional impairment of vision has been reported after exposure to long ultraviolet radiation, defects which have been more obviously noted in lensless eyes -- color or central scotomata, an impairment of dark-adaptation, and a lowering of the differential sensitivity of both rod and cone vision in both the central and peripheral areas of the retina. These findings have been indefinite and the occurrence of ill effects due specifically to ultraviolet radiation has not been generally corroborated in experimental conditions. "On the whole it is probably safe to say that the ultraviolet radiations which might harm the retina do not reach it, and that those radiations of the spectral region which do reach it have not been shown to do organic or functional harm of any practical importance to this tissue." Blum (1) has said, "There seems every reason to regard photophthalmia as

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sunburn of the eye. Unlike sunburn of the skin, however, it does not confer protection against subsequent exposure. The condition results frequently from exposure to artificial sources of radiation, particularly welding arcs, but may also occur as the result of prolonged exposure to intense sunlight." Blum (1) pointed out, further, that about half the energy of sunlight reaching the retina is perceived by the eye and about half lies in the infrared, the ultraviolet portion being negligible. He, like Duke-Elder (177), considered it highly improbable that ultraviolet light shorter than 3200 Å reaches the retina in sufficient quantity to have deleterious effects, since it is absorbed so strongly by the proteins of the ocular media.

Cogan (178) has also discussed lesions of the eye resulting from radiant energy and has, on the whole, agreed with the conclusions of Duke-Elder and Blum as referred to above. He has noted that keratitis, resulting from ultraviolet radiation of the sun, occasionally occurs with prolonged exposures at high altitudes. The dose necessary to elicit a threshold reaction in the cornea (of rabbits) is of order of 2 times 10^6 ergs when the whole ultraviolet portion of the spectrum is used and 0.15 times 10^6 ergs when the relatively monochromatic band at the peak sensitivity is used. Symptoms come on after a latent period of 5 to 12 hours, the subject having had no awareness that he was being exposed to harmful radiation at the time of exposure. Cogan also noted that effects are cumulative during an interval of 24 hours and that, unlike the skin reaction, no tolerance is acquired by repeated exposures. The wave band responsible for keratitis or the so-called action spectrum of ultraviolet keratitis, has a sharp peak at 2880 Å, falling off to approximately zero at 3050 Å on the long end and reaching a minimum at 2600 Å on the short end. The sensitivity curve has the same shape as that for erythema of the skin but the peak of the latter is shifted somewhat toward the longer end, probably owing to the differential scattering effect of the superficial layer (keratinized) of the skin. Cogan noted that the lens is peculiarly susceptible to radiant energy. He pointed out that the lens is a unique structure in that it is not only avascular but also is removed by several mm. from blood vessels. This would have the effect, so far as radiation is concerned, of making the lens less effective in dissipating heat as compared with tissues elsewhere in the body. The lens also has a peculiar method of growth in that the actively metabolizing part of the lens (epithelium) is present only beneath the anterior capsule while the lens fibers which are derived from the epithelial cells and make up the bulk of the lens, extend from the equator toward the anterior and posterior poles. Genetic effect in the lens epithelium might therefore be expected to become clinically manifest in the lens fibers only when these cells have reached the equator. Such an explanation may account for the latent period but the locus of the opacity at the posterior pole or, less often, at the anterior pole, does not appear to have any

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obvious explanation. Cogan (178), in agreement with other investigators, states that ultraviolet has not been shown to produce any organic lesion of the retina. Earlier claims that ultraviolet radiation caused macular degeneration has not been corroborated. The long ultraviolet rays (4000 to 3200 Å) are said to induce transient inhibition of dark-adaptation but, if true, this action is of no great practical importance. "It seems unlikely that any harmful effects could result to the retina without complete destruction of the anterior portion of the eye." Kutscher (179) has discussed the ocular effects of radiant energy and has agreed with the observations and conclusions of Duke-Elder, Blum and Cogan. He has noted that in thermal burns (resulting from long ultraviolet wavelengths), the cornea reacts throughout its entire area with the greatest effect in the posterior regions whereas, with short ultraviolet the reaction is most intense in the central region of the anterior surface. In the opinion of Kutscher, the retina may be damaged by ultraviolet rays in the aphakic eye.

Wald (180) maintains that there is justification for excluding the near ultraviolet from the aphakic eye. The yellow pigmentation of the human lens serves to exclude from the retina a band of intrinsically visible ultraviolet radiation for which the eye has a particularly high chromatic aberration. The human lens is not only a lens but an efficient cutoff filter. When this is replaced in aphakics by a clear glass lens, the retina is exposed to radiation which it can see but which is not in good focus. It is very possible that if aphakics were given a lens resembling in transmission the human lens, the attendant loss in brightness might be more than compensated for by an improvement in visual acuity. Wald concluded that when the eye is subjected to strong sources of ultraviolet radiation, a filter which excludes wavelengths shorter than about 3150 Å can protect the cornea and conjunctiva from damage and a filter which excludes wavelengths shorter than 4000 Å can prevent the lens from fluorescing. In the aphakic eye the latter type of filter may improve also visual acuity by keeping from the retina radiations for which the chromatic aberration of the eye is very high, which are normally excluded by the pigmentation of the human lens.

The effects of ultraviolet irradiation on the corneal epithelium subjected to monochromatic radiation, with particular reference to inhibition of mitosis, nuclear fragmentation, and loosening of the epithelium, have been studied by Friedenwald, Buschke, Crowell and Hollaender (181). The three phenomena compared occur at different levels in the corneal epithelium. Nuclear fragmentation after exposure to ultraviolet radiation occurs almost exclusively in the superficial layers of the corneal epithelium. Normal mitotic activity, on the other hand, is confined to basal cell layers and this is consequently the layer at which inhibition of mitosis occurs. Loosening of the corneal epithelium occurs at the boundary between epithelium and

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underlying connective tissue. If it is assumed that the photochemical reaction for each of these effects occurs at the place where the effects are subsequently found, then the intensity of the incident radiation must be corrected for the absorption of the overlying layers. The results of the experiments indicated that upwards of 50 times as much of the incident radiation reached into the bottom of the epithelial layer with wavelength 2900 Å as with wavelength 2400 Å. Correction of the various factors influencing the absorption of ultraviolet light by the cornea and application of these factors to the results obtained, produced curves which showed that mitosis inhibition, epithelial loosening and nuclear fragmentation all reached a peak at a wavelength of 2800 Å. The fact that all three curves reached a high level at wavelength 2800 Å suggested that the absorbing substances might be protein or nucleoprotein in nature.

Cogan and Kinsey (173) have contributed to the mass of experimental evidence regarding the action spectrum of keratitis produced by ultraviolet radiation. They exposed the eyes of rabbits to separate bands of ultraviolet radiation at energy levels having a sufficient spread to determine the threshold dose necessary to produce keratitis. The amount of energy that any one wavelength, just necessary to produce a corneal change that was visible with the biomicroscope, was considered the threshold dose for that wavelength. The cornea was found to have a peak sensitivity to ultraviolet radiation at wavelengths of about 2880 Å, with a sharp decline in sensitivity to either side of the peak. The amount of energy necessary to elicit an ocular reaction at 2880 Å was approximately 0.15 times 10^6 ergs per sq. cm. Although the absorption peak of the corneal epithelium corresponded to that of nucleoprotein (2650 Å), the peak of the action spectrum corresponded more nearly to the absorption peak of albumin and globulin (2800 Å). They inferred, therefore, that the photochemical reaction in the cornea is due not to an indiscriminate absorption by nucleoprotein but rather to a selective absorption by a substance having a peak in a wavelength longer than that of nucleoprotein or by certain constituents only of the nucleoprotein molecule. The photosensitive substance in skin responsible for erythema after ultraviolet irradiation has been assumed to be protein in nature and the lack of correspondence between the action spectrum of erythema and the absorption curve of the skin has been assumed to be due to the absorption and differential scattering effects of the superficial, inert layer of the skin. Diminishing the scattering effect of the superficial layer by suitable clearing agents resulted in an absorption curve for the whole epidermis similar to that for protein (serum albumin). Presumably, if it were possible to eliminate the effect of the superficial inert layer of the skin, the erythema curve would be shifted toward the shorter wavelength to have a peak sensitivity similar to that of protein at about 2800 Å. In the case of skin, therefore, there is reasonably good correlation between absorption by the

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epidermis and the erythema reaction. The cornea has no superficial layer corresponding to that in the skin. It is interesting, therefore, to find that the peak of the action spectrum for keratitis corresponds approximately to that of the "corrected" erythema spectrum. But, whereas the corrected erythema spectrum corresponds approximately to the corrected absorption curve for skin, both having peaks at about 2800 Å, the keratitis peak does not correspond to the absorption peak of the corneal epithelium. While all the values for erythema spectrum are compatible with assumption that ultraviolet radiation causes a general protein denaturation in the skin, absorption and effect coinciding, the values for the cornea indicate a selective effect at wavelengths other than those which are absorbed maximally by the cornea. The fact that the maximum absorption of the cornea occurs at 2650 Å suggests that nucleoprotein is chiefly responsible for the absorption whereas keratitis occurs with a maximum that more nearly corresponds to that of the cytoplasmic proteins, albumin and globulin.

In view of the more recent investigations referred to above, it is of some interest to note that Martin (182) conducted experiments to determine whether the lens was affected by ultraviolet radiation. His results are of interest because they have not been materially challenged by the majority of subsequent investigations. He utilized rabbits and guinea pigs and his results were as follows: In a group exposed for 1 to 3 hours weekly at a distance of 3 feet for 3 to 9 months there was slight chronic conjunctivitis, the media were clear, and the anterior lens capsule was normal onmicroscopic examination; in animals exposed for 1 hour every 10 days at 4 feet for 3 to 12 months, there was marked chronic conjunctivitis with thickening of the lids and slight ectropion with moderate corneal but no lenticular opacity; in four rabbits exposed for 3 hours every 2 weeks at 4 inches for 3, 9, 10, and 11 months respectively, there was chronic conjunctivitis with marked ectropion, dense opacity of the cornea which had undergone vascularization but the lens was clear and its anterior capsule normal. With one exception, which was described in considerable detail and in which there was a beginning cataract of the lens, the results of repeated exposure to the Kromayer lamp failed to produce changes in the lens. The pathological action of light on the lens was discussed by Duke-Elder (183). He said, "It is with the absorbed portions of incident energy that we are primarily interested in the consideration of its possible pathological effects upon the lens since only these can exert an effect, deleterious or otherwise, upon it. In the average normal adult a large proportion of the rays between 14,000 and 11,000 Å and between 4000 and 3200 Å and practically all the rays between 3200 and 3000 Å are absorbed. Those of the first two spectral regions will produce a potentially pathological thermal effect while those of the third will produce an abiotic effect as well as a mild thermal one. The fact that abiotically active rays

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are so absorbed has been indicated by the fact that exposure to ultraviolet which in the normal rabbit left the retina unaffected, produced typical abiotic changes in the retina of an aphakic rabbit. Abiotic effects on the lens are not obtained unless a critical exposure is given because, with great intensities of radiation, the cornea turns opaque and protects the underlying structures. After short exposures swelling of the cells is the only change noted and further intensities involve the appearance of the typical eosinophilic and basophilic cells which are ordinarily associated with abiotic reactions. More prolonged and intense exposures induce nuclear pyknosis. Around the periphery of the exposed area, just underneath the papillary margin, there is a zone of cellular proliferation surrounded by an area of marked karyokinetic activity, all this in the lens capsule. In all probability the changes are due to stimulation of cellular activity by the heat which is absorbed in quantity by the iris pigment, which in this region comes into direct contact with the lens capsule."

Duke-Elder and Duke-Elder (184) wrote, "Although they seem to have almost invariably eluded the observations of previous workers, changes in the retina can be demonstrated after radiations with short wavelengths in such intensities as were employed in these (their) experiments." They noted that Birch-Hirschfeld (1904) seemed to have been the only one to have described such changes, his results and conclusions never having been confirmed but frequently having been denied. In the experiments described by Duke-Elder and Duke-Elder the most marked changes were evident 8 to 20 hours after radiation. The abiotic changes they found in the retina affected mainly the ganglion cells and the internuclear layers. The changes consisted of chromotolysis and an acidophil tendency. They concluded that the changes were rather of the nature of a pathological intensification of physiological processes of vision than a direct abiotic response although the occurrence of the latter in a specially sensitized tissue is not altogether impossible. They suggested an analogy between the nuclear appearances of abiotically traumatized tissues and inclusion bodies described as occurring in the lesions caused by herpetic and other viruses and possibly in trachoma. The analogy supported the opinion that the appearances were degenerative in nature and nonspecific in origin.

Burge (185, 186, 187) carried out a series of experiments designed to test the hypothesis that certain salts may modify lens proteins in such a way that they are precipitated by ultraviolet light. The evidence which he accumulated, indicated that potassium salts are not involved in the production of nuclear cataracts but, on the other hand, there were definite indications that calcium, magnesium and silicate may play an important role in the production of cataracts in that these substances do modify the lens protein in such a way that ultraviolet radiation can precipitate it. Another interesting

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observation by Burge concerned cataracts as a complication of diabetes. In this disease sugar is increased in the blood and body fluids and presumably in the eye media. The increase of sugar is not of sufficient magnitude to produce opacity of the lens by itself so, according to Burge, there must be another factor involved. His experiments showing the effect of ultraviolet radiation on the protein of the lens modified by sugar would suggest that ultraviolet radiation is the other factor. The normal lens absorbs wavelengths between 3500 and 3000 Å and transmits longer than these. These absorbed short wavelengths do not normally produce opacity in the lens. Burge's experiments showed that very weak solutions of sugar can modify the protein of the lens so that the absorbed short wavelengths are able to precipitate the protein. The assumption that might be made in the case of diabetic cataract is that the sugar present in the humors of the eye modifies the lens proteins so that the short wavelengths can bring about precipitation.

A unique investigation by Petrovich (188) was designed to determine the effect of ultraviolet irradiation on the permeability to protein, radio calcium, and radio phosphorous of the capillaries of the anterior portion of the eye. Experimental "electro-ophthalmia" was produced by irradiating the left eye for 30 minutes with a mercury vapor lamp. The distance from the light source to the eye was 65 cm. or 40 cm., depending upon the intensity of the two light sources used. The right eye was shielded from the light of the lamps. The results were as follows: Conjunctivitis and blepharopasm were observed on the next day and also 2 to 3 days after irradiation. These sequelae were no longer visible after 1 to 3 weeks. The protein content of the aqueous humor of the irradiated eye rose abruptly after 1 to 3 days. Whereas the protein content of aqueous of control animals varied from 39 to 84 milligrams percent, the range after irradiation was 200 to 1840 milligrams percent (but it should be noted that there was only one determination in the range of 1840 with the next highest determination being 520). At the stage of maximum rise in protein content (1 to 3 days) the aqueous humor of the affected eye, as the result of intravenous injection of radioactive isotopes, showed a higher concentration of the isotopes than did the unaffected eye. The mean calcium 45 content on the irradiated side was 136.8 impulses per minute as compared with only 101.4 impulses per minute on the opposite side. The corresponding values for P^{32} were 75.2 and 51.2 impulses per minute respectively. The experimental findings were considered to be evidence of increased capillary permeability for all the substances examined. The ascorbic acid level fell in many cases to less than half the value found for aqueous humor of the intact eye at the height of the rise in capillary permeability. The results were checked by using the same quartz mercury vapor lamp with the interposition of a glass filter which eliminated all radiation of wavelengths less than 3340 Å. The animals in which the filter was interposed

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showed no changes in protein content of the aqueous humor nor in ascorbic acid levels such as were found in those eyes exposed to the ultraviolet wavelengths. Ultraviolet irradiation of tissues "leads to release of histamine and histamine-like substances. The increased permeability of the capillaries and of the anterior chamber observed in these experiments may depend upon the formation of such biologically active products. This view is supported by the observation that repeated subconjunctival injections of histamine lead to a raised protein content of the aqueous humor with a simultaneous fall in its vitamin C content."

Munich (189) investigated the ultraviolet absorption of normal aqueous humor and that of aqueous humor altered by inflammatory processes. The absorption by human aqueous humor was examined with respect to ultraviolet light within the range of 2200 to 3200 Å before and after being incubated at 37 degrees C. as well as before and after addition of yeast-nucleic acid. The normal human aqueous humor showed an ultraviolet absorption curve similar to that which can be observed in all fluids containing proteins. Aqueous humor derived from hypopyon iritis showed a marked maximum of absorption at 2800 Å which increased after incubation at 37 degrees C. Addition of yeast-nucleic acid to the aqueous humor derived from hypopyon iritis resulted in the appearance of maximal absorption at 2600 Å. An increase of this maximum occurred with incubation at 37 degrees C. It was deduced from these experiments that enzymes which could be of importance in removing cellular elements play an important part in aqueous humor.

The most positive statements concerning damage to the retina by ultraviolet light have been made by Kahnemann (190). In a previous article Kahnemann described twelve cases of exudative retinitis in the majority of which it was possible to find in the patients' histories a factor of strong sensitivities to light. The hypothesis was advanced that in these cases retinal edema was caused by ultraviolet rays. In the subsequent report Kahnemann described experiments with two anthropomorphic monkeys. The animals were blinded with arc lamps, excluding certain wavelengths and limiting the strength in order to damage the cornea as little as possible. The eye was atropinized and the time of exposure was 20 minutes. On the day following exposure to the arc lamp there was ophthalmoscopic evidence of slight edema at the posterior pole of the eye. One eye was protected from the light and used as a control. Histological examination of the posterior segments of the exposed eye revealed changes as follows: The choroid, which is characteristically intensely pigmented in monkeys, appeared somewhat thicker in the area of the posterior pole and the blood vessels were dilated and thickly filled with red corpuscles. There were no infiltrative processes of any kind nor were there any abnormal cellular elements in this stroma. The retina appeared normal at the periphery while, in

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the region of the macula and around the papilla there were multiple changes which were most accentuated toward the posterior pole, reaching their high point in the area of the fovea. In the peri-foveal region the most notable observation was a thickening of the external plexiform layer through dissociation of the Henle fibers which assumed a sinuous appearance. Beyond the limits of the macula, where the Henle fibers give way to the fibrillar reticulum which constitutes the external plexiform layer, there was a clear area between the external granules of the layer and the rods and cones which apparently resulted from fragmentation of the fibrils. The fragmentation was thought to be due to marked edema which expanded the fibrils beyond their elasticity and caused them to break. In the more peripheral retina where the edema was not present, the plexiform layer appeared normal. Small vessels at the periphery of the macula were moderately dilated and packed with red corpuscles. On the medial side of the fovea a thickening of the retina appeared to be due to a greater density of Henle fibers while, on the temporal side, the greater thickening of the retina was caused by accumulation of cone cells almost to the point of forming a nodule; this caused a marked asymmetry between the two halves of the foveal framework. Edematous inhibition, presenting as a collection of droplets interposed between the internal segments of the individual cones led to a situation such that these elements were represented only by a palisade made up of the external processes. The alterations found in the retina were considered to be due to a process of intense edema manifested especially in the area of the external plexiform layer. The presence of edema tended to separate the stratum of visual cells from the internal granular layer. When this collection of liquid is excessive, the fibers are stretched to the point that they break. The complex changes noted in the retina are "no doubt" produced by intense concentration of ultra-violet rays on the retina at the posterior pole of the eye. A second monkey was blinded and killed after 20 minutes. The findings observed were essentially the same as in the first monkey. In discussing his observations Kahnemann (190) said: "The first thing to consider is there were no corneal and no conjunctival lesions and no lesions of the lens." He went on to say that the retina of the monkey is not in any way dissimilar to the retina of the human so that it is easy to argue that both the monkey and man use central vision for the daily necessities of their life, a secondary factor for other animals. Because of this it follows that the luminous rays are concentrated on the macula and fovea which is the part most affected. The author maintains that the most important finding in his work was the genesis of retinal edema by ultraviolet rays.

Ocular lesions following the atomic bombing of Hiroshima and Nagasaki have been reported by Flick (191), Cogan et al (192), and Benkwith (193). Flick found that ocular lesions associated with, or secondary to, irradiation were limited to the retina and were found in 46 cases in a total of about 400.

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Of the patients showing retinal lesions, 75% had one or more other clinical symptoms of irradiation at the time the study was made. The types of hemorrhage included flame hemorrhage situated in the nerve fiber layer of the retina, preretinal hemorrhage in which the blood had leaked into the subhyaloid space producing an elevated rounded hematoma immediately adjacent to, but not invading, the vitreous, vitreous hemorrhage, exudation into the retina taking the form of round, snow white, slightly elevated lesions obscuring the retinal vessels. Dozens of eyes were examined with the pupil dilated but no lens opacities were observed. It must be remembered, however, that there is a long latent period between lens injury and the development of cataracts; these patients were examined in 1945, months after their exposure to irradiation. At no time was a view of the fundus hampered by opacities of the media that could be localized in the lens. It was concluded by Flick (191) that the ocular lesions described were directly related to the deficiency in blood elements and in no way directly related to the action of radiant energy on the eye. The lesions were observed to disappear in those individuals recovering from the hematopoietic depression with no residual damage to the eye. It was felt that this healing occurred in all patients who survived the irradiation and that no permanent residual damage resulted from the retinal lesions. "As to the question of radiation damage to the lens, time alone will tell." It should be noted that Flick made no attempt to differentiate between ocular effects of ionizing and ultraviolet irradiation. Cogan, Martin, Kimura and Ikui (192) conducted an ophthalmalogic survey of atomic bomb survivors in Japan in 1949, 4 years after the atomic explosions. They stated that radiations were of four types -- visible rays, ultraviolet rays, gamma rays, and neutrons -- and that injuries to the eyes were theoretically attributable to any or all of these. They considered that the only significant source was direct radiation from the bomb at the time of the explosion; induced radiation on the ground or deposition of fission products were insufficient to have had a harmful effect. It was not clear what effect the visible and ultraviolet radiation had on the body as a whole but reference is made to possible ocular effects. It is commonly stated that the spectrum of electromagnetic radiation resulting from the bomb had the same general distribution as that of the sun. Keratoconjunctivitis lasting only a few days was presumed to be due to ultraviolet radiation. The number of patients who gave a history of bilateral keratoconjunctivitis within a few days or a few weeks after the atomic bombing, not obviously caused by trauma or burns, was 56. Of these 42 gave a history of keratitis coming on within the first day and lasting for several hours or several days. These patients were 1200 to 2000 meters from the hypocenter and in none were there permanent sequelae. Discounting a few who believed they had symptoms for as long as a month, the cause of this early and temporary keratitis was almost surely ultraviolet radiation. A similar history of keratitis is known to result from exposure to other sources

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of ultraviolet radiation. Presumably there were many more with keratoconjunctivitis from ultraviolet radiation in whom the ocular symptoms were masked by thermal injuries. Benkwith (193) reported retinal hemorrhage in an atomic bomb casualty. The subject was 1 mile from ground zero at the time of the explosion. She had a leucopenia of 2200 white blood cells, prolonged bleeding time, decreased platelets, and 2.2 million red blood cells. Very little evidence of pathologic change was visible after 4 months. The patient's general physical condition showed improvement which coincided with the improved ocular findings. In this case the retinal hemorrhages appeared to be associated with ionizing radiation resulting in depression of the blood forming organs rather than to ultraviolet radiation. Hollaender (2) has noted that the eye per se is quite resistant to x-ray exposure. The effects of ultraviolet on the eye are usually the first to be noted (on prolonged exposure) because radiation of 2537 Å is considerably less effective in the production of erythema than are longer ultraviolet wavelengths.

Ultraviolet radiation of wavelengths less than 3200 Å has been found to induce neoplasms and other lesions of the eyes of mice (194). The pathologic changes are relatively superficial, being confined primarily to the cornea. Hyperplasia of epithelial and connective tissue elements and increased vascularization of the cornea were observed. The iris and lens appeared to be involved only secondarily. The tumors observed were sarcomas and hemangio-endotheliomas of the substantia propria. Changes in the epithelium of the cornea suggest that carcinomas may occur at times. Vascularization of the cornea was not considered to be a specific response to ultraviolet radiation. The same phenomenon has been observed in vitamin A deficient rate and in rats and mice with riboflavin deficiencies; in all of these deficient animals extensive growth of vessels from the limbus was observed. It has been suggested that the vascularization results from local asphyxia in the case of these vitamin deficiencies but it seems improbable that this is the cause in the ultraviolet radiated animals. Products resulting from destruction of cell constituents including histamine-like substances are usually formed when tissues are exposed to ultraviolet radiation and these may provide the stimulus to vascularization in these cases, rather than asphyxia. As a result of continued exposures to ultraviolet radiation all stages of transition from single focal non-neoplastic vascularization to true hemangio-endotheliomas have been observed. Huldschinsky (195) reported the production of sarcomas of the eye in all of a group of five rats irradiated with a quartz lamp for 2 hours daily for almost 1 year. All these tumors appeared to have their origin in the cornea. Huldschinsky noted that the quartz lamp had its greatest irradiation in the neighborhood of 2700 Å. The sarcomas which were produced were described as being of the single-cell type. Ash and Wilder (196) studied a series of 93 epithelial tumors arising at or near the limbus in humans. Eighty-three of the

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patients were white persons and only 10 were Negroes. Twenty-six of the 93 patients were from Texas and the remainder, including 15 from Tennessee, were distributed among 25 other states, the Panama Canal Zone, and Hawaii. Although it was thought by the authors that conclusions on the basis of geographic distribution were not justified because their largest number of biopsies of ocular adnexae came from Texas and Tennessee, one gains the impression that such tumors are particularly prone to occur in those areas where there is a relatively high percent of ultraviolet in sunlight.

Kelner and Taft (118) irradiated mice 5 times per week for a total of 280 to 297 days. The mice were then observed for 145 to 157 days longer without further irradiation. The total dose of 2537 Å ultraviolet given the animals over the entire experiment was approximately 1.24 times 10^{10} ergs per sq. cm. For the first 24 weeks the intensity was approximately 1.5 times 10^{4} ergs per sq. cm. per second for a weekly dose of 1 times 10^{8} ergs per sq. cm. When no signs of tumors had appeared after the first 24 weeks of irradiation, the intensity was increased to approximately 3 times 10^{4} ergs per sq. cm. per second and the weekly dose to approximately 5.35 times 10^{8} ergs per sq. cm. for the remainder of the irradiation period. In the group irradiated with 2800 to 3100 Å ultraviolet light the minimal intensity and weekly dosage was used in the beginning but, after 142 days when the animals had received a total of 5.7 times 10^{8} ergs per sq. cm. without appearance of tumors, the dose for the remainder of the experiment was increased to 1.75 times 10^{8} ergs per sq. cm. per week. The total dose in this group was about 3.8 times 10^{9} ergs per sq. cm. For photoreactivation, the animals to be photoreactivated were, after each daily ultraviolet dose, immediately subjected for an hour to as intense reactivating light as they could tolerate. The reactivating light source was a General Electric mercury arc supplemented by three 15 watt blue fluorescent bulbs and two 500 watt tungsten lamps. Soon after irradiation with wavelength 2537 Å, the eyes of the rats became irritated. The corneas became dull, conjunctivitis developed, followed by ulcerations, and later opacities were apparent. Animals kept in the dark and those exposed to reactivating light appeared similar except that the eye defects in the light animals were less severe. With increased dosage eye damage became more severe. Eye reactions were less severe in the 2800 to 3100 Å group than in the 2537 Å groups and there was little obvious difference between those kept in the dark and those exposed to reactivating light. The typical ocular lesion in the 2800 to 3100 Å group was a tightly closed eye with serious exudation. With increased dosage of the 2800 to 3100 Å wavelengths, eye lesions became worse and were definitely worse in the group exposed to reactivating light than in those kept in the dark. All tumors produced in these experiments occurred in the ear except for the following eye tumors: a carcinoma in an animal irradiated with 2537 Å light and kept in the dark; a carcinoma in a 2800 to 3100 Å animal kept in

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the dark; and a tumor, not microscopically studied, in a second 2800 to 3100 Å animal kept in the dark. No eye tumors were found in the light-reactivated group.

The photosensitivity of visual purple and the scotopic sensitivity of the eye in the ultraviolet have been investigated by Goodeve, Lythgoe and Schneider (197). The rate of bleaching of visual purple solutions at any particular wavelength is governed by the photosensitivity. The photosensitivity of visual purple corresponds to the scotopic sensitivity of the eye on the red side of the spectrum but on the blue side, the scotopic sensitivity is less than the photosensitivity. This discrepancy has been attributed to the presence of yellow substances in the eye which absorb light and thus reduce the intensity of the light absorbed by the visual purple. Hosoya (198) found that visual purple is sensitive to ultraviolet light of wavelengths 3650, 3130, 3020, and 2970 Å. Visual purple is defined as a light sensitive chromophore with a maximum extinction at 5020 Å. The absorption at 3650 Å and again at 2540 Å may be due to the same chromophore as that at 5020 Å. On the other hand, a light quantum responsible for bleaching by the latter wavelength may be absorbed by a neighboring or even an entirely independent chromophore which then acts as a photosensitizer, destroying the visual purple chromophore. From the experiments described by Goodeve et al, it appears that light of any wavelength down to 2540 Å causes the same photochemical change in visual purple and indicates, therefore, that the retina is sensitive to this range of ultraviolet light. Because it is known that there is a sharp limit of vision between 3020 and 3130 Å, attributed to the absorption of the crystalline lens, and because preliminary experiments showed that the relative sensitivity of the eye to 3650 Å was very much less than would be expected from the photosensitivity of visual purple at this wavelength, experiments were undertaken to obtain quantitative information. Interest in these measurements was heightened by the observation that aphakic eyes are more sensitive to ultraviolet than ordinary eyes. It was found that the relative scotopic sensitivity of the aphakic eye at 3650 Å is that to be expected from the photosensitivity of visual purple. This indicates that in vivo as in vitro the photochemical process is the same in the ultraviolet as in the visible part of the spectrum and emphasizes the fact that a photochemical change is the initiating process in vision. The simplest explanation for the greatly reduced sensitivity of normal eyes to ultraviolet is that there is present in normal eyes a substance which strongly absorbs the ultraviolet but which is not present in the aphakic eye. The presence of a yellow pigment in another part of the eye (lens) must therefore be assumed. Goodeve et al (197) concluded that vision in the normal eye is limited by the sharp rise in the absorption of the lens whereas vision in the aphakic eye is limited by that of the cornea. The absorption of the lens and of the cornea are due to proteins which make up their structure. "Therefore,

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although visual purple is sensitive to 2540 Å, vision at that wavelength is not possible."

Recommendations for Further Study

There should be more definitive investigation of the effects of whole body irradiation, exclusive of the tumor-induction effects. The literature implies that there are many possible generalized effects of ultraviolet irradiation but these have been studied relatively little and the many theories which have been expounded seem to be based on little real information. Of particular interest are the studies on consensual blood vessel reactions after combined ultraviolet-infrared radiation by Schulz and Amelung (17). These authors assumed that ultraviolet radiation or ultraviolet radiation combined with infrared irradiation was responsible for the production or mobilization of a vaso-active substance in one part of the body which resulted in a generalized effect. Further work is indicated to rule out infrared effects. It is particularly interesting that this article was published last year. If there is in fact a mobilization of a "vaso-active" substance, whose histamine-like character has been repeatedly supported and denied, an attempt should certainly be made to determine its nature, chemically and pharmacologically.

There appears to be no real light microscopic evidence of injury to the retina by ultraviolet irradiation, although Kahnemann (190) presented what he considered to be very conclusive evidence of such injury. Kahnemann's method of application of ultraviolet light to the eye of the monkey was poorly controlled and probably resulted in a thermal effect, rather than a radiation effect. The fact that ultraviolet irradiation results in no injury to the retina has been attributed to the absorption of the abiotic wavelengths by the cornea and the lens. Electron microscopic studies of the retina following irradiation with carefully controlled wavelengths in the ultraviolet spectrum may provide information which is not available through use of light microscopy. Electron microscopic studies of the cornea and of the lens following ultraviolet irradiation may provide information which has not been available to the use of light microscopy. Inasmuch as the iris and ciliary body are absorbers of infrared radiation and, possibly, of ultraviolet irradiation as well, electron microscopic studies of them might prove to be productive.

Hemoglobin is an absorber of ultraviolet irradiation, as are other components of the blood. There have been no definitive investigations of possible hematopoietic effects. Such effects would certainly not be due to penetration of ultraviolet

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irradiation to the bone marrow, but might be secondary to a relatively superficial effect upon blood elements, followed by secondary systemic effects.

The work of Sarachek and Townsend (16) on the disruption of mitochondria by ultraviolet irradiation is highly suggestive. Definitive electron microscopic studies of mitochondria, the granular and agranular reticulum of epidermal cells and of the cells of the cornea, following ultraviolet irradiation, might be productive of information which has not been obtainable through the use of light microscopic studies. These cytoplasmic constituents have been studied extensively with the electron microscope in recent years. There seems to have been no attempt to determine whether there are constant modifications of them by ultraviolet irradiation. Cellular metabolism may well be modified by ultraviolet irradiation and such metabolic modifications might be traced to the mitochondria within which the respiratory enzymes of the cell are located.

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BIBLIOGRAPHY

1. Blum, H. F.: "The physiological effects of sunlight on man." Physiol. Rev., 25, 483-530, 1945.

2. Hollaender, A.: "Effects of ultraviolet radiation." Ann. Rev. Physiol., 8, 1-16, 1946.

3. Hollaender, A. and Oliphant, J. W.: "The inactivating effects of monochromatic ultraviolet radiation on influence of virus." J. Bact., 48, 447-454, 1944.

4. Giese, A. C.: "Ultraviolet radiations and life." Physiol. Zool., 18, 223-250, 1945.

5. Swanson, C. P.: "A comparison of chromosomal aberrations induced by x-ray and ultraviolet radiation." Proc. Nat. Acad. Sci., 26, 366-373, 1940.

6. Hill, L.: "Action of ultraviolet rays." Brit. J. Phys. Med., 9, 7, 1934.

7. Dobson, R. L. and Lawrence, J. H.: "Physiological effects of radiant energy." Ann. Rev. Physiol., 10, 479-500, 1948.

8. Thomson, M. L.: "Delayed effects of ultraviolet burns in man." Lancet, 1, 1347-1348, 1951.

9. Brumberg, E. M. and Larionow, L. T.: "Ultraviolet absorption in living and dead cells." Nature, 158, 664-664, 1946.

10. Clark, J. H.: "The temperature coefficient of the production of erythema by ultraviolet radiation." Am. J. Hyg., 24, 334-342, 1936.

11. Giese, A. C.: "Protozoa in photobiological research." Physiol. Zool., 26, 1-22, 1953.

12. Weinstein, I.: "Quantative biological effects of monochromatic ultraviolet light." J. Opt. Soc. Amer., 20, 433-456, 1930.

13. Giese, A. C. and Leighton, P. A.: "Quantative studies on the photolethal effects of quartz ultraviolet radiation on paramecium." J. Gen. Phys., 18, 557-571, 1935.

14. Kline, B. E. and Rusch, H. P.: "Effects of prolonged irradiation of mice with fluorescent light." Proc. Soc. Exp. Biol. Med., 60, 254-255, 1945.

Approved for Public Release

Contrails

15. Montgomery, P. O'B. and Bonner, W. A.: "A 'flying-spot' microscope." Scientific American, May 1958.

16. Sarachek, A. and Townsend, G. F.: "The disruption of mitochondria of saccharomyces by ultraviolet radiation." Science, 117, 31-33, 1953.

17. Schultz, W. and Amelung, W.: "Untersuchungen uber konsensuelle Gefassreaktionen nach kombinierter Ultraviolett-Infrarot-Bestrahlung." Medizinische, 22, 310-312, 1958.

18. Hirshfield, H. I., Zimmerman, A. M., Landau, J. V. and Marsland, D.: "Sensitivity of ultraviolet-irradiated blepharsma undulans to high pressure lysis." J. Cell. Physiol., 49, 287-294, 1957.

19. Hvidberg, E., Kvorning, Sv. A., Schmidt, A. and Schou, J.: "Effect of ultraviolet irradiation on hyaluronic acid in vitro." Acta pharmacol. et toxicol., 15, 356-364, 1959.

20. Hvidberg, E., Kvorning, Sv. A., Schmidt, A. and Schou, J.: "Effect of ultraviolet irradiation on connective tissue." Acta pharmacol. et toxicol., 15, 365-372, 1959.

21. Blum, H. F., Grady, H. G. and Kirby-Smith, J. S.: "The effect of ultraviolet radiation on body weight of mice." Fed. Proc., 1, 8, 1942.

22. Blum, H. F., Grady, H. G. and Kirby-Smith, J. S.: "Limits of accuracy in experimental carcinogenesis as exemplified by tumor induction with ultraviolet radiation." J. Nat. Canc. Inst., 3, 83-89, 1942.

23. Blum, H. F., Grady, H. G. and Kirby-Smith, J. S.: "Relationships between dosage and rate of tumor induction by ultraviolet radiation." J. Nat. Canc. Inst., 3, 91-97, 1942.

24. Demina, D. M.: "Bewertung der Wirksameit von longwelliger Ultravioletttstrahlung im Vergleich zur Wirkung von Vitamin D2 in der Prophylaxe des D-Vitaminmangels." J. Hyg., Epidem., Microbiol. Immunol. (Praha), 1, 459-471, 1957.

25. Gusarova, A. S.: "Changes in the reactivity of the organism of patients with pyodermatitis under the influence of ultraviolet irradiation." Vestnik dermatologii i venerologii (Moskva), 32, 16-19, 1958.

26. Levkovich, F. N., Goldfeld, A. Ya. and Rzhakhova, O. E.: "The effect of short wavelength ultraviolet rays on the infectiosity and antigenic properties of the viruses of Russian tick-borne and Japanese encephalitis." Bull. Exper. Biol. Med. USSR, 43, 843-847, 1957.

Approved for Public Release

Contrails

27. Blum, H. F.: "The photochemical basis of photopathology." Proc. 1st Int. Photobiological Congr., Amsterdam, 359-368, 1954.

28. Dürken, A. and Graul, E. H.: "Experimentelle Beiträge zur Frage der UV-Einwirkung auf die lehende Zelle des Erwachstenen Organismus und des Embryos." Strahlentherapie, 80, 427-452, 1949.

29. Bradfield, J. R. G. and Errera, M.: "Ultraviolet absorption of living cells." Nature, 164, 532-533, 1949.

30. Montgomery, P. O'B., Bonner, W. A. and Roberts, F. F.: "Ultraviolet flying spot television microscopy. Study of living HeLa cells." Proc. Soc. Exp. Biol. Med., 93, 409-412, 1956.

31. Blum, H. F., Robinson, J. C. and Loos, G. M.: "The loci of action of ultraviolet and x-radiation, and of photorecovery, in the egg and sperm of the sea urchin." Proc. Nat. Acad. Sci., 36, 623-627, 1950.

32. Kelner, A.: "Effect of visible light on the recovery of streptomyces griseus conidia from ultraviolet irradiation injury." Proc. Nat. Acad. Sci., 35, 73-79, 1949.

33. Kelner, A.: "Photoreactivation of ultraviolet-irradiated escherichia coli, with special reference to the dose-reduction principle and to ultraviolet-induced mutation." J. Bact., 58, 511-522, 1949.

34. Carlson, J. G. and McMaster, R. D.: "Nucleolar changes induced in the grasshopper neuroblast by different wavelengths of ultraviolet radiation and their capacity for photorecovery." Exper. Cell. Res., 2, 434-444, 1951.

35. Errera, M. and Vanderhaege, F.: "Effects des rayons UV sur acetabularia Mediterranea." Exp. Cell. Res., 13, 1-10, 1957.

36. Mazia, D. and Hirshfield, H. I.: "Nucleus-cytoplasm relationships in the action of ultraviolet radiation on amoeba proteus." Exp. Cell. Res., 2, 58-72, 1951.

37. Zimmerman, A. M., Landau, J. V. and Marsland, D.: "The effects of a adensione triphosphate and dinitro-o-cresol upon the form and number of amoeba prateus: A pressure-temperature study." Exp. Cell. Res., 15, 484-495, 1958.

38. Iverson, R. M.: "Nuclear transfer studies on ultraviolet-irradiated amoeba proteus." Exp. Cell. Res., 15, 268-270, 1958.

Approved for Public Release

Contrails

39. Lythgoe, R. J.: "The absorption spectra of visual purple and of indicator yellow." J. Physiol., 89, 331-358, 1937.

40. Montgomery, P. O'B., Bonner, W. A. and Roberts, F.: "Ultraviolet flying spot time-lapse motion picture observations of living cells." Texas Rep. Biol. Med., 15, 386-395, 1957.

41. Montgomery, P. O'B., Bonner, W. A. and Roberts, F.: "Ultraviolet damage to living HeLa cells as recorded by time-lapse motion picture studies." Proc. Soc. Exp. Biol. & Med., 95, 589-591, 1957.

42. Blum, H. F., Cook, J. S. and Loos, G. M.: "A comparison of five effects of ultraviolet light on the arbacia egg." J. Gen. Physiol., 37, 313-324, 1954.

43. Sonne, C.: "The biological effects of the ultraviolet rays and investigation as to what part of the spectrum they lie in." Arch. Phys. Ther., 10, 239-252, 1929.

44. Zirkle, R. E., Bloom, W. and Uretz, R. B.: "Use of partial-cell irradiation studies of cell division." Proc. Internat. Conf. on Peaceful Uses of Atomic Energy, 11, 273-282, 1956.

45. Labaw, L. W., Mosley, V. M. and Wyckoff, R. W. G.: "Electron microscopy of ultraviolet irradiated bacteria and their interaction with bacteriophage." Biochim. et Biophys. Acta, 5, 327-334, 1950.

46. Elkind, M. M. and Sutton, H.: "Ultraviolet mitigation of x-ray lethality in dividing yeast cells." Science, 128, 1082-1083, 1958.

47. Hirshfield, H. I. and Pecora, P.: "Reconstitutive events in blepharisma undulans as affected by cochicine." Exp. Cell. Res., 9, 414-427, 1955.

48. Edwards, R. G.: "The experimental induction of gynogenesis in the mouse. I. Irradiation of the sperm by x-rays." Proc. Roy. Soc., B, 146, 469-487, 1957.

49. Edwards, R. G.: "The experimental induction of gynogenesis in the mouse. II. Ultraviolet irradiation of the sperm." Proc. Roy. Soc., B, 146, 488-504, 1957.

50. Henshaw, P. S.: "Studies of the effect of roentgen rays on the time of the first cleavage in some marine invertebrate eggs. I. Recovery from roentgen-ray effects in arbacia eggs." Am. J. Roentgenol., 27, 890-898, 1932.

Approved for Public Release

Contrails

51. Carlson, J. G. and Hollaender, A.: "Immediate effects of low doses of ultraviolet radiation of wavelength 2537 Å on mitosis in the grasshopper nemoblast." J. Cell. Physiol., 23, 157-169, 1944.

52. Carlson, J. G. and Hollaender, A.: "Intensity effects of ultraviolet radiations of wavelength 2537 Å on mitosis in the grasshopper nemoblast." J. Cell. Physiol., 26, 165-173, 1945.

53. Kelner, A., Bellamy, W. D., Stapleton, G. E. and Zelle, M. R.: "Symposium on radiation effects on cells and bacteria." Bact. Rev., 19, 22-44, 1955.

54. Lewis, M. R.: "The injurious effect of light upon dividing cells in tissue cultures containing fluorescent substances." Anat. Rec., 91, 199-208, 1945.

55. McLaren, A. D.: "Photochemistry of proteins. XVIII. Some observations with enzymes and nucleic acids, and discussion." Enzymologia, 18, 81-96, 1957.

56. McLaren, A. D.: "Some comparisons of the actions of ionizing radiations and ultraviolet light on enzymes and other proteins." Second Internat. Congr. of Photobiology, Turin, 1957.

57. Iverson, R. M. and Giese, A. C.: "Nucleic acid content and susceptibility of tetrahymena pyriformis." Exp. Cell. Res., 13, 213-223, 1957.

58. Alechinsky, A.: "L'erytheme actinique et vitamine P. P." Extract from Documentation Medicale, No. 13, 1948.

59. Bourdillon, R. B., Gaddum, J. H. and Jenkins, R. G. C.: "The production of histamine from histidine by ultraviolet light and the absorption spectra of these substances." Proc. Roy. Soc., B, 106, 388-398, 1930.

60. Canzanelli, A., Sossen, R. and Rapport, D.: "Succinic and cytochrome oxidase activity of rat liver mitochondria after in vitro irradiation with ultraviolet light." Am. J. Physiol., 188, 547-549, 1957.

61. Beyer, R. E. and Kennison, R. D.: "The release of nucleotides from mitochondria during ultraviolet irradiation." Biochim. Biophys. Acta, 28, 432-433, 1958.

62. Billen, D.: Modification of the release of cellular constituents by irradiated escherichia coli. Arch. Biochem. Biophys., 67, 333-340, 1957.

Approved for Public Release

Contrails

63. Findlay, G. H.: "Light eruptions and the ATP mechanism." Brit. J. Derm., 70, 242-244, 1958.

64. Hargreaves, A. B.: "Acetylcholinesterase response to ultraviolet radiation and its relation with sulfhydryl groups." Arch. Biochem. Biophys., 57, 41-51, 1955.

65. Flesch, P. and Rothman, S.: "Role of sulfhydryl compounds in pigmentation." Science, 108, 505-506, 1948.

66. Mayneord, W. V. and Roe, E. M. F.: "The activation of cholesterol by radiation." Am. J. Canc., 31, 476-483, 1937.

67. Wickwire, G. C. and Burge, W. E.: "The mode of action of ultraviolet radiation in decreasing sugar metabolism." Am. J. Physiol., 81, 514-551, 1927.

68. Estermann, E. F., Luse, R. A. and McLaren, A. D.: "Some observations on the photolysis of acetylalanine at 2537 Å." Rad. Res., 5, 1-8, 1956.

69. Eremeev, V. F.: "Significance of mitogenetic rays in the synthesis of peptides in the liver." Bull. Exp. Biol. Med., 45, 581-584, 1958.

70. Friedman, B. A. and Ceponis, M. J.: "Effect of ultraviolet light on pectolytic enzyme production and pathogenicity of pseudomonas." Science, 129, 720-721, 1959.

71. Harold, F. M. and Ziporin, Z. Z.: "Synthesis of protein and DNA in escherichia coli irradiated with ultraviolet light." Biochim. Biophys. Acta, 29, 439-440, 1958.

72. Kimball, R. F.: "Nongenetic effects of radiation on microorganisms." Ann. Rev. Microbiol., 11, 199-220, 1957.

73. Dulbecco, R.: "Experiments on photoreactivation of bacteriophages inactivated by ultraviolet radiation." J. Bact., 59, 329-347, 1950.

74. Blum, H. F., Loos, G. M., Price, J. P. and Robinson, J. C.: "Enhancement by 'visible' light of recovery from ultraviolet irradiation in animal cells." Nature, 164, 1011, 1949.

75. Blum, H. F., Loos, G. M., and Robinson, J. C.: "Accelerating action of illumination in recovery of arbacia eggs from exposure to ultraviolet radiation." J. Gen. Physiol., 34, 167-181, 1950.

76. Johnson, F. H., Flagler, E. A. and Blum, H. F.: "Relation of bacteria after ultraviolet radiation." Proc. Soc. Exp. Biol. Med., 74, 32-35, 1950.

Approved for Public Release

Contrails

77. Blum, H. F. and Mathews, M. M.: "Photorecovery after ultraviolet radiation in amphibian larvae." Biol. Bull., 99, 330, 1950.

78. Wells, P. H. and Giese, A. C.: "Photoreactivation of ultraviolet light injury in gametes of the sea urchin, strongylocentrotus purpuratus." Biol. Bull., 99, 163-172, 1950.

79. Novick, A. and Szilard, L.: "Experiments on light-reactivation of ultraviolet inactivated bacteria." Proc. Nat. Acad. Sci., 35, 591-600, 1949.

80. Giese, A. C., Brandt, C. L., Iverson, R. and Wells, P. H.: "Photoreactivation in colpidium colpodia." Biol. Bull., 103, 336-344, 1952.

81. Christensen, E. and Giese, A. C.: "Increased photoreversal of ultraviolet injury by flashing light." J. Gen. Physiol., 39, 513-526, 1956.

82. Rieck, A. F. and Carlson, S. D.: "Photorecovery from the effects of ultraviolet radiation in the albino mouse.: J. Cell. Physiol., 46, 301-305, 1955.

83. Schechtmann, A. M.: "Morphogenetic effects of ultraviolet radiation." Proc. Soc. Exp. Biol. Med., 56, 233-235, 1944.

84. Dürken, A.: "Das Uerhalten des Triton Auges inshesondere der Linsenbildung nach UV Bestrahlung vor Enstehung der Linse." Arch. F. Entw. Mech., 144, 278-300, 1950.

85. Rieck, A. F.: "The effects of ultraviolet, and of photorecovery, on the developing forelimb of amblystoma." J. Morphol., 94, 367-447, 1954.

86. Butler, E. G. and Blum, H. F.: "Regenerative growth in the urodele forelimb following ultraviolet radiation." J. Nat. Canc. Inst., 15, 877-888, 1955.

87. Blum, H. F., Butler, E. G., Chang, J. J., Mawe, R. C. and Schmidt, S. E.: "Studies on regression and regeneration in the urodele forelimb after localized ultraviolet radiation." J. Cell. Physiol., 49, 153-170, 1957.

88. Butler, E. G., Blum, H. F. and Schmidt, S. E.: "The localized character of ultraviolet effects on the urodele forelimb." J. Cell. Physiol., 50, 381-388, 1957.

89. Thornton, C. S.: "The inhibition of limb regeneration in urodele larvae by localized irradiation with ultraviolet light." J. Exp. Zool., 137, 153-180, 1958.

Approved for Public Release

Contrails

90. Audiat, J.: "Action des rayons ultra-violets sur l'excitabilite du nerf." Compt. rend. Soc. Biol., 107, 931-934, 1931.

91. Audiat, J.: "Etude de quelques variables intervenant dans l'excitabilite' du nerf soumis aux radiations ultra-violettes." Compt. rend. Soc. Biol., 107, 935-937, 1931.

92. Audiat, J., Auger, D. and Fessard, A.: "Etude des courants d'action du nerf soumis au rayonnement ultra-violet." Compt. rend. Soc. Biol., 107, 1218-1221, 1931.

93. Booth, J., von Muralt, A. and Stämpfli, R.: "The photochemical action of ultraviolet light on isolated single-nerve fibers." Helv. Physiol. Acta, 8, 110-127, 1950.

94. Boyarsky, L. L.: "Effect of ultraviolet on electrical properties of nerve." Proc. Soc. Exp. Biol. Med., 79, 213-214, 1952.

95. von Muralt, A. and Stämpfli, R.: "Die photochemische wirkung von Ultraviolettlicht auf den erregten Ranvierschen Knoten der einzelnen Nervenfaser." Helv. Physiol. et Pharmacol. Acta, 11, 182-193, 1953.

96. Gasteiger, E. L.: "Effects of ultraviolet on electrical properties of invertebrate nerve." Fed. Proc., 12, 48-49, 1953.

97. Pierce, S. and Giese, A. C.: "Photoreversal of ultraviolet injury to frog and crab nerves." J. Cell. Physiol., 49, 303-317, 1957.

98. Punt, A. and Schippers, B.: "The influence of light on the acetylcholine-contracture." Acta Physiol. Pharmacol. Neerl., 5, 117-262, 1956.

99. Punt, A., Nijhof-Rombach, F. and Schippers, B.: "Muscle contracture elicited by ultraviolet irradiation." Acta Physiol. Pharmacol. Neerl., 6, 551-555, 1957.

100. Seidl, E.: "Ultraviolettbestrahlung und Muskeltraining." Strahlentherapie, 101, 630-633, 1956.

101. Sigmund, R.: "Die Wirkung ultravioletter Strahlen auf die Reactionzeit des Menschen." Strahlentherapie, 101, 623-629, 1956.

102. Lawrence, H.: "The relative low humidity of the atmosphere and much sunshine as casual factor for the great prevalence of skin cancer in Australia." Med. J. Australia, 2, 403-410, 1928.

Approved for Public Release

Contrails

103. Findlay, G. M.: "Ultraviolet light and skin cancer." Lancet, 2, 1070-1073, 1928.

104. Rusch, H. P. and Baumann, C. A.: "Tumor production in mice with ultraviolet irradiation." Am. J. Canc., 35, 55-62, 1939.

105. Rusch, H. P., Baumann, C. A. and Kline, B. E.: "The effect of local applications on development of ultraviolet tumors." Proc. Soc. Exp. Biol. Med., 42, 508-512, 1939.

106. Rusch, H. P., Kline, B. E. and Baumann, C. A.: "Carcinogenesis by ultraviolet rays with reference to wavelength and energy." A.M.A. Arch. Path., 31, 135-146, 1941.

107. Bain, J. A. and Rusch, H. P.: "Carinogenesis with ultraviolet radiation of wavelength 2800 to 3400 Å." Cancer Res., 3, 425-430, 1943.

108. Baumann, C. A. and Rusch, H. P.: "Effect of diet on tumors induced by ultraviolet light." Am. J. Canc., 35, 213-221, 1939.

109. Bain, J. A., Rusch, H. P. and Kline, B. E.: "The effect of temperature upon ultraviolet carcinogenesis with wavelengths 2800 to 3400 Å." Cancer Res., 3, 610-612, 1943.

110. Blum, H. F.: "Sunlight and cancer of the skin." J. Nat. Canc. Inst., 1, 397-421, 1940.

111. Blum, H. F.: "On the mechanism of cancer induction by ultraviolet radiation." J. Nat. Canc. Inst., 11, 463-495, 1950.

112. Blum, H. F., Kirby-Smith, J. S. and Grady, H. G.: "Quantitative induction of tumors in mice with ultraviolet radiation." J. Nat. Canc. Inst., 2, 259-268, 1941.

113. Grady, H. G., Blum, H. F. and Kirby-Smith, J. S.: "Pathology of tumors of the external ear in mice induced by ultraviolet radiation." J. Nat. Canc. Inst., 2, 269-276, 1941.

114. Blum, H. F. and Lippincott, S. W.: "Carcinogenic effectiveness of ultraviolet radiation of wavelength 2537 Å." J. Nat. Canc. Inst., 3, 211-216, 1942.

115. Kirby-Smith, J. S., Blum, H. F. and Grady, H. G.: "Penetration of ultraviolet radiation into skin, as a factor in carcinogenesis." J. Nat. Canc. Inst., 2, 403-412, 1942.

Approved for Public Release

Contrails

116. Clark, J. H., Luce-Clausen, E. M. and Mider, G. B.: "The effect of ultraviolet radiation on the production of spontaneous mammary tumors in C3H mice." Cancer Res., 12, 451-453, 1952.

117. Griffin, A. C., Dolman, V. S., Bohlke, E. B., Bouvart, P. and Tatum, E. L.: "The effect of visible light on the carcinogenicity of ultraviolet light." Cancer Res., 15, 532-538, 1955.

118. Kelner, A. and Taft, E. B.: "The influence of photo-reactivating light on the type and frequency of tumors induced by ultraviolet radiation." Cancer Res., 16, 860-866, 1956.

119. Blum, H. F.: "Sur l'aspect quantitatif de l'action cancerogene des radiations ultraviolettes." Bull. Assoc. fr. Cancer, 45, 163-166, 1958.

120. Hyde, J. N.: "On the influence of light in the production of cancer of the skin." Am. J. Med. Sci., 131, 1-22, 1906.

121. McCoy, J. N.: "The solar keratoses and cutaneous cancer." Arch. Derm. Syph., 1, 175-181, 1920.

122. Lewis, T. and Zotterman, Y.: "Vascular reactions of the skin to injury. VI. Some effects of ultraviolet light." Heart, 13, 203-217, 1926.

123. Macht, D. I., Anderson, W. T. and Bell, F. K.: "The penetration of ultraviolet rays into live animal tissues." JAMA, 90, 161-165, 1928.

124. Bachem, A. and Reed, C. I.: "The transparency of live and dead animal tissue to ultraviolet light." Am. J. Physiol., 90, 600-606, 1929.

125. Bachem, A. and Kunz, J.: "The transmission of ultraviolet light through human skin." Arch. Phys. Therap., 10, 50-59, 1929.

126. Freund, L.: "Beitrag zur Physiologie der Epidermis mit Bezug auf deren Durchlaessigkeit fur Licht." Arch. f. Dermatol. u. Syph., v. 58, 1901.

127. Hasselbalch, K. A.: "Quantitative Untersuchungen uber die Absorption der menschlichen Haut von ultraviolleten Strahlen." Skand. Arch. s Physiol., 25, 55-68, 1911.

128. Miescher, G.: "Das Problem des Lichtschutzes und der Lichtgewöhnung." Strahlentherapie, 35, 403-443, 1930.

Approved for Public Release

Contrails

129. Bachem, A. and Reed, C. I.: "The penetration of light through human skin." Am. J. Physiol., 97, 86-91, 1931.

130. Bachem, A. and Reed, C. I.: "Skin and radiation." Arch. Phys. Therap., 12, 581-590, 1931.

131. Lucas, N. S.: "The permeability of human epidermis to ultraviolet irradiation." Biochem. J., 25, 57-70, 1931.

132. Roffo, A. H.: "Heliotropism of cholesterol in relation to skin cancer." Am. J. Canc., 17, 42-57, 1933.

133. Mitchell, J. S.: "The origin of the erythema curve and the pharmacological action of ultraviolet radiation." Proc. Roy. Soc., B, 126, 241-261, 1938.

134. Abramson, H. A.: "Whealing response of human skin to ultraviolet light and the histamine theory of allergic reactions." Proc. Soc. Exp. Biol. Med., 43, 410-412, 1940.

135. Blum, H. F., Baer, R. L. and Sulzberger, M. B.: "Studies in hypersensitivity to light. II. Urticaria solare (λ<3700)." J. Invest. Derm., 7, 99-107, 1946.

136. Blum, H. F., Barksdale, E. E. and Green, H. G.: "Urticaria solare (λ4000-5000 Å)." J. Invest. Derm., 7, 109-115, 1946.

137. Blum, H. F. and Terus, W. S.: "The erythemal threshold for sunburn." Am. J. Physiol., 146, 107-117, 1946.

138. Blum, H. F. and Terus, W. S.: "Inhibition of the erythema of sunburn by large doses of ultraviolet radiation." Am. J. Physiol., 146, 97-106, 1946.

139. Blum, H. F., Eicher, M. and Terus, W. S.: "Evaluation of protective measures against sunburn." Am. J. Physiol., 146, 118-125, 1946.

140. Rothman, S., Krysa, H. F. and Smiljanic, A. M.: "Inhibitory action of human epidermis on melanin formation." Proc. Soc. Exp. Biol. Med., 62, 208-209, 1946.

141. Fitzpatrick, T. B., Becker, S. W., Jr., Lerner, A. B. and Montgomery, H.: "Tyrosinase in human skin: Demonstration of its presence and its role in human melanin formation." Science, 112, 223-225, 1950.

142. Lamb, J. H., Shelmire, B., Cooper, Z., Morgan, R. H. and Keaty, C.: "Solar dermatitis." Arch. Derm. Syph., 62, 1-27, 1950.

Approved for Public Release

Contrails

143. Rottier, P. B. and Mullink, J. A.: "Localization of erythemal processes caused by ultraviolet in human skin." Nature, 170, 574-575, 1952.

144. Cahn, M. M., Levy, E. J., Shaffer, B. and Beerman, H.: "Lupus erythematosis and polymorphous light eruption. An experimental study on their possible relationship." J. Invest. Derm., 21, 375-396, 1953.

145. Cahn, M. M., Levy, E. J. and Shaffer, B.: "The use of chloroquine diphosphate (Aralen) and quinacrine (Atabrine) hydrochloride in the prevention of polymorphous light eruption." J. Invest. Derm., 22, 93-96, 1954.

146. Partington, M. W.: "The vascular response of the skin to ultraviolet light." Clin. Sci., 13, 425-439, 1954.

147. Maizelis, M. Ya.: "The influence of ultraviolet irradiation on the permeability and on some other indicators of the functional condition of animal and human skin." Bull. Exp. Biol. Med., USSR, 42, 917-921, 1956.

148. Jarvinen, K. A. J.: "Effect of cortisone on reaction of skin to ultraviolet light." Brit. Med. J., 2, 1377-1378, 1951.

149. Holti, G.: "The effect of cortisone upon the skin reactions to local histamine and ultraviolet irradiation." Clin. Sci., 15, 41-53, 1956.

150. Claesson, S., Juhlin, L. and Wettermark, G.: "The reciprocity law of ultraviolet-irradiation effects; damage on mouse skin exposed to ultraviolet light varied over a 10^7-fold intensity range." Acta derm. vener., Stock., 38, 123-136, 1958.

151. Lipkin, M., Bailey, O. and Hardy, J. D.: The effect of ultraviolet irradiation upon the cutaneous pain threshold. Rept. No. NADC-MA-5414, 22 Oct. 1954.

152. Becker, S. W., Jr.: "Effects of 8-methoxypsoralen and ultraviolet in human skin." Science, 127, 878, 1958.

153. El-Mofty, A. M.: "Observations on the use of Ammi Majus Linn in vitiligo." Brit. J. Derm., 64, 431-441, 1952.

154. Lerner, A. B., Denton, C. R. and Fitzpatrick, T. B.: "Clinical and experimental studies with 8-methoxypsoralen in vitiligo." J. Invest. Derm., 20, 299-314, 1953.

155. Fitzpatrick, T. B., Hopkins, C. E., Blickenstaff, D. D. and Swift, S.: "Augmented pigmentation and other responses of

Approved for Public Release

Contrails

normal skin to solar radiation following oral administration of 8-methoxypsoralen." J. Invest. Derm., 25, 187-190, 1955.

156. Fitzpatrick, T. B., Imbrie, J. and Labby, D.: "Effects of methoxsalen on liver function." J.A.M.A., 167, 1586-1589, 1958.

157. Griffin, A. C., Hakim, R. E. and Knox, J.: "The wavelength effects upon erythemal and carcinogenic response in psoralen treated mice." J. Invest. Derm., 31, 289-295, 1958.

158. Wald, G.: "Human vision and the spectrum." Science, 101, 653-658, 1945.

159. Goodeve, C. F.: "Vision in the ultraviolet." Nature, 134, 416-417, 1934.

160. Abe, T.: "Recherchez sur les prapretes optiques der corps vitre." Arch. de Phys. Biol., 6, 1-21, 1927.

161. Wolf, E.: "Effects of exposure to ultraviolet light on visual thresholds." Proc. Nat. Acad. Sci., 31, 236-241, 1945.

162. Wolf, E.: "Effects of exposure to ultraviolet light on subsequent dark adaptation." Proc. Nat. Acad. Sci., 31, 349-355, 1945.

163. Le Grand, Y.: "La protection des yeux contre l'ultra-violet." Ann. D'Ocul., 191, 193-208, 1958.

164. Ludvigh, E. and Kinsey, V. E.: "Effect of long ultraviolet radiation on the human eye." Science, 104, 246-247, 1946.

165. Wolf, E.: "Effects of ultraviolet radiation on visual thresholds." Science, 105, 366, 1947.

166. Wolf, E. and Zigler, M. J.: "Cause of dark adaptation under various conditions of pre-exposure and testing." J. Opt. Soc. Am., 45, 696-702, 1955.

167. Brandenburg, K. C.: "Ultraviolet effects on night vision." Ann. West. Med. Surg., 4, 302, 1950.

168. Sexton, M., Malone, F. and Farnsworth, D.: "The effect of ultraviolet radiation from fluorescent lights on dark adaptation and visual acuity." MRL No. 169, v. 109, 301-317, 1950.

169. Hecht, S., Hendley, C. D. and Ross, S.: "Influence of exposure to intense sunlight on subsequent night vision." Arch. Ophth., 44, 625, 1950.

Approved for Public Release

Contrails

170. Kinsey, V. E., Cogan, D. G. and Drinker, P.: "Measuring eye flash from arc welding." J.A.M.A., 123, 403-404, 1943.

171. Coblentz, W. W.: "Experimental production of cancer of the skin by ultraviolet radiation: Its implications in use of sunlamps." Report of Council on Physical Medicine, J.A.M.A., 36, 1040-1043, 1948.

172. Bachem, A.: "Ophthalmic ultraviolet action spectra." Am. J. Ophth., 41, 969-975, 1956.

173. Cogan, D. G. and Kinsey, V. E.: "Action spectrum of keratitis produced by ultraviolet radiation." Arch. Ophth., 35, 670-677, 1946.

174. Kinsey, V. E.: "Spectral transmission of the eye to ultraviolet radiations." A.M.A. Arch. Ophthal., 39, 508-513, 1948.

175. Verhoeff, F. H. and Bell, Z.: Proceedings of the American Academy of Arts and Sciences, 51, 630, 1916.

176. Ludvigh, E. and McCarthy, E. F.: "Absorption of visible light by the refractive media of the human eye." A.M.A. Arch. Ophthal., 20, 37-51, 1938.

177. Duke-Elder, S.: Textbook of Ophthalmology, Vol. 6, Injuries, C. V. Mosby Company, St. Louis, 1954.

178. Cogan, D. G.: "Lesions of the eye from radiant energy." J.A.M.A., 142, 145-151, 1950.

179. Kutscher, C. F.: "Ocular effects of radiant energy." Trans. Am. Acad. Ophth. Otolaryngol., 50, 230-241, 1946.

180. Wald, G.: "Alleged effects of the near ultraviolet on human vision." J. Optical. Soc. Amer., 42, 171-177, 1952.

181. Friedenwald, J. S., Buschke, W., Crowell, J. and Hollaender, A.: "The effects of ultraviolet irradiation on the corneal epithelium. II. Exposure to monochromatic radiation." J. Cell. Physiol., 32, 161-173, 1948.

182. Martin, E. K.: "The effects of ultraviolet rays upon the eye." Proc. Roy. Soc., B, 85, 319-330, 1912.

183. Duke-Elder, W. S.: "The pathological action of light upon the eye. Part II. Action uponthe lens: Theory of the genesis of cataract." Lanset, 1, 1188-1191; 1250-1254, 1926.

Approved for Public Release

Contrails

184. Duke-Elder, W. S. and Duke-Elder, P. M.: "A histological study on the action of short-waved light upon the eye, with a note on 'inclusion bodies'." Brit. J. Ophth., 13, 1-37, 1929.

185. Burge, W. E.: "The effect of radiant energy on the lens and the humors of the eye." Am. J. Physiol., 36, 21-36, 1914.

186. Burge, W. E.: "The mode of action of ultraviolet radiation in injuring living cells with special reference to those constituting the eye." Am. J. Physiol., 39, 335-344, 1916.

187. Burge, W. E., Wickwire, G. C. and Schamp, H. M.: "Cause of calcification of the crystalline lens with advance in age and in cataract." Arch. Ophthal., 17, 234-240, 1937.

188. Petrovich, Yu. A.: "Effect of ultraviolet irradiation on the permeability to proteins, radiocalcium, and radiophosphorous of the capillaries of the anterior portion of the eye." Bull. Exp. Biol. Med. (Moskva), 44, 1088-1092, 1957.

189. Munich, W.: "Research on the ultraviolet absorption of normal and inflammatorily altered aqueous humor." Docum. Ophthalm. (den Haag), 11, 257-263, 1957.

190. Kahnemann, F.: "Richerche sperimentali sulle lesioni retiniche da raggi a breve lunghessa d'onda." Ann. ottalm., Parma, 83, 471-481, 1957.

191. Flick, J. J.: "Ocular lesions following the atomic bombing of Hiroshima and Nagasaki." Am. J. Ophth., 31, 137-154, 1948.

192. Cogan, D. G., Martin, S. F., Kimura, S. J., and Ikui, H.: "Ophthalmologic survey of atomic bomb survivors in Japan, 1949." Trans. Am. Ophth. Soc., 48, 62-87, 1950.

193. Benkwith, K. B.: "Retinal hemorrhage as seen in an atomic-bomb casualty." Am. J. Ophth., 29, 799-800, 1946.

194. Lippincott, S. W. and Blum, H. F.: "Neoplasms and other lesions of the eye induced by ultraviolet radiation in strain A mice." J. Nat. Canc. Inst., 3, 545-554, 1943.

195. Huldschinsky, K.: "Augensarkome bei Ratten, hervorgerufen durch abnormlange ultravioletteinwirkung." Deutsche med. Wchnschr., 59, 530-531, 1933.

196. Ash, J. E. and Wilder, H. C.: "Epithelial tumors of the limbus." Trans. Am. Acad. Ophthal., 46, 215-222, 1941.

Approved for Public Release

Contrails

197. Goodeve, C. F., Lythgoe, R. J. and Schneider, E. E.: "The photosensitivity of visual purple solutions and the scotopic sensitivity of the eye in the ultraviolet." Proc. Roy. Soc., B, 130, 380-395, 1942.

198. Hosoya, Y.: "Veränderung der Absorption und der Farbe des Sehpurpurs durch Bestrahlung im sichtbaren und ultravioletten Spektralgebiet." Jap. J. Med. Sci., III, Biophys., IV:2, 49-52, 1937.

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SECTION II

BIOMEDICAL EFFECTS OF ULTRAVIOLET RADIATION

Dr. H. C. Heim

Since the discovery, in 1877, that the lethal effects of sunlight are due to the ultraviolet component, a great many studies have been undertaken in order to determine the manner by which ultraviolet radiation exerts a deleterious effect on living cells. A search of the literature reveals that, for the most part, investigators have confined their efforts to that area of the ultraviolet spectrum in which the wavelengths lie between 2000 Å and 3100 Å. This region is frequently termed the abiotic region because radiation at these wavelengths kills or injures cells most readily. From 3100 Å to 4000 Å the biological effects are not so prominent. Relatively little attention has been paid to the biological effects elicited by the lower end of the ultraviolet spectrum, i.e., the so-called "Schumann region" which is comprised of wavelengths between about 140 Å and 2000 Å.

Effects on Proteins

Numerous studies have shown that proteins are changed by exposure to ultraviolet radiation. The exact mechanisms involved in the changes, however, are not well understood (1). It has been suggested that alterations of proteins, as induced by ultraviolet light, may be the basis for cell injury resulting from such radiation (2). Changes induced by ultraviolet have been studied by both physical and chemical methods. Among the changes which have been reported to occur in proteins after exposure to ultraviolet radiation are:

1 - Alteration in odor.

Gates (3) observed that the irradiation of solutions of crystalline pepsin produced an odor in the solutions which he described as resembling that of "stale straw." Irradiation of egg albumen produced a definite change in odor (4). According to one investigator (5) the development of odor in proteins results from photoxidation of components of the protein by the radiation.

2 - Development of color.

It has been stated that all proteins, upon irradiation, develop a yellowish color which parallels the production of

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odor (6). In some proteins it appears that development of color is correlated with loss of activity but in others the changes in color have not been found to parallel changes in specific activity (5). The development of yellowish discoloration has been suggested to result from the formation of compounds related to dihydroxy phenylalanine (DOPA) (7). Some proteins, however, develop definite brownish color upon irradiation but the nature of the colored derivatives as yet has not been elaborated.

3 - Changes in optical rotation.

In general, irradiation of proteins causes an increase in levorotation. This is probably related to the pH of the solutions being irradiated and has been related to such proteins as albumin, gliadin, edestin, and gelatin. At least one study indicates that the increase in optical rotation is the result of structural changes induced by irradiation (8).

4 - Altered ultraviolet absorption spectra.

One of the most prominent changes induced in proteins by ultraviolet irradiation is an alteration of the absorption spectrum of the particular protein. Such changes appear to be more marked with albumins than with globulins. It has been reported that, after irradiation of pepsin solution, pronounced absorption bands appeared at 2500 Å and 3500 Å (9). These bands are thought to be indicative of the liberation of cystine and cysteine from the protein as a result of prolonged irradiation. The products of photolysis of amino acids and of proteins are not well understood and much also needs to be learned about absorption spectra of individual amino acids before an adequate explanation can be offered for the effects produced by the irradiation of proteins. There is some evidence (10) that the presence of oxygen is not necessary in order for ultraviolet denaturation of proteins to occur and this indicates that oxidation by gaseous oxygen is not necessarily involved in the process.

5 - Effects on viscosity

The viscosity of gelatin solutions decreases upon exposure to ultraviolet radiation (11). Other studies have shown that similar effects are produced with globulin and albumin (13). It was suggested that a degradation occurred during the irradiation which reduced the number of centers of asymmetry and that this was possibly accompanied by dehydration of the protein molecules. In other proteins increased viscosity has been observed as a result of ultraviolet irradiation. At 2537 Å horse serum shows an increase in viscosity which is proportional to the time of exposure. Very long exposure times were used, however, so that the changes in viscosity might well be secondary effects. It has been suggested (5) that such experiments should be repeated with purified enzyme preparations in order to determine whether a

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change in viscosity "parallels a loss of activity."

6 - Effect on surface phenomena.

The viscosity of monolayers of pepsin is altered by irradiation at 2537 Å (13). Both visible and ultraviolet radiation alter the surface tension of proteins (14). The decrease in surface tension is greatest with the euglobulins and least with the albumins. It has been suggested that ultraviolet induces a degradation of albumins and globulins with the formation of soluble fragments with reduced surface activity (15). The irradiation of surface layers of gliadin, zein, and ovalbumin showed that at 2537 Å egg albumin layers liquefied and that ultraviolet causes a hydrolysis of the -CONH- linkages of the polypeptides (16).

7 - Effects on hydrogen ion concentration.

Changes in pH following irradiation of protein solutions have been reported by several investigators (12, 17). Acid solutions of albumin become less acid and alkaline solutions become more acid.

8 - Effects on electrical conductivity.

Ultraviolet radiation causes an increase in conductivity of gelatin solutions (11). It has been suggested (5) that this is because the radiation causes depolymerization and liberation of ammonia.

9 - Effects on molecular weight.

Studies have shown (18) that very large protein molecules are split into "half molecules" by radiation from a quartz mercury lamp and that further splitting of the "half molecules" does not occur. After prolonged irradiation denaturation and precipitation occurred. Smaller protein molecules, it appears, do not exhibit this property of dissociation under the influence of ultraviolet and it is suggested that ultraviolet causes rupture of the weakest bond in the protein molecule, i.e., the bond holding the two halves of very large molecules together. Such photochemical reactions apparently occur only with wavelengths shorter than 2800 Å. The irradiation of serum albumin solutions, at pH 3.5, causes a marked increase in sedimentation rate, indicating aggregation of protein molecules. Evidence has been reported which indicates (19) that irradiation brings about a primary process of depolymerization and which is followed by an aggregation of the depolymerized material.

10 - Other effects of ultraviolet.

Investigators have shown that irradiation causes a precipitation of serum albumin and horse pseudoglobulin solutions

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(20) and that egg albumin becomes less soluble after exposure to ultraviolet. It has been shown also that the coagulation temperature of globulins and fibrinogen is elevated by ultraviolet irradiation.

In addition to the phenomena listed above, ultraviolet radiation has shown that nucleic acids are altered by even small doses (21). It has been postulated that this is at least a partial basis for the tissue damage induced by such radiation. It appears that the uracil moiety of the nucleic acid is altered by ultraviolet (22). Other studies (23) indicate that the component of nucleic acid which is most susceptible to ultraviolet is the pyrimidine ring. Irradiation of solutions of sodium desoxyribonucleate and sodium ribonucleate caused changes in absorption spectra, phosphate linkages, and electrometric titration curves. It was found that the pyrimidine rings of the consitituent purines were most labile. Absorption spectra were diminished by one-sixth to one-third before any significant effect was observed in the internucleotide linkages. With further irradiation inorganic phosphate began to appear indicating hydrolysis of up to one-third of the nucleotides. By studying the affinity of the cell nucleus for methyl green before and after irradiation it was concluded (24) that the total deoxyribonucleic acid (DNA) in the nucleus was unchanged by irradiation but a possible structural change in the DNA was induced by the radiation. A change in the absorption spectrum of nucleic acids was observed after irradiation at 2537 Å. Sodium ribonucleate, sodium deoxyribonucleate, adenosine triphosphate, 3-adenylic acid, adenosine, adenine, xanthine, hypoxanthine, uric acid, guanine, uracil, and caffeine all showed disruption of the pyrimidine moiety of the molecules with consequent loss of characteristic absorption spectra (25). These authors point out that the irradiation of concentrated solutions may not cause any apparent change in the absorption spectrum because of a masking by unchanged material. In dilute solutions, however, the concentration of unchanged material is not sufficiently great to produce the washing effect. Both free and nucleotide-bound carbohydrates are readily destroyed by ultraviolet. Adenylic acid, cytidilic acid, and yeast nucleic acid all showed decomposition from less than 30 minutes exposure to ultraviolet. Even glucose and ribose buffered at pH 7.4 were rendered incapable of reducing Benedict's reagent after 60 minutes irradiation (26). Dry deoxyribonucleic acid (DNA), after irradiation, forms an insoluble gel. The amount of gel formed depends upon the incident energy per square centimeter of surface. The rate of formation of the insoluble gel as a function of incident energy per square centimeter of surface area enables comparisons to be made relative to the efficiencies of ultraviolet light in inducing gel formation. From 1850 Å to 3000 Å the efficiency for the gel formation parallels the absorption spectrum of DNA. Formation of the gel is not decreased if irradiation is carried out at the temperature of liquid air (27). The nucleic acids in the skin appear to be

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altered after irradiation with ultraviolet with wavelengths below 3150 Å. Alteration was not found to occur with longer wavelengths (28).

In a study of the effect of ultraviolet on serum albumin (29) it was found that, in an atmosphere of oxygen a degradation of the protein occurred and that this degradation was due to the photoxidation of hydroxyl groups of the albumin. When the irradiation was carried out in an atmosphere of nitrogen, polymerization of the protein occurred. Other studies have shown that ultraviolet produces two parallel effects on proteins -- a denaturation caused only by rays of short wavelengths and which is not an oxidative process; and a photoxidation caused by either long or short wavelengths in the presence of oxygen. It was postulated that oxygen was activated by the radiation and was mainly bound to the protein, but it could also conceivably serve as a hydrogen acceptor with the resulting formation of hydrogen peroxide. It was further suggested that prolonged exposure to ultraviolet probably results in partial splitting of the protein molecule (30).

The exposure of frog muscle to ultraviolet radiation evokes a change in the x-ray diffraction pattern of the muscle and the magnitude of change depends upon the intensity of the radiation (31). When aqueous solutions of blood serum or serum proteins are exposed to ultraviolet radiation the surface tension drops rapidly for the first few minutes, then slowly rises and sometimes after 30 to 90 minutes exposure exceeds the initial value. The preliminary drop in surface tension is not due to liberation of lipids from the lipid-protein complexes, but rather is due to some change in the structure of the protein (15). Exposure of aqueous solution of ovalbumin at 25 degrees C. to ultraviolet radiation from a mercury vapor lamp produces secondary protein derivatives which can be dialyzed at pH 4-8. The formation of denatured protein and soluble protein derivatives are first order processes. Oxygen does not influence the denaturation but it does increase the formation of the soluble protein derivatives (32). Another investigator has reported that the effects of ultraviolet on proteins depends to some extent upon the temperature of the solutions being irradiated. Coagulation of salt-free albumin by ultraviolet is believed to involve three processes -- light denaturation; a chemical reaction (with a high temperature coefficient) between the denatured molecule and water; and finally flocculation. It is suggested also that heating after irradiation increases the effect of ultraviolet on tissues and that heating before irradiation may also increase the effect but to a considerably less extent (33).

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The most important effects of ultraviolet radiation on amino acids are decomposition reactions, rearrangements, and sensitized reactions (5). From consideration of the bond energies in proteins and amino acids, it can be shown that there is sufficient energy in wavelengths shorter than 2850 Å to break any single bond present in proteins or amino acids. Irradiation of glycine solutions with rays from a mercury arc produces an increase in conductivity and pH. This is thought to be due to a hydrolytic cleavage triggered by the radiation and which converts glycine to glycolic acid and ammonia. At the same time side reactions appear to occur because carbon monoxide is liberated (34). Wavelengths shorter than 2265 Å are required to produce decomposition and the decomposition is maximal at pH 3 with minimal effects at pH 6. The literature reveals that probably all of the naturally occurring amino acids can be readily altered by ultraviolet radiation, in vitro, yet it is difficult to correlate such in vitro observations with tissue damage produced in vivo. In a few instances, however, agents with profound pharmacologic activity could conceivably be produced as a result of photolysis of amino acids. It has been shown (35) that irradiation of histidine solutions produces a substance with a histamine-like action. Irradiation of this product with longer wavelengths (2970 Å and 3020 Å) causes a loss in pharmacologic activity. The irradiation of histidine was studied by another investigator (36), who was able to isolate histamine from solutions of histidine after the irradiation. It would appear that histidine is converted to histamine by decarboxylation and that this process can be initiated through the influence of ultraviolet radiation. The irradiation of histidine solutions causes a brown discoloration and this occurs independently of the formation of histamine. The addition of nicotinaminde retards the conversion of histidine to histamine (37). In neutral or alkaline solution it has been shown that 70 to 80% of the histidine in solution is destroyed by irradiation, while if the irradiation occurs in acid solution only about 12% is destroyed. It can be demonstrated that the ultraviolet irradiation of histidine yields histamine, imidazoleacetic acid, urocanic acid, asparagine, and serine (38). The ultraviolet absorption curves of indole, indole-3-propionic acid, tryplophane, tryptamine, chloracetyl-alpha-tryptophane, and kynurenine were determined in aqueous solution at pH 4 and pH 11 before and after irradiation. The curves before irradiation varied slightly with pH. In every case ultraviolet irradiation produced changes in the absorption curves and, with the exception of chloroacetyl-alpha-tryptophane, the changes in the absorption curves were much greater in irradiated alkaline solutions than in acid solutions (39). The irradiation of certain amino acids in air, nitrogen, and in argon produced the same absorption curves regardless of the gas used, but in each instance the radiation evoked changes in the curves. These results would

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indicate that the changes were not due to an uptake of oxygen, but rather to a splitting off of hydrogen (40). Irradiation of uracil has been reported to produce oxamide and parabanic acid (41). When l-tryptophane is irradiated by ultraviolet, kynurenine is first formed and then is converted into a substance of unknown composition together with alanine, aspartic acid, and glycine (42).

The irradiation of proteins at 2400 Å produced changes in the extinction coefficients. The results have been interpreted as due to modifications of the aromatic portions of tyrosine, phenylalanine, and tryptophane, probably as a result of oxidation. Irradiation times of up to 24 hours were used in this study (43). It has been reported that a phenol is formed by irradiation of phenylalanine and some indications were noted of the presence of tyrosine. It was further observed that dihydroxy phenylalanine was formed, possibly from the action of the radiation on tyrosine. Irradiation of dry phenylalanine for long periods caused approximately 20% destruction of the amino acid (44). In a study of the effects elicited upon proteins and certain amino acids by ultraviolet radiation, it was concluded that the absorption spectra are altered and that the alterations do not appear to be caused by ozone produced during the irradiation (45).

Irradiation of solutions of cystine, cysteine, and methionine with a quartz mercury lamp showed that cystine is reduced to cysteine and, as the time of irradiation is lengthened, increasing amounts of the cysteine are destroyed. On the irradiation of cysteine, at first only oxidation to cystine occurred without destruction, then with increasing time of irradiation more destruction occurred. The formation of hydrogen sulfide during irradiation was noticeable by odor. At 2250 Å methionine was approximately 10% destroyed after 8 hours irradiation (46). Irradiation of tyrosine, tryptophane and phenylalanine yields melanin-like compounds. The products were isolated, then purified, and were found to differ slightly in solubility, optical absorption, and in reactions with hydrogen peroxide (47). In solutions of pepsin dihydroxy phenylalanine (DOPA) was produced upon irradiation and it has been suggested that the inactivation of pepsin by ultraviolet is due to the formation of DOPA from tyrosine molecules (48).

Effects on Hormones

The inactivation of insulin by ultraviolet has been reported (51) to be accompanied by the liberation of tyrosine and by an increase in ultraviolet absorption. It was suggested that the increased absorption is probably associated with photolysis of cystine and tyrosine residues within the insulin molecule. The liberation of tyrosine from insulin apparently does not parallel

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the loss of biological activity. It is interesting to note, however, that in an earlier study it was shown that irradiated insulin caused a greater drop in blood sugar than did nonirradiated insulin (50). Treatment of insulin with rays from a mercury vapor lamp for 1 minute was reported to increase the activity by 4 times and after irradiation for 5 minutes the activity became 3 times that of nonirradiated insulin. Insulin irradiated for 30 minutes exhibited no difference in potency when compared to nonirradiated insulin, but after 60 minutes irradiation the activity diminished quite markedly. These results would seem to indicate slight alteration of the insulin molecule after brief exposure to ultraviolet and possible destruction after prolonged exposure.

Irradiation of thyrotropic and gonadotropic hormones of the hypophysis has been reported to inactivate these hormones, possibly by causing release of oxygen and subsequent formation of hydrogen peroxide (51). Ultraviolet light has been reported to produce chemical changes in steroid hormones during the drying of a paper chromatogram (52).

The oxidation of a 1:10,000 solution of epinephrine at pH 5.6 by air is promoted only by ultraviolet radiation corresponding to the absorption band of epinephrine at 2890 Å and by diffuse daylight passing through glass. In the latter case it is felt that the upper part of the visible spectrum is probably responsible (53). Irradiation has been reported to destroy the estrogenic activity of estrone (54) and of alpha-estradiol (57). A loss of potency is observed after irradiation of crystalline androgens (56). It has been reported (57) that irradiation at 3130 Å converts androsterone and estrone to "lumi" derivatives.

Effects on Enzymes and Coenzymes

Diphosphopyridine nucleotide (DPN) is partially inactivated by irradiation at 2537 Å. Reactivation of the DPN can be accomplished by exposing it to visible light. The ability of DPN to accelerate hydrogen transfer in rat liver homogenates was used as a criterion of activity and the actual measurement of rate of hydrogen transfer was carried out in Thunberg tubes containing methylene blue together with the homogenate, DPN, etc. DPN is not inactivated by visible light (58). It has been reported that irradiation of DPN causes photochemical changes in the pyridinium moiety of the molecule together with rupture of the nucleoside and nucleotide linkages. It is suggested that the loss of coenzyme function of DPN is probably associated with the photochemical changes in the pyridinium structure. Adenosine triphosphate (ATP) was found to be degraded by ultraviolet with the liberation of denine. Neither adenine nor adenosine were degraded by

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ultraviolet. Because adenine was also identified in the products of ultraviolet decomposition of DPN, it appears that the pyrophosphate bond is labilized by ultraviolet in both ATP and DPN (59). In another study (60) it was found that irradiation of DPN solutions at 2537 Å destroyed cozymase activity. This inactivation was not due to an accumulation of inhibitors. It was accompanied by the formation of four products; adenosine diphosphate, adenylic acid, adenine, and nicotinamide. Adenosine diphosphate (ADP) was the major decomposition product. Some of the nicotinamide rings are ruptured and the ribose moiety is partially destroyed. Ultraviolet irradiation of solutions of triphosphopyridine nucleotide (TPN) resulted in loss of coenzymatic activity as studied by the glucose-6-phosphate dehydrogenase system. The inactivation was due to the splitting of the molecule. The decomposition products were tentatively identified as nicotinamide and probably nicotinamide linked to unidentified breakdown products of ribose; adenosine 2'5'-diphosphate; and probably 2' phospho adenosine diphosphate (63). Adenosine triphosphate (ATP) shows an absorption peak at 2600 Å and this suggests that ATP may be one site of ultraviolet action on living systems. Prolonged exposure to ultraviolet radiation causes marked loss of selective absorption, and recovery does not occur spontaneously or after exposure to visible light. Exposure to intense visible light does not alter the absorption maximum, but causes the release of inorganic phosphate (62). When rat liver mitochondria were exposed to ultraviolet radiation at 2537 Å a decrease in the P:O ratio was observed with beta-hydroxy butyrate as the substrate. When vitamin K was added to irradiated mitochondria the P:O ratios were restored almost to control levels. Vitamin K had no effect on control mitochondria and irradiated vitamin K had no effect in restoring P:O ratios of irradiated mitochondria. Ultraviolet irradiation did not affect the P:O ratio obtained with the use of cytochrome C as substrate (63).

Both ribonuclease and lysozyme are altered by exposure to ultraviolet. Cysteine does not protect ribonuclease nor lysozyme from inactivation but affords some protection to triose phosphate dehydrogenase. It is postulated that cysteine can, under certain conditions, act as an "internal filter" to strongly absorb the radiation, thus protecting the enzyme. No free amino acids are liberated by irradiation of either enzyme (64).

Dry trypsin at temperatures between 90°K and 450°K was irradiated at 2537 Å. The sensitivity at 90°K was found to be about one-third that at room temperature. Sensitivity appeared to remain constant at room temperature, but at the higher temperatures the sensitivity became as much as 6 times that at room temperature. It was suggested that the increased sensitivity at high temperatures is partly the result of the rapid heat inactivation of a fraction of the irradiated molecules, and that the inactivation of trypsin by ultraviolet is not the result of the

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absorption of energy by a specific aromatic residue of the molecule (65). Ultraviolet radiation rapidly and completely destroys crystalline urease, and the irradiated enzyme cannot be reactivated by reducing agents. Urease and oxidized urease produce similar antibodies, but no anti-urease is produced by irradiated urease. This indicates that irradiation denatures the enzyme and causes a marked change in its molecule (66). Crystalline liver catalase and noncrystalline red cell catalase were irradiated at 2537 Å and at temperatures between 90°K and 366°K. It was found that liver catalase is more sensitive to ultraviolet than is red cell catalase. The sensitivity at 366°K was found to be more than 10 times as great as at 90°K (67). Cytochrome oxidase is markedly inhibited by ultraviolet irradiation (68). Irradiation at 2537 Å of a purified acetylcholinesterase preparation obtained from Electrophorus electricus produced inactivation but quite high energy levels were required in order to obtain inactivation. Cysteine was not found to reactivate the enzyme. It is postulated that the inactivation of the enzyme is due to a direct action of the radiation rather than to an indirect oxidation of the sulfhydryl groups in the enzyme molecule (69). The irradiation of dilute solutions of vitamin B-12 caused a loss in potency of about 35%. Approximately the same extent of decomposition was observed upon irradiation with intense visible light. No attempts were made to determine the products formed as a result of the irradiation (70).

Recommendations for Further Study

A search of the literature reveals that much effort has been devoted to the study of the effects elicited by ultraviolet irradiation on biological processes. Work in this field has been extended to encompass the effects of irradiation on cell particulates including many different proteins and enzymes; coenzymes, hormones, vitamins, and amino acids. In almost every instance it appears that ultraviolet evokes structural changes in these compounds. Most of the studies reported in the literature seem to have been performed with radiation at wavelengths in the vicinity of 2500 Å. In order to augment existing knowledge relative to the effects elicited by ultraviolet radiation on biological processes, studies in the following areas would appear pertinent:

1 - An investigation of the effects of ultraviolet on amino acids with particular emphasis placed on the identification of the degradation products. Such a study should include the effects of radiations ranging downward from about 2500 Å. It is conceivable that some degradation products of amino acids might have behavioral effects somewhat analogous to those elicited by adrenochrome and adrenoxine, both of which are metabolic products of

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epinephrine. Particular emphasis should be placed on the effects of ultraviolet on tyrosine, tryptophane, phenylalanine, proline, hydroxyproline, and thyroxine.

2 - Although the effects elicited by ultraviolet on a number of enzymes have been studied, it would be most informative to investigate, in greater detail, the effects on respiratory enzymes and upon the enzymes involved in phosphorylation. Such a study should also include effects on the cofactors which are necessary for the function of these enzymes.

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BIBLIOGRAPHY

1. Giese, A. C.: "Action of ultraviolet radiation on protoplasm." Physiol. Rev., 30, 437, 1950.

2. Arnow, L. E.: "Effects produced by the irradiation of proteins and amino acids." Physiol. Rev., 16, 671, 1936.

3. Gates, F. L.: "Absorption of ultraviolet radiation by crystalline pepsin." J. Gen. Physiol., 18, 265, 1933.

4. Agren, G.: "The effect of ultraviolet light on proteins." Skand. Arch. Physiol., 69, 59, 1934.

5. McLaren, A. D.: "Photochemistry of enzymes, proteins, and viruses." Advances in Enzymology, 9, 109, 1949.

6. Clark, J. H.: "The biological effects of radiation." Ann. Rev. Physiol., 1, 21, 1939.

7. Arnow, L. E.: "The formation of DOPA by the exposure of tyrosine solutions to ultraviolet radiation." J. Biol. Chem., 120, 151, 1937.

8. Clark, J. H.: "Denaturation changes in egg albumin with urea, radiation, and heat." J. Gen. Physiol., 27, 101, 1943.

9. Gates, F. L.: "Absorption of ultraviolet by crystalline pepsin. Temperature coefficient of inactivation by ultraviolet radiation." Jour. Gen. Physiol., 18, 265, 1934.

10. Spiegel-Adolph, M.: "Physico chemical studies on radiated proteins." Biochem. Zeit., 197, 197, 1928.

11. Ponthus, P.: "Action of ultraviolet radiation on aqueous solutions of gelatin." Compt. rend., 196, 1248, 1933.

12. Mond, R.: "The action of ultraviolet light on protein solutions." J. Chem. Soc., 123, 868, 1923.

13. Schaefer, J. V.: "Expansion patterns of protein monolayers on water." J. Phys. Chem., 42, 1089, 1938.

14. Clark, J. H.: "Radiated proteins. Coagulation of egg albumin by ultraviolet light and heat." Am. J. Physiol., 23, 649, 1925.

15. Dognon, A. and Gongerot, L.: "The action of ultraviolet rays on protein surface films." Bull. Soc. Chim. Biol., 25, 183, 1943.

Approved for Public Release

Contrails

16. Mitchell, J. S. and Rideal, E. K.: "Photochemical reactions in monolayers. Photochemistry of proteins." Proc. Roy. Soc. London, A167, 342, 1938.

17. McLaren, A. D. and Lewis, C.: "Concerning the inactivation-denaturation of pepsin." J. Polymer. Sci., 5, 379, 1950.

18. Svedberg, T. and Brohult, S.: "Splitting of protein molecules by ultraviolet light." Nature, 142, 830, 1938.

19. McLaren, A. D., Shepard, E., and Wagman, J.: "A study of the action of ultraviolet light on urease by means of the ultracentrifuge." Nature, 162, 370, 1948.

20. Spiegel-Adolph, M.: "Effect of ultraviolet radiation and heat upon protein solutions." Biochem. J., 28, 372, 1934.

21. Hollaender, A. and Greenstein, J. P.: "Alteration of nucleic acids by ultraviolet radiation." J. Nat. Cancer Inst., 2, 23, 1941.

22. Christensen, E. and Giese, A. C.: "Changes in absorption spectra of nucleic acids and their derivatives following exposure to ultraviolet irradiation." Arch. Biochem. Biophys., 51, 208, 1954.

23. Seraydarian, M., Canzanelli, A., and Rapport, D.: "Correlation of changes in electrometric titration, absorption spectrum and phosphate content of nucleic acid produced by ultraviolet light." Am. J. Physiol., 172, 42, 1953.

24. Errera, M.: "Action of ultraviolet light on affinity of cell nucleus for methyl green." Biochem. Biophys. Acta, 7, 605, 1951.

25. Rapport, D. and Canzanelli, A.: "Photochemical action of ultraviolet on absorption spectra of nucleic acids and related substances." Science, 112, 469, 1950.

26. Rice, E. W.: "The action of ultraviolet light on the pentose moiety of nucleic acids and related compounds." Science, 115, 92, 1952.

27. Setlow, R. and Doyle, B.: "Action of radiation on dry deoxyrebonucleic acid." Biochem. Biophys. Acta, 15, 117, 1954.

28. Hamperl, H., Henschke, U., and Schulze, R.: "Primary process in erythema production by ultraviolet radiation." Naturwiss., 27, 486, 1939.

Approved for Public Release

Contrails

29. Roberts, R.: "Action of ultraviolet radiation on proteins." J. Soc. Dyers and Colourists, 65, 669, 1949.

30. Fiala, S.: "Denaturation of proteins by ultraviolet radiations." Biochem. Zeit., 318, 67, 1947.

31. Spiegel-Adolph, M., Henney, G. C., and Ashkenaz, E.: "X-ray diffraction studies on frog muscle." J. Gen. Physiol., 28, 151, 1944.

32. Bernhart, F. W.: "Denaturation and molecular splitting of ovalbumin by ultraviolet radiation." J. Biol. Chem., 128, 289, 1939.

33. Clark, J. H.: "Temperature coefficient of the effect of radiation on proteins and its relation to injury of the living cell." Am. J. Roentgenol. Radium Therapy, 40, 501, 1938.

34. Henri, V., Weizmann, C., and Hirshberg, Y.: "Photochemic al decomposition of glycine." Comp. rend., 198, 168, 1934.

35. Ellinger, F.: "Production from histidine by means of ultraviolet irradiation of a substance which stimulates the intestine and causes a fall in blood pressure." Archiv. eiptl Path. Pharmakol., 175, 97, 1934.

36. Holtz, P.: "Formation of histamine by the irradiation of histidine." Arch. epitl Path. Pharmakol., 175, 97, 1934.

37. Alechinsky, A. and Rense, J.: "Nicotinamide and the ultraviolet irradiation of histidine." Comp. rend. Soc. Biol., 140, 1224, 1946.

38. Tomohiro, Y.: "The photolysis of histidine." Nagasaki Igakki Zassi, 28, 1117, 1953.

39. Florence, G., Drilbon, A., and Wang, D. S.: "Action of ultraviolet rays on the indole nucleus." Bull. Soc. Chim. Biol., 21, 298, 1939.

40. Mischenko, P. I.: "Action of ultraviolet rays on absorption spectra of amino acids." Biokhimiya, 19, 268, 1954.

41. Conrad, W. E.: "Isolation of oxamide and parabanic acid from the products of ultraviolet irradiated uracil." Radiation Research, 1, 523, 1954.

42. Deschreider, A. and Renard, M.: "Degradation products of l-tryptophane produced by ultraviolet light." Bull. inst. agron. et stas recherches Gembloux, 23, 269, 1955.

Approved for Public Release

Contrails

43. McLean, D. J. and Giese, A. C.: "Absorption spectra of proteins and amino acids after ultraviolet irradiation." J. Biol. Chem., 187, 537, 1950.

44. Arnow, L. E.: "Destruction of phenylalanine by ultraviolet radiant energy." Proc. Soc. Exp. Biol. Med., 49, 578, 1942.

45. Giese, A. C., Leighton, H. L., and Bailey, R.: "Changes in absorption spectra of proteins and representative amino acids induced by ultraviolet radiation and ozone." Arch. Biochem. Biophys., 30, 431, 1950.

46. Schocken, K.: "The effect of ultraviolet radiation on sulfhydryl and disulfide containing amino acids." Science, 116, 544, 1952.

47. Spiegel-Adolph, M.: "Photosynthetic melanins." Biochem. J., 31, 1303, 1937.

48. Imahori, K.: "Ultraviolet inactivation of pepsin." Biochem. Biophys. Acta, 18, 216, 1955.

49. Kaplan, E. H., Campbell, E. D., and McLaren, A. D.: "Inactivation of insulin by ultraviolet light." Biochem. Biophys. Acta, 4, 493, 1950.

50. Ito, R.: "Action of insulin irradiated by ultraviolet and x-rays on blood sugar." J. Oriental Med., 29, 271, 1938.

51. Binder, E.: "Effects of diverse agents on the activity of hormones of the hypophysis." Rev. suisse zool., 54, 236, 1947.

52. Savard, K., Wotiz, H. W., Marcus, P., and Lemon, H. M.: "Effect of ultraviolet light on steroids during paper chromatography." J.A.C.S., 75, 6327, 1953.

53. Chatonnet, J. and Vial, J.: "Influence of various radiations on the oxidation of adrenalin." Comp. rend. Soc. Biol., 139, 112, 1945.

54. Herbant, M. and Thomas, J.: "Influence of ultraviolet radiation on the biological activity of estrone." Ann. endocrinol., 9, 12, 1948.

55. Trentin, J. J. and Turner, C. W.: "Effects of ultraviolet irradiation on biological activity of alpha-estradiol benzoate." Endocrinology, 42, 448, 1948.

56. Cantor, M. M. and Huston, M. J.: "Effect of ultraviolet light and chlorine on androgens." Can. Pharm. J., 81, 746, 1948.

Approved for Public Release

Contrails

57. Butenandt, A. and Poschmann, L.: "Lumiandrosterone." Ber., 77B, 392, 1944.

58. Wells, P. H.: "Photoreactivation of ultraviolet-inactivated diphosphopyridine nucleotide." Science, 124, 31, 1956.

59. Carter, C. E.: "Decomposition of DPN and ATP by ultraviolet light." J.A.C.S., 72, 1835, 1950.

60. Seraydarian, M., Cohen, A. I., and Sable, H.: "Inactivation of DPN by ultraviolet irradiation and identification of the degradation products." Am. J. Physiol., 177, 150, 1954.

61. Seraydarian, M.: "Inactivation of TPN by ultraviolet irradiation and identification of decomposition products." Am. J. Physiol., 181, 291, 1955.

62. Wells, P. H. and Johnson, H.: "Action of ultraviolet and visible light on adenosinetriphosphate." Anat. Record, 117, 644, 1953.

63. Anderson, W. N., and Dallam, R. D.: "Effect of vitamin K on oxidative phosphorylation of rat liver mitochondria irradiated with ultraviolet light." J. Biol. Chem., 234, 409, 1959.

64. Shugar, D. and Rzendowska, F.: "The ultraviolet photolysis of ribonuclease." Bull. Acad. Polon. Sci., 4, 293, 1956.

65. Setlow, R. and Doyle, B.: "Effect of temperature on the ultraviolet inactivation of trypsin." Arch. Biochem. Biophys., 48, 441, 1954.

66. Pillemer, L., Ecker, E. E., Myers, V. C., and Muntwyler, E.: "Chemical and immunological studies of the effects of radiant energy and of the oxidation of crystalline urease." J. Biol. Chem., 123, 365, 1938.

67. Setlow, R. and Doyle, B.: "Combined effect of temperature and ultraviolet radiation on dry catalase." Arch. Biochem. Biophys., 46, 31, 1953.

68. Yost, H. T., Robson, H., and Spiegelman, I. M.: "Effects of irradiation of cell particulates. Inhibition of cytochrome oxidase." Biol. Bull., 110, 96, 1956.

69. Hargreaves, A. B.: "Acetylcholinesterase response to ultraviolet radiation and its relation with sulfhydryl groups." Arch. Biochem. Biophys., 57, 41, 1955.

70. DeMerre, L. J. and Wilson, C.: "Photolysis of vitamin B-12." J.A.Ph.A., 45, 129, 1956.

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SECTION III

BEHAVIORAL EFFECTS OF ULTRAVIOLET RADIATION

Dr. D. W. Stilson

This report is focused on the effects upon behavior of electromagnetic radiation of between 4000 Å and 3000 Å in wavelength. The term "ultraviolet radiation" will refer to wavelengths in this region throughout the report.

The articles reviewed can be divided into four rough classes as follows:

A. The visibility and hue of ultraviolet radiation.

B. The effects of solar radiation on dark adaptation and visual acuity.

C. The effects of ultraviolet radiation on dark adaptation, differential intensity sensitivity, and visual acuity.

D. The effects of ultraviolet radiation on the "reactivity" of the organism (e.g., reaction time, conditionability).

The report is organized according to this classification.

The Visibility and Hue of Ultraviolet Radiation

More than 100 years ago, the visibility of electromagnetic radiation below 4000 Å was reported by Herschel (1), Mathiesson (2), Stokes (3), and Helmholtz (4). In spite of this, the lower limit of the "visible spectrum" has been fixed traditionally at about 4000 Å (5, 6, 7). The papers reviewed below indicate that about 3100 Å would be a more reasonable lower limit. (It might be added that the traditional upper limit of the "visible spectrum" -- about 7000 Å -- can be extended to between 8000 and 10,000 Å with sufficient intensities (8, 9).

Two reasons for this restriction of the "visible spectrum" can be suggested. First, it has been customary to express luminosity functions in percent of maximum sensitivity (5, 10), and if relative sensitivities below 0.0001 are considered "negligible," then the photopic luminosity function can be bounded by about 4100 Å and 7200 Å while scotopic visibility

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extends from near 3850 Å to about 6450 Å (11). Most investigators seem to have viewed sensitivities below about 0.001 of maximum as inconsequential.

A second reason for the traditional limits of the "visible spectrum" may be the known absorbtion characteristics of the ocular media (12, 13, 14, 15, 16, 17, 18, 19, 20). Judd (16, 17) has combined an extrapolation of the data of Ludvigh and McCarthy (14) and the data of Wald (18) to estimate that about 5% of the incident energy at 3650 Å passes through the human cornea, lens, aqueous, vitreous, and macula lutea to the retina. This estimate is quite close to that given by Kinsey (15) for the rabbit eye. However, in interpreting these data it must be remembered that the transmission of the ocular media falls short of 100% for the wavelengths of maximum sensitivity. At the maximum for scotopic sensitivity (about 5050 Å), Judd (16, 17) estimates the transmittance of cornea, lens, aqueous, vitreous, and macula lutea combined is less than 40%. Thus, transmittance at 3650 Å is about 12% of the transmittance at the wavelength of maximum scotopic sensitivity. From this it can be seen that the possibility of visual sensation in the near ultraviolet cannot be excluded on the basis of the energy transmission characteristics of the ocular media. Further, it has been maintained by some (21, 22) that some ultraviolet wavelengths reach the retina with sufficient intensity to produce retinal damage.

In 1840 Herschel (1) described radiation in the ultraviolet as "lavender gray." Mathiesson (2) reported the visibility of 3180 Å in 1844, and in 1852 Stokes (3) described ultraviolet wavelengths as lacking "the luminousness of the blue and the rudiness of violet." Helmholtz (4) reported observations of ultraviolet in considerable detail. He described a "reversal of the spectrum" from violet toward indigo and blue as the stimulating wavelength decreased from 4000 Å. Helmholtz also reported a hue change as a function of ultraviolet intensity. Increasing intensity was found to shift the color experience from lavender toward blue-white, whereas a decrease of intensity produced a shift toward indigo and blue. Mascart (23) found 3130 Å to be visible at high intensities, and he described the hue as lavender-gray. Chardonnet (24) examined two aphakic Os and found that they could see wavelengths between 3010 and 3430 Å, though normal Os could not detect the presence of these wavelengths at the intensities used.

With sufficiently high intensities, Glancy (25) found 3175 Å to be visible. However, Saidman and Dufestel (26) were unable to find evidence for visibility below 3650 Å. (Later, Saidman (27) published results showing vision at 3130 Å for normal Os. It is probable that the intensities used by Saidman and Dufestel were insufficient to produce visual experience below 3650 Å.) The hue at 3650 Å was described by the Os of

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Saidman and Dufestel as identical to that at 4040 Å, but the sensitivity to the latter wavelength was estimated to be 900-1000 times that of the former. Goodeve (28) reported "normal vision" down to 3125 Å where color experience was described as similar to that at 4047 Å for the light-adapted eye. At 3023 Å, Goodeve found no evidence for visual experience for either light-adapted or dark-adapted eyes. de Groot (29) found that all but one of 21 Os could see 3650 Å and that 3130 Å was visible to all but three. However, none were able to detect 3076 Å. de Groot's Os described 3130 Å and 3650 Å as "clear blue" whereas 4047 Å and 4057 Å were called "violet." "It seemed to them," says de Groot, "as if the succession of the spectrum was reversed." This appears to be the same reversal described by Helmholtz (4).

Saidman (27) published data on the ultraviolet sensitivity for 102 Os. All Os 33 years of age or less described 3130 Å as "violet," "mauve," or "blue" with the exception of one woman. However, there appeared to be a lack of sensitivity to the shorter wavelengths among older Os.

Pinegan (30, 31, 32, 33) was able to demonstrate both photopic and scotopic sensitivity to 3020 Å for all Os less than 38 years old. He found sensitivity at 3020 Å to be about one-one hundredth of sensitivity to 3130 Å. Most Os described 3130 Å as "bluish" whereas 3020 Å was most often described as "pale blue" or "gray." Goodeve et al (34) consider 3090 Å to constitute the lower limit of normal scotopic vision, though they found that aphakic Os could detect wavelengths as short as 2980 Å. Bachem (35) obtained scotopic luminosity data for both normal and aphakic Os, also. The aphakic Os and "a few others" reported 3910 Å to be more highly saturated with blue than 4050 Å. At low intensities, saturation appeared to increase from 4000 Å to 3650 Å. Thus, Bachem describes the spectrum reversal previously found by Helmholtz (4) and de Groot. With the exception of one aphakic O, none of Bachem's Os could detect wavelengths below 3130 Å. Data published by the Institute of Optics (9) indicate that the lower limit of the visible spectrum is 3130 Å, also.

Schneider (36) has described a "second chromatic threshold" at 3650 Å for an aphakic O. A violet hue is reported just above the absolute detection threshold, but as intensity is increased, a shift towards blue occurs. Helmholtz found a similar shift for a normal O.

Friedrich and Schreiber (37) were unable to establish any agreement on the hue of 3650 Å for their Os. They interpreted this as indicating that previous descriptions of hue were due to stray light in the optical systems employed or to spectral impurities in the source. However, it may be that the intensity used by Friedrich and Schreiber was near the detection threshold for some Os and well above the threshold for others. If this were true, then the shift in hue as a function of intensity

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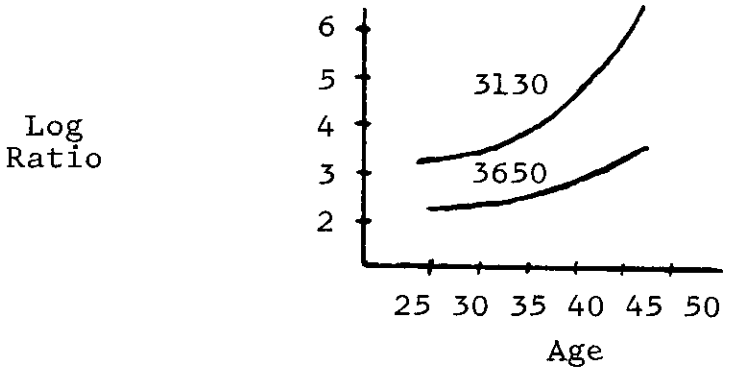
(previously described by Helmholtz and Schneider) may account for the lack of agreement concerning the hue of 3650 Å. This possibility is supported by fact that individuals show a great deal of variation in sensitivity to ultraviolet wavelengths.

Widmark (38) reported that the lower limit of the visible spectrum increases with age. He established 3839 Å as the mean lower limit for Os from 11 to 30 years of age. For Os between 62 and 74, the mean lower limit was 4018 Å.

Taylor (39) found that "younger men" saw 3130 Å as "dark violet" not differing much from 3650 Å, whereas "older" persons were unable to see below 3650 Å. de Groot (40) studied systematically the relationship between ultraviolet sensitivity and age. For Os ranging from about 25 to 45 years of age, he obtained the ratio of the energy threshold for 3130 Å to that of 4047 Å (T_{3130}/T_{4047}) and the ratio for 3650 Å to 4047 Å (T_{3650}/T_{4047}). The figure below shows the relationship between log ratio and age. Though interindividual variability was large



within age groups, there does appear to be a systematic decline in sensitivity as a function of age. At 25 years, sensitivity at 4047 Å is about 100 times that at 3650 Å and about 1000 times that at 3130 Å. At 3650 Å, the 45-year old has experienced nearly a 10-fold decline in sensitivity and at 3130 Å nearly a 1000-fold decline over the 25-year old relative sensitivity levels. It is known that the yellow lipins of the eye increase with age (34), which probably reduces sensitivity to 4047 Å (as well as to 3130 Å and 3650 Å), so that the relative sensitivity data reported by de Groot probably underestimates the decrement in absolute sensitivity to ultraviolet wavelengths which accrues with age.

Wright (41) found that the sensitivity of normal Os to 3650 Å varied widely, and that a substantial portion of the variability could not be attributed to age. Wright suggests that variations in ultraviolet sensitivity may be a consequence of variations in the level of some toxic product which produces yellowing of the ocular media. (He observes that the three Os having the least sensitivity to 3650 Å were "persistent smokers.") Related to this is Salomon's report (42) of large variability in the absorbtion of 3650 Å by the lens. Perhaps

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it is variations in lens coloring which is primarily responsible for individual differences in sensitivity to ultraviolet.

Wawilow (43) has estimated the number of photons reaching the retina at the absolute intensity threshold for scotopic vision. Between 4000 Å and 6500 Å the scotopic luminosity function obtained by Wawilow has essentially the same shape as the relative luminosity functions obtained by others. However, for one of Wawilow's Os, the curve was extended downward to 3040 Å. For this O, a sharp local maximum in retinal sensitivity was found near 3800 Å with a local minimum occurring at about 4100 Å. Since the lens is largely responsible for absorbtion in the region around 3800 Å, one might expect a local peaking or at least a flattening of the aphakic luminosity function in this region. Gaydon (44) has given a subjective description of an apparent maximum in this region, but attributed it to the peculiarities of the iron arc spectrum (the iron arc was being used as a source for ultraviolet). Goodeve et al (34) found a local maximum in the aphakic scotopic luminosity function near 3650 Å (sensitivity at 3600 Å and 5460 Å were identical) with a minimum between 4000 and 4100 Å. These results are probably within the limits of error one would expect in Wawilow's calculation of retinal sensitivity. For the normal eye, Goodeve et al found a slight flattening of the scotopic luminosity function in the 3500 Å region. Pinegan (30) reported a similar flattening of the scotopic function, but found no such effect for the chromatic curve.

These results are quite interesting in relation to the photosensitivity of visual purple. Hosoya (45) found visual purple photosensitive to wavelengths of 3650, 3130, 3020, and 2970 Å. Wald (46) showed that the photo absorbtion spectrum for rhodopsin has essentially the same shape as the scotopic luminosity function down to 4000 Å. However, Wald did not obtain data extending into the ultraviolet. Schneider et al (47) found that the photosensitivity of rhodopsin corresponded well with the scotopic luminosity function in the red region of the spectrum, but showed that rhodopsin is more sensitive at the blue end of the spectrum than scotopic luminosity data suggest. This was accounted for in terms of the selective absorbtion of the shorter wavelengths by the yellow pigments known to exist in the ocular media. The important point here is that Schneider et al and Goodeve et al describe a local maximum in the photosensitivity of visual purple near 3600 Å. Wald (48) found a local maximum in the absorbtion spectrum of visual purple near 3500 Å, also. Thus, the peaking of the aphakic scotopic luminosity function and the flattening of the corresponding function for the normal eye appear to be closely paralleled by the photosensitivity spectrum of rhodopsin. However, Wald (49) did not find either flattening or peaking in scotopic sensitivity for the normal eye and only slight flattening was reported for the aphakic O. It is relevant,

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perhaps, that Wald used one-twenty fifth second exposure times whereas Goodeve et al (34) used 1 second and that Wald's data do not extend below 3500 Å.

Laurens (50) has discussed claims of visual sensitivity to wavelengths as short as 3130 Å as due solely to the fluorescence of the anterior parts of the eye, particularly of the lens. However, there is considerable evidence indicating that the visibility of ultraviolet is not merely a misinterpretation of the well-known fluorescence effect.

Helmholtz was quite aware of the fluorescence effect, and he used it in attempting to explain the reversal of the apparent spectrum below 4000 Å. He believed the reversal to be a result of the combination of the violet produced by direct retinal stimulation by ultraviolet and the greenish-white sensation produced by fjuorescence. As wavelength is shortened, the former effect decreases and the latter increases. The resultant, thought Helmholtz, is the observed spectrum reversal. More recently, Le Grand (51) found that the dominant wavelengths in the fluorescence of sheep and rabbit eyes is about 4680 Å when the excitation wavelength was either 3650 Å or 3130 Å. On the basis of these results, Le Grand adopted the same explanation of the spectrum reversal as that given by Helmholtz. However, Ogilvie (19) has argued that the reversal occurs in the aphakic O, also, and Ogilvie doubts that the fluorescence of the cornea or retina are of sufficient intensity to produce the effect. Bachem (35) has questioned the interpretation of Helmholtz and Le Grand, also.

Saidman (27) described the difference between the sensation due to fluorescence and the visibility of the energy source. Goodeve reports noticeable fluorescence at 3023 Å and to a slight extent at 3125 Å, but in addition, a definite "retinal impression" was recorded at 3125 Å though none was observed at 3023 Å. Goodeve considered the two effects "quite separable." Pinegan (30) also considered the two sensations distinguishable. Gaydon (44) argued on three grounds that his report of visibility down to 3100 Å for aphakic vision is not attributable to fluorescence. First, he describes visual acuity as about the same at 3100 Å as in the "visible spectrum." Acuity would be expected to be poor at the shorter wavelengths if only fluorescence were being observed. Second, sensitivity was essentially the same from 3100 Å to 4300 Å, according to Gaydon, whereas the fluorescence of organic compounds is known to vary over this range. Finally, fluorescence of organic compounds usually appears greenish-white or white whereas the ultraviolet wavelengths were seen as violet or blue. Gaydon described the sensation between 3300 and 3600 Å as matching 4530 Å, and, more generally, the spectrum from 3100 to 4800 Å is described as "blue."

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Bachem (35) measured the fluorescence threshold by psychophysical methods using an "ultraviolet blind" O (probably an older person). He found these subjective estimates to correspond well with physical measurements. The stimulating wavelengths which produced the lowest fluorescence threshold were found near 3800 Å. It is possible that the local maximum in scotopic sensitivity described earlier is a reflection of the low fluorescence threshold in this region. However, if this were true, then the peaking effect in the 3500 to 3800 Å region should be greater for the normal O than for the aphakic (since the lens is known to fluoresce with greater intensity than the other ocular media) (19, 52). Contrary to this interpretation, Goodeve et al (34) found greater peaking in the 3600 Å region for their aphakic Os than for normal Os. As indicated earlier, Wald (49) found no evidence of peaking or flattening in the 3600 Å region for either aphakic or normal scotopic luminosity functions.

Goodeve et al found that the ratio of sensitivity at 5640 Å to that at 3650 Å remained constant throughout the course of dark adaptation. This was interpreted as indicating that the mechanism of vision is the same at both wavelengths, and consequently, that vision at 3650 Å is not due solely to fluorescence of the eye.

Finally Klang (52) reports that ocular fluorescence increases with age whereas ultraviolet sensitivity decreases with age. If the visibility of ultraviolet was simply due to ocular fluorescence, the opposite relationship would be expected to occur.

In summary, the following conclusions concerning the visibility of ultraviolet radiation to the human observer seem warranted.

1. The lower limit for both scotopic and photopic visibility in the normal young adult is about 3100 Å.

   a. For scotopic vision, sensitivity at 3650 Å is about one-twenty thousandth of maximum sensitivity (49).

   b. For photopic vision, sensitivity at 3650 Å is between one-forty five hundredth (32) and one-ten thousandth (49) of maximum sensitivity, and at 3130 Å, sensitivity is about one-fifty five thousandth of maximum (32).

2. The lower limit of the visible spectrum increases with age. However, there is substantial interindividual variation in sensitivity to wavelengths below 4000 Å which is probably attributable in part to variations in the pigmentation of the lens.

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3. As wavelength is decreased below 4000 Å, there is an apparent reversal of the spectrum from violet towards blue.

4. There may be a local maximum in retinal sensitivity in the region 3800 to 3500 Å which corresponds to a local maximum in the photosensitivity of rhodopsin.

Effects of Solar Radiation on Dark Adaptation and Visual Acuity

The effects of exposure to sunlight on dark adaptation have been investigated by Hecht et al (53, 54). They describe the onset of rod adaptation as delayed for 10 minutes following a 2-3 hour period in bright sunlight. In addition, the normal asymptotic level of dark adaptation is not reached for "several hours" following a single exposure. After repeated daily exposure to sunlight, the night vision threshold remained about 0.11 log units above normal. This is described as amounting to a 50% deterioration in visual acuity, range of visibility, contrast discrimination, and frequency of seeing. The effect is described as cumulative over a 10-day period and is said to persist for as long as 10 days following protection from sunlight.

Clark et al (55) placed a patch over one of O's eyes and exposed the other eye to sunlight for 4½ hours each day. A threshold increase of 0.34 log units was observed following 30 minutes of dark adaptation and a threshold elevation of 0.13 log units remained the following morning. However, this elevation completely disappeared when the previously exposed eye was completely protected from light for 7-8 hours. Thus, the cumulative effect described by Hecht et al was not observed when complete protection was provided.

Clark et al found, also, that 12% transmission polarizing glasses eliminated the detrimental effects of sunlight on dark adaptation. Clark et al and Hecht et al indicate that radiation in the visible spectrum is probably responsible for the observed effects, though Hecht et al (54) suggest that the role of ultraviolet radiation be investigated further.

Peckham and Harley (56) studied the effects of exposure to sunlight on photopic "retinal sensitivity" as measured by critical flicker frequency and minimum contrast. Minimum contrast acuity was observed to decline over the entire summer for the 70 lifeguards serving as Os. In the study of c.f.f. acuity, Os were divided into three groups. The first group wore 10-15% transmission glasses, a second group wore commercial lenses transmitting 35-40%, and members of the third group were

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unprotected. The group wearing the 10-15% glasses showed less daily loss in acuity and greater overnight recovery than either of the other two groups. Since the 10-15% transmission lenses offered considerably more effective protection than the commercial lens, and since the latter absorb wavelengths in the ultraviolet and infrared whereas the former are most effective in the "visible spectrum," it was concluded that energy in the visible spectrum was primarily responsible for the observed detrimental effects of sunlight on visual acuity.

Whiteside (57) describes a persistent haze which is present in the visual field during flight at high altitudes. This haze has been observed to interfere with instrument reading within the shadow of the airplane cockpit. It was suspected that this haze might be due to increased fluorescence of the ocular media due to greater intensities of ultraviolet in solar radiation at high altitudes. At 40,000 feet above sea level, it has been estimated that the intensity of wavelengths below 3100 Å is about 3 times that at sea level. Intensities in the region of maximum lens fluorescence (about 3600-3700 Å) are increased at high altitudes, though to a lesser degree.

Whiteside conducted a preliminary investigation in which sunlight was passed through a woods glass filter which admits wavelengths between 3000 and 4000 Å with a maximum at 3600 Å. Under these conditions, sunlight was seen with a gray-blue haze over the entire visual field when the background was sky. This appearance was gone when full sunlight was admitted to the eye. It was concluded that fluorescence was "masked" by the presence of other wavelengths in full sunlight.

Os were then equipped with filters cutting out most of the radiant energy below 4000 Å and required to read a test target located in the cockpit of an airplane flying at an altitude of 40,000 feet. The haze was found to persist in spite of the presence of the filter. Consequently, it was concluded that the haze condition was not a result of increased ocular fluorescence.

The Effects of Ultraviolet Radiation on Dark Adaptation, Differential Intensity Sensitivity, and Visual Acuity

Heine (58) reported a number of case histories in which impaired dark adaptation was a symptom. In each case, Euphos glass spectacles were prescribed, and improved dark adaptation was reported to follow in each instance. It was concluded that ultraviolet radiation was responsible for the initial impairment of dark adaptation, since Euphos glass is an effective filter of ultraviolet wavelengths.

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Siegfried (59) attempted to verify Heine's observations in the laboratory. One eye of each O was exposed to an incandescent lamp and the other to a mercury vapor lamp. All wavelengths below 5000 Å were filtered from the incandescent source whereas only the abiotic rays were filtered from the mercury vapor source. Following pre-exposure to these lamps, dark adaptation was tested. It was found that there was no difference between the two pre-exposure conditions in their effects on dark adaptation. However, the two pre-exposure conditions were not equated for brightness so that no conclusions can be drawn from these results.

Similarly negative results have been reported by Langstroth et al (60). However, Ogilvie (19) has dismissed these results altogether stating that the experimental design employed was inadequate.

Keil (61) found that exposure to a standard Army Air Corps ultraviolet lamp raised the dark adaptation threshold for form recognition by from 0.3 to 1.0 log units. Recovery from the effect of the lamp was considered complete within 2.5 minutes. It was concluded that the observed effects were attributable to the visible light produced by the lamp and to ocular fluorescence produced by the ultraviolet wavelengths.

Wolf (62, 63) found that the dark-adaptation curve of the chick was raised by a 10-minute pre-exposure to wavelengths below 3650 Å (see also Stafford, 64). If pre-exposure was restricted to wavelengths longer than 3650 Å, no subsequent effects on dark adaptation were detectable. With pre-exposure wavelengths of 3550 Å and above, a 0.3 log unit threshold elevation was observable after 30 minutes of dark adaptation. For 3150 Å and above, 0.6 log units elevation occurred, and for 2900 Å and above, the elevation was 1.1 log units. Wolf interpreted these results as indicating that wavelengths below 3650 Å have some special desensitizing effect on the retinal receptors. Luckiesh has criticized Wolf's interpretation on the ground that the ultraviolet doses employed were sufficiently high to produce retinal damage. This criticism cannot be evaluated adequately in the absence of data on the absorbtion of ultraviolet wavelengths by the anterior parts of the chick's eye. However, on the basis of the antibiotic properties generally attributed to certain ultraviolet wavelengths, it is possible that retinal damage was produced during exposure to wavelengths down to 2900 Å, though such a conclusion requires the assumption that the absorbtion for these wavelengths in the anterior eye of the chick is far less than for several other animals and for human adults. On the other hand, it appears unlikely that retinal damage would result through exposure to wavelengths no shorter than 3550 Å, though dark adaptation was found to be impaired by pre-exposure to radiation of 3550 Å or longer.

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Wolf (65) reported similar effects on human dark adaptation. Six Os were given a 10-minute pre-exposure to a mercury vapor lamp at a luminance of 6250 ml. with only wavelengths below 2850 Å filtered. Then, the progress of dark adaptation was recorded over about 35 minutes. Dark adaptation was also recorded following a 10-minute pre-exposure with all ultraviolet wavelengths filtered out. Dark adaptation was measured using a square 12.5 degrees test field exposed centrally for one-twenty fifth second for each determination. The onset of the rod portion of the dark-adaptation curves was delayed for about 1.5 minutes by pre-exposure which included the ultraviolet wavelengths. At the end of about 30 minutes of dark adaptation, the curve obtained following pre-exposure including ultraviolet wavelengths remained from 0.23 to 0.29 log units higher than the curve obtained following pre-exposure not including ultraviolet wavelengths. An O with one aphakic eye was tested, also. For the normal eye, an elevation of 0.20 log units was recorded after 30 minutes of dark adaptation following ultraviolet pre-exposure, whereas the elevation was 0.68 log units for the aphakic eye. A second aphakic O showed a 0.46 log unit elevation with ultraviolet pre-exposure.

Wolf (65) concluded that ultraviolet may have some direct desensitizing effect on the rods or that the fluorescence of the anterior parts of the eye during ultraviolet exposure increased the light-adaptation level of the rods.

Ludvigh and Kinsey (66) investigated the effects of ultraviolet pre-exposure on the foveal differential brightness threshold and critical flicker frequency (c.f.f.). Radiation from a 1000 watt mercury arc was passed through a filter system transmitting only wavelengths between 3200 Å and 4000 Å. One eye was covered and O was exposed to this radiation for 5 minutes. The differential threshold and c.f.f. were tested before exposure and at intervals 5 minutes and 1 hour following ultraviolet exposure. No differences were found between exposed and unexposed eyes after exposure nor between tests given before and after exposure for either c.f.f. or differential threshold.

This result lends support to the conclusion reached by Peckham and Harley (56) to the effect that reduced foveal sensitivity following exposure to solar radiation is the consequence of "visible" portions of the solar spectrum rather than ultraviolet wavelengths.

Ludvigh and Kinsey interpreted their results as inconsistent with those obtained by Wolf using chicks, and they suggest that the inconsistency is due to differences between the human and chick eye.

Wolf (67, 68) has criticized this interpretation. He points out that in his work with chicks (62, 63) and with humans (65), only the rod portion of the dark-adaptation curve was affected

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by pre-exposure to ultraviolet. He states that the results of Ludvigh and Kinsey pertain to cone sensitivity only and are therefore unrelated to his earlier work. As described below, Wolf and his co-workers later concluded that ultraviolet pre-exposure does affect cone sensitivity (69, 70).

In a 1949 paper, Wolf (68) discusses the effects of exposure to solar radiation on subsequent dark adaptation and reports a series of studies of the effects of the ultraviolet components of fluorescent illumination on dark adaptation.

Referring to the work of Clark et al (55), Wolf argues that the elevation of the terminal dark-adaptation threshold following exposure to sunlight may be due primarily to ultraviolet components of the solar spectrum rather than to visible light. Clark et al found that 12% transmission polarizing lenses eliminated the effects of solar exposure on dark adaptation, and Wolf points out that these lenses screen most of the energy below 3650 Å. Since Wolf's previous work indicated that wavelengths below 3650 Å are most detrimental to subsequent dark adaptation, the results of Clark et al seem to support Wolf's contention. It is perhaps relevant that Peckham and Harley (56) found lenses transmitting little in the ultraviolet region but a relatively high proportion of energy in the visible spectrum were less effective than 10-15% transmission lenses which are relatively inefficient ultraviolet filters in preventing reduced photopic sensitivity following exposure to sunlight.

The empirical results reported by Wolf (68) in this paper involved the use of a fluorescent tube as an ultraviolet source. Os were exposed for 10 minutes to the illumination of a 29 watt fluorescent lamp which was passed through either a Noviol A filter (which screens wavelengths below 3650 Å) or through crown glass (which transmits wavelengths down to about 3000 Å). The dark-adaptation test procedure used was essentially the same as that employed by Wolf earlier (65).

Using a "daylight" fluorescent tube, the rod portion of the dark-adaptation curve was between 0.20 and 0.34 log units higher 30 minutes after ultraviolet exposure (crown glass filter) than it was 30 minutes after the control exposure (Noviol A filter). Cone adaptation appeared to be unaffected by ultraviolet pre-exposure.

Further tests were made using a 2 degrees square 6 degrees temporal from the fixation point (previous tests were with a 12.5 degrees test field centrally fixated). O was required to detect three vertical bars in the test field. With this test procedure, ultraviolet produced a 0.21 to 0.34 log unit elevation of the dark-adaptation curve 30 minutes after pre-exposure.

Similar though lesser effects were found using "softwhite" and "white" fluorescent tubes. However, when a fluorescent

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"gold" lamp was used as a source, the crown glass and Noviol A filters had no differential effect on subsequent dark adaptation. This was anticipated by Wolf since the "gold" tube produces very little energy below 5000 Å.

Wolf maintains that in each of these studies, the brightnesses of the two pre-exposure fields were equal in the "visible spectrum." However, as indicated in the first section of this report, wavelengths well below 4000 Å are visible, and it may be that the brightness of the field using crown glass was sufficiently greater than with the Noviol A filter to account for the elevation of dark adaptation described by Wolf. If this were true, one would expect the elevation of the dark-adaptation threshold due to greater brightness to be apparent early in the dark-adaptation period, whereas Wolf reports that no differential effects are evident until the break separating the cone and rod portions of dark adaptation.

Sexton et al (71) studied the effects of ultraviolet on dark adaptation and visual acuity. Os received 1 hour of pre-exposure to one of three types of 20-foot lambert illumination: (1) fluorescent, (2) ultraviolet-shielded fluorescent, or (3) incandescent. No statistically significant differences were found between the three pre-exposure conditions for either dark adaptation or visual acuity (measured using the Weston Cancellation Test involving rings with gaps of 0.84, 1.26, and 1.68 minutes of visual arc). Dark-adaptation measurements were continued for 1 hour following pre-exposure. One additional O was exposed for 10 minutes to a 165-foot lambert incandescent lamp plus 10 minutes of exposure to an unfiltered fluorescent tube. The entire dark-adaptation curve (30 minutes) following this 20-minute pre-exposure was above a curve obtained following a 10-minute exposure to a 165-foot lambert incandescent source alone. However, since it is known that the onset and rate of dark adaptation are retarded with increased pre-exposure time, it is difficult to see how this result has any bearing on the effects of ultraviolet on dark adaptation.

Zigler, Wolf and King (69) investigated the effects of ultraviolet radiation on the subsequent sensitivity of foveal and parafoveal (6 degrees temporal of fixation point) cones. Sensitivity was tested against background luminances ranging from 0 to 40 ml. The pre-exposure source was a 20 watt fluorescent tube screened by either a crown glass or Noviol A filter. The "visual brightness" of these two fields is described as equal. For the lowest background luminances, the elevation of the rod portion of the dark-adaptation curve previously found by Wolf to follow ultraviolet exposure was observed. For higher luminances, the rod portion of adaptation did not appear, but the "asymptotic" level of cone adaptation was higher following ultraviolet exposure for both foveal and parafoveal cones. Zigler et al argue that if only increased brightness produced

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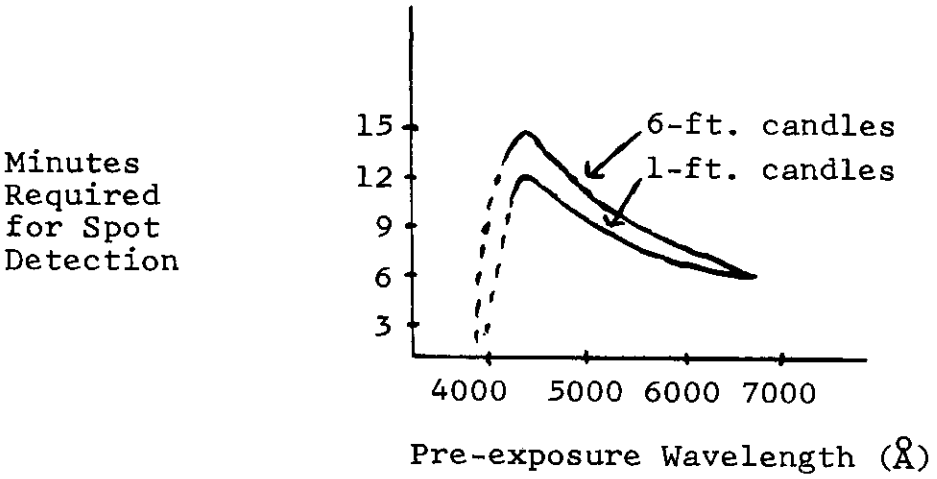
this differential effect, then delayed onset and a reduced rate of adaptation should have been observed rather than an asymptotic elevation.

Though Wolf had earlier criticized Ludvigh and Kinsey (66) for "expecting" to find that ultraviolet exposure affected subsequent cone sensitivity, it appears that he and his colleagues now consider desensitization of both rod and cone vision to be a consequence of ultraviolet exposure.

Hulbert (72) measured the rate of dark adaptation as a function of the wavelength of pre-exposure illumination. He used 3-minute pre-exposures to monochromatic wavelengths of 4360, 5460, and 5890 Å; filtered wavelengths of 5200, 6300, 6500, and 3660 Å, and white light at 2900 K. The time required to detect a black 1 degree spot located on a 24 degrees screen of 8 mμ.l. luminance was used as a measure of the light-adaptation efficiency of the pre-exposure wavelength. The results are sketched in the figure below for intensities of 1- and 6-foot candles. (It should be noted that several other intensity levels were studied by Hulbert.) The ultraviolet pre-exposure stimulus was not equated with the other wavelengths in brightness.



Hulbert reports spot detection required only 5-10 seconds following exposure to 3660 Å for 3 minutes. Thus, according to this result, ultraviolet radiation alone has very little effect on subsequent dark adaptation.

Peckham (73) has devised an ingenious scheme for utilizing Hulbert's results to interpret the data obtained by Zigler, Wolf and King (69). Using available data onthe energy spectrum of fluorescent tubes and the Hulbert data, he was able to estimate the "total light-adaptation efficiency" of each of the pre-exposure conditions used by Zigler et al. First, the energy of

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each wavelength in the spectrum of the fluorescent tube was multiplied by Hulbert's measure of detection time for that wavelength. Each of these products was then multiplied by the transmittance of the Noviol A filter at each wavelength. The sum of these three-factor products gives the total light-adaptation efficiency of the control pre-exposure field in the Zigler et al (69) experiments. Following the same procedure, but entering the transmittance of crown glass as the third factor in each product gives the total light-adaptation efficiency of the ultraviolet pre-exposure condition. Since the latter value exceeded the former by about 25%, it was concluded by Peckham (73) that greater brightness in the ultraviolet pre-exposure field accounted for the elevated adaptation thresholds rather than any special effects of the ultraviolet energy.

Though it must be admitted that there are several possible sources of error in Peckham's calculations, the results do suggest an explanation of the effects reported by Wolf and his co-workers.

Further evidence supporting Peckham's interpretation has been presented by Wald (18). Wald begins by pointing out that the transmittance of the ocular media for wavelengths shorter than 3650 Å is such that only a very small fraction of the incident energy at these wavelengths can reach the retina. He doubts that the energy reaching the retina can have any effect beyond the stimulation of vision. In addition, Wald shows that the crown glass filter used by Wolf and his associates has about the same absorbtion spectrum as the cornea and that the absorbtion spectrum of the Noviol A filter is essentially the same as that of the lens. Wald concludes that as far as the retina is concerned, these filters change nothing.

Wald (18) goes on to describe the results of a series of experiments designed to test Wolf's conclusions. Os were given simultaneous pre-exposure of both eyes, but with one eye covered by crown glass and the other by a Noviol A filter. Dark-adaptation measurements were then made alternately on the right and left eye using a red 2 degrees test field which was exposed for one-twenty fifth second at a point 8 degrees from the fixation point. Wald points out that the macula lutea, which has high absorbtion for ultraviolet wavelengths, was included in the centrally fixated 12.5 degrees test field used by Wolf. With normal Os, Wald found that ultraviolet exposure had no differential effects on subsequent dark adaptation. For two binocular aphakic Os, the initial portions of the dark-adaptation curves for the ultraviolet-exposed eye were elevated, but the curves for the two eyes converged within about 25 minutes of dark adaptation. This is contrary to Wolf's finding that the effects of ultraviolet pre-exposure were most noticeable in the terminal stages of a 30-40 minute dark-adaptation period. When one eye of a binocular aphakic was pre-exposed to high

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intensity in the visible spectrum, the initial (cone) portion of the dark-adaptation curve for this eye was higher than that for the other eye which was pre-exposed simultaneously to ultraviolet plus visible light of lower intensity. The same effect was produced more clearly when normal Os were tested under the same conditions.

Wald (18) observes that all of these results can be interpreted in terms of brightness differences between the ultraviolet and control pre-exposure fields. He concludes that ultraviolet has no special effects upon subsequent dark adaptation other than those effects which are attributable to the visibility and intensity of the ultraviolet wavelengths.

In response to Wald's criticisms, Wolf and Zigler (70) have pointed to several differences between Wald's experimental procedures and their own. Wald exposed both eyes simultaneously, one to the ultraviolet field and one to the control field, and then tested right and left eyes alternately during dark adaptation. On the other hand, Wolf and his co-workers had always pre-exposed and tested one eye at-a-time. Wolf and Zigler state that eyes preference is not perfectly correlated with sensitivity, and furthermore, that in dark adaptation the final cone level, final rod level, and the break-point may each differ for the two eyes of a single individual. Perhaps more important, Wolf and Zigler also suggest that the effect of ultraviolet pre-exposure may depend on the wavelength of the test light. Whereas they always had used a white test light, Wald used red, which might account for the differences in results. In connection with this, Adair (74) found that when monochromatic light was used for both pre-exposure and test light, the initial dark-adaptation threshold was elevated and the rate of adaptation retarded if both lights were of the same wavelength. She also found that the magnitude of the differential effect of using the same or difference pre-exposure and test wavelengths depended upon the wavelength of the pre-exposure light.

Wolf and Zigler go on to report a series of experiments pertinent to the questions they raised concerning Wald's (18) work. One eye was exposed to a field of 1510 ml. luminance using a 20 watt fluorescent tube as a source. For each O, pre-exposure included ultraviolet wavelengths during one experimental period (crown glass filter) and no ultraviolet during another (Noviol A filter). Following each pre-exposure condition, dark adaptation was tested for the exposed eye using a 2 degrees test field flashed 6 degrees below the fixation point for one-twenty fifth second. The differences previously reported by Wolf and his co-workers were observed. However, when Wald's procedure of pre-exposing both eyes simultaneously and testing dark adaptation alternating tests of the right and left eye, only very small differences between the ultraviolet exposed eye and the control eye were observed. To test further the effects of

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the procedure used by Wald, Wolf and Zigler exposed one of O's eyes to a 1510 ml. field and simultaneously exposed the other eye to 710 ml. Testing dark adaptation alternately for each eye, no differences were found between the two eyes. However, when simultaneous pre-exposure to 1510 and 151 ml. was used, the eye exposed to the greater luminance showed an elevated dark-adaptation curve, as might be expected. These results indicate that the level of light adaptation reached by stimulating one eye with a particular luminance simultaneously affects the adaptation level of the other eye, provided that the luminance difference between the two eyes is not "too great." Wolf and Zigler's (70) results seem to imply that such a spread of adaptation effects operates in the case of ultraviolet exposure. If so, then Wald's results (18) do not necessarily contradict the earlier findings of Wolf and his colleagues. However, it should be mentioned that a controversial literature already exists concerning the facilitation of light adaptation in one eye through exposure of the other eye to higher luminances.

Wolf and Zigler also investigated the effect of ultraviolet on dark adaptation as a function of the wavelength of the test light. It was found that when the test light was red (6690 Å), ultraviolet pre-exposure had no differential effect on dark adaptation. With a green (5447 Å) test light, the rod portion of the adaptation curve was elevated with ultraviolet pre-exposure, and with blue (4880 Å) or white test lights, both rod and cone adaptation thresholds were elevated. Since Wald (18) used a red test light, this result provides a second basis for the conclusion that Wald's results do not necessarily contradict the effects of ultraviolet reported by Wolf and his associates.

In order to interpret these effects of test light wavelength, Wolf and Zigler hypothesized that ultraviolet radiation forms a temporary yellow pigment in the ocular media. This interpretation appears to be consistent with their data on the effects of test light wavelength. However, this hypothesis says nothing about the different results obtained as a function of the two methods of pre-exposure and testing described above.

Moeller et al (75) have studied the effects of ultraviolet exposure on subsequent dark adaptation, also. A Hecht-Shlaer adaptometer was modified so that illumination from either a mercury arc or from an incandescent lamp could be added to the adaptation field. The brightness of the field with the mercury arc added was then equated visually to that of the field plus the incandescent lamp. Each adaptation field was used at two brightness levels, 3.6 and 288 ml. Dark-adaptation measurements were obtained following a 10-minute exposure to one of the four adaptometer field conditions. The test field appeared on a 9.5 degrees area 10 degrees temporal of the fixation point for one-twenty fifth second. No differences between ultraviolet

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and control fields were found at either of the two brightnesses employed.

A second similar experiment was performed by Moeller et al (75) in which the intensity of the ultraviolet component in the pre-exposure field was about 6 times that employed by Wolf (65). Again, the results were interpreted as showing no differential effects of ultraviolet on subsequent dark adaptation.

Ogilvie and Ryan (76) investigated the effects of the presence of ultraviolet in the visual field on light sensitivity. The test field was illuminated by either a 3650 Å source or by a 4050 Å source of equal brightness. A 3 degrees circular test field of white light was flashed for one-fifth second 7 degrees nasal from the fixation point. For 8 normal Os, a mean threshold elevation of 0.7 log units was observed with ultraviolet present. A similar result was observed when pupil size was controlled by artificial dilation.

It was suspected that this effect was due to the increased retinal illumination the ultraviolet field induced by ocular fluorescence. According to Klang (52), the lens of the eye is the only part which fluoresces significantly. From this, Ogilvie and Ryan reasoned that aphakic Os or Os with artificial lenses implanted would not show the threshold elevation with ultraviolet present which was found for normal Os. For 2 Os with synthetic lenses, the presence of ultraviolet had no effect on the threshold. However, some threshold elevation was found for the 5 aphakic Os. It may be that the synthetic lens absorbed a sufficient proportion of the energy at 3650 Å to reduce the fluorescence of the interior parts of the eye, and, thus, account for the differences between the results for aphakics and Os with synthetic lenses (Ogilvie and Ryan estimate the transmittance of the synthetic lenses at 3650 Å to be about 70-80%). Ogilvie and Ryan concluded that the threshold elevation accompanying the presence of ultraviolet in the visual field is attributable to the effects of ocular fluorescence.

Brandenburg (77) surveying the work of Wolf prior to 1950, concludes that fluorescent sources (e.g., television screens) may interfere with subsequent dark adaptation, and in so doing, contribute to the high incidence of traffic accidents occurring at dusk.

Harmon (78) pointed out that vitamin A, one of the constituents of rhodopsin, is rapidly made ineffective by light having an energy spectrum quite similar to that of some commercial fluorescent tubes. He also describes the possibility that some fluorescent lighting installations may generate ultraviolet wavelengths of sufficient intensity to produce ocular fluorescence.

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Morgan (79) has described the ocular fluorescence produced by a fluorescent lamp. Filtering wavelengths below 3150 Å from a fluorescent tube source, he found that ocular fluorescence produced by fixating the lamp was blinding when the eye was adapted to 15-foot candles or less. Though the "blinding" effect was not noticeable at higher illumination levels, fluorescence may still occur but be masked by visible light. (This was suggested by Whiteside, also, 57.) Morgan concludes that constant adjustments necessitated by ocular fluorescence may form the basis for eyestrain.

Luckiesh and Taylor (80) criticize the work of Harmon and Morgan. They argue that the ultraviolet intensities generated by commercial fluorescent tubes are insufficient to produce biologically harmful effects. To support this, they show that sunlight contains far greater ultraviolet intensities than found in the energy spectrum of fluorescent tubes. This comparison, however, does not appear to be relevant to the determination of the effects of the ultraviolet components of fluorescent illumination on vision.

An attempt will be made at this point to summarize the research surveyed in the preceding two sections of this report.

1. Most of the work surveyed in this section has been focused on the following question: "Does exposure to wavelengths in the ultraviolet (primarily below 3650 Å) affect subsequent dark adaptation by means of a mechanism which is distinct from that which operates for wavelengths in the visible spectrum?" At this point, the weight of the evidence seems to dictate the answer, "No."

One approach in attempting to reach a definitive answer to this question can be suggested. This involves the use of a pre-exposure field illuminated only by a narrow band of ultraviolet wavelengths and equated for subjective brightness, and as nearly as possible, for hue, with a control field illuminated by a narrow band of wavelengths above 4000 Å. Following exposure to one of these two adaptation fields, dark adaptation would be tested, and the curves obtained following each of the two pre-exposure conditions compared. If young Os (with high ultraviolet sensitivity) were used, and if, as several papers reviewed in the first section of this paper suggest, ultraviolet wavelengths can be matched in hue with wavelengths above 4000 Å, this approach might provide an efficient means of answering the question.

2. Prolonged exposure to sunlight delays and decreases the rate of dark adaptation, and, in addition, reduces photopic retinal sensitivity. Tentatively, it appears that this is primarily attributable to visible radiation in the solar spectrum.

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3. When sufficient intensities of ultraviolet wavelengths are present in the visual field, the threshold for white light is elevated. This is probably due to the fluorescence of the ocular media (primarily the lens), and can be expected to be most pronounced with wavelengths near 3650 Å. It can be inferred that this effect is likely to raise differential brightness thresholds and reduce visual acuity, at least when the eye is adapted to low illumination levels.

The Effects of Ultraviolet Radiation on the "Reactivity" of the Organism

This section deals with research which indicates that ultraviolet irradiation of the organism produces increased "sensitivity," "reactivity," or capacity for certain behaviors. They seem to be more important for their suggestion of the possible effects of ultraviolet radiation than for the specific results they describe.

Sigmund (81) states that ultraviolet radiation can be viewed as a stimulant, and has conducted experiments showing that periodic ultraviolet radiation is accompanied by substantial reductions in reaction time. Since Sigmund's work is based on observations made by Dull (82), the pertinent results of Dull will be introduced first.

Dull conducted extensive studies of the seasonal variations in auditory and visual reaction time. He found that during the late summer months (August through October), mean reaction times were about 7% lower than during July or December. It is suggested that this effect is connected with the seasonal change in the intensity of ultraviolet wavelengths reaching the earth during these months, though the nature of the relationship is not explored empirically.

Sigmund noted that skin sensitivity to ultraviolet showed a pattern of seasonal variation very similar in form to the variations in reaction time reported by Dull, and he proceeded to investigate this relationship experimentally.

According to Dull's data, reaction time ordinarily increases during October, and Sigmund conducted his research during this period of "normal" seasonal increase. Two groups of subjects were used: (1) children 5-6 years of age and (2) adults. Both adults and children were divided into experimental and control groups. Children in the experimental group were irradiated with a medium erythemal dose of ultraviolet 6 times a week for 3 weeks. The head and neck were protected from the radiation. The experimental group of adults were exposed to a "complete"

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erythema 3 times a week for 3 weeks. The control groups received no irradiation. Visual reaction time was tested once each week beginning at the end of the first week of irradiation of the experimental subjects and continuing for 6 weeks. During this 6-week period, the control subjects showed a steady increase in mean visual reaction time, as Dull's data would lead one to predict. However, during the 3-week ultraviolet irradiation period, the experimental group of children showed a decrease in reaction time of about 25%. During the 3 weeks of testing which followed the termination of ultraviolet exposures, mean reaction time for these children increased to near the initial value. The same pattern was observed for the irradiated adults, though the maximum decrease in mean reaction time for them was about 15%. The differential magnitude of the effect for experimental children and adults may be due to the fact that the children received twice the number of ultraviolet exposures received by the adults.

Ronge (83) studied the effects of sub-erythemal ultraviolet exposure on the work capacity of 10-11 year old school children. The children were divided into two groups, one a control group and the other group exposed to mild ultraviolet irradiation in their school classroom. Work capacity of both groups was measured by the amount of work done on an ergonometer prior to the pulse-rate-pulse pressure product reaching 10,000. The data was gathered in a region in which there is little exposure to sunlight during the winter months.

The control group showed systematic seasonal fluctuations in work capacity, a maximum being reached early in the fall and a minimum in the early spring. The experimental subjects were exposed to ultraviolet from mid-January through mid-May the first year of the study and between September and May of the second year. Their maximum work capacity appeared about 3 months after the beginning of ultraviolet irradiation each year. It was found that the ultraviolet effect was greatest for those children having the lowest initial work capacity.

In December of the second year of the study, the control group was split, and half of the controls were given 250,000 I.U. of vitamin D (calciferol) each day while the other half served as placebo controls. Within 3 months the work capacity of the vitamin D group was comparable to that of the ultraviolet irradiated experimental subjects. During this same period, the work capacity of the placebo control group decreased.

It was concluded that exposure to sub-erythemal ultraviolet increases vitamin D utilization, and that this is the basis for the observed increase in work capacity.

Seidl (84) found that following 7 weekly erythemal doses of ultraviolet radiation work capacity was increased by 15-20% and pulse rate decreased by about 10%. No attempt was made to trace the mechanism leading to this result.

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It is possible that increased vitamin D utilization is responsible for the effects of ultraviolet exposure on reaction time reported by Sigmund (81). The fact that work capacity and reaction time follow similar patterns of seasonal variation, that both are affected by ultraviolet exposure, and that at least one (work capacity) is affected by supplementary vitamin D when ultraviolet exposure is minimal suggests that the other (reaction time) may be affected by vitamin D, also. This possibility could be adequately explored using essentially the same experimental design as that employed by Sigmund and the addition of placebo controls. (Perhaps Sigmund's results would have been more convincing had a placebo procedure -- e.g., irradiation with visible wavelengths only -- been employed.)

Altukhov (85) investigated the effects of red light and ultraviolet radiation (wavelengths not given) on conditioned response latencies and stimulus differentiation for both auditory and visual CS. The experiment was carried out at normal atmospheric pressure and at a pressure equivalent to an altitude of about 13,000 feet. Subjects were exposed to either red light or ultraviolet for 2 hours at one of the two atmospheric pressures. The brightness of ultraviolet was 0.08 ml. and of the red light, 0.10 ml. Conditioning was carried on during the 2-hour exposure to light alternating an "irritable noise" and a light CS. At the end of the 2 hours of conditioning and light exposure, 25 test trials were given. Also, differentiation on both light and sound CS was tested. For both light and sound CS, response latencies were greater for the ultraviolet condition than for the red light condition at both atmospheric pressures. In addition, differentiation was poorer under the ultraviolet condition.

These results are based on visual inspection of the conditioning curves for five subjects. Most of the effects described above appeared to be small and difficult to evaluate in the absence of a statistical analysis. However, the general inference which might be made is that ultraviolet exposure reduces the strength of the CR and impairs differentiation relative to red light.

At this time, little is known of the behavioral consequences of physiological changes which are induced by ultraviolet radiation between 3000 and 4000 Å. In the case of x-irradiation, it is known that sub-lethal doses produce relatively few known behavioral effects (86). However, a few studies of the behavioral effects of x-irradiation suggest that behavior may be affected by the consequences of relatively small x-ray dosages. Perhaps these observations will suggest possible areas of investigation for ultraviolet radiation.

Kektcheew (87) and Lenoir (88) each report that dermal x-irradiation was followed by elevation of the dark-adaptation

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threshold. Lenoir used dosages of from 2400-6240 r. The effect persisted for several days.

On several occasions, reports have appeared in the medical literature describing improved auditory acuity following x-irradiation (e.g., Richardson, 89). Girden (90) investigated the effect of several x-ray doses on the auditory threshold of dogs. He found that acuity increased an average of 5.5 db following x-irradiation. A latent period of 7 to 11 days was also observed, and the effect persisted for about 2 to 5 weeks.

Brogden and Culler (91) replicated Girden's results and obtained further information on the effect. They observed acuity gains of from 3.84 to 7.87 db. The duration of these gains was found to be independent of x-ray dosage. However, the latency of the appearance of improved acuity was inversely related to dosage. In a series of experiments, Brogden and Culler were led to the conclusion that x-irradiation of the head reduces the pituitary inhibition of the insulin-secreting tissues of the pancreas, and oversecretion of insulin follows. This produces a mild hypoglycemia. The low blood sugar level leads to reduced density and viscosity of the blood, which, after some time, spreads to the cerebrospinal and cochlear fluids. In some way, this change in the cochlear fluid appears to mediate the improved auditory acuity following x-irradiation.

Both x-rays and ultraviolet wavelengths have been described by some investigators as "stressors" (86, 92). It may be that changes associated with the "stress response" systematically alter the "sensitivity" or "reactivity" of the organism.

The results described above are summarized in the following paragraphs, and certain implications for further study are indicated:

1. It has been reported that repeated exposures to "medium" erythemal ultraviolet irradiation lowers visual reaction time. This might be investigated to find (a) whether the effect is reproducible, (b) if so, whether changes in vitamin D utilization are associated with the changes in reaction time, (c) if not related to vitamin D utilization, what mechanism is operating to produce the effect.

2. Studies are reported indicating, (a) that x-radiation of the head decreases the absolute auditory threshold, (b) that x-radiation reduces the efficiency of dark adaptation, (c) that mild ultraviolet exposure increases the capacity for work, apparently through its effects on vitamin D utilization, and (d) that mild ultraviolet exposure reduces visual reaction time. These results suggest the possibility that the "sensitivity" and/or "reactivity" of the organism may be altered by relatively mild ultraviolet exposure. Exploratory investigations might be

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initiated to determine whether or not mild ultraviolet exposure alters absolute sensory sensitivity or sensory discrimination ability in vision and/or audition, and also, whether mild ultraviolet exposure effects the rate and/or precision of motor performance.

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BIBLIOGRAPHY

1. Herschel, J. F. W.: "On the chemical action of the rays of the solar spectrum on preparations of silver and other substances, both metallic and non-metallic and on some photographic processes." Phil. Trans. Roy. Soc., 1-59 (see pp. 19-20 on "Extension of the visible prismatic spectrum: A new prismatic colour"), 1840.

2. Mathiessen: "Memoire sur le spectre solaire optique; sure le lentiprisme perfectionne." Compt. rend., 9, 112, 1844.

3. Stokes, G. G.: "On the change of refrangibility of light." Phil. Trans. Roy. Soc., 463-562 (see Note B, 558 ff.), 1852.

4. Helmholz, H.: "Uber die Empfindlichkeit der menschlichen Netzhaut fin die brechbaren Strahlen des Sonnenlichtes." Pogg. Ann. d Phys. u. Chem., 94, 205-211, 1855.

5. Coblentz, W. W. and Emerson, W. B.: Bull. Nat. Bur. Stand., 14, 168-234, 1818-1919.

6. Crawford, B. H.: "The scotopic visibility function." Proc. Phys. Soc., 62, 321-334, 1959.

7. Weaver, K. S.: "A provisional standard observer for low-level photometry." J. Optic. Soc. Amer., 39, 278-291, 1949.

8. Bartley, S. H.: "The psycholphysiological vision." in Stevens S.S. (ed.), Handbook of Experimental Psychology, 921-984, 1951.

9. Institute of Optics, Rochester University: "Extension of the scotopic visibility curve to 3130 Å and 8000 Å." Bibliography of Scientific and Industrial Reports Publication Board, No. 28539, Office of Scientific Research and Development, Report No. 1431, March 1, 1943.

10. Gibson, K. S. and Tyndall, E. P. T.: "Visibility of radiant energy." National Bureau of Standards Scientific Paper No. 475, 19, 131-191, 1923.

11. Le Grand, Y.: "La protection des yeux contre l'ultraviolet." Annals. of the Oculist, Paris, 191, 193-208, 1958.

12. Verhoeff, F. H. and Bell, Z.: "The pathological effects of radiant energy on the eye: An experimental investigation." Proc. Am. Acad. Arts and Sci., 51, 629-759, 1916.

Approved for Public Release

Contrails

13. Graham, W. P.: "The absorption of the eye for ultraviolet." Am. J. Physiol. Optics, 4, 151-160, 1923.

14. Ludvigh, E. and McCarthy, E. F.: "Absorption of visible light by the refractive media of the human eye." Arch. Ophth., 20, 37-51, 1938.

15. Kinsey, V. E.: "Spectral transmission of the eye to ultraviolet radiation." Arch. Ophth., 39, 508-513, 1948.

16. Judd, D. B.: "Colorimety." National Bureau of Standards, Circular 478, 1950.

17. Judd. D. B.: "Basic correlates of the visual stimulus." in Stevens S. S. (ed.), Handbook of Experimental Psychology, 811-867, 1951.

18. Wald, G.: "Alleged effects of the neon ultraviolet on human vision." J. Optic. Soc. Amer., 42, 171-177, 1952.

19. Ogilvie, J. C.: "Ultraviolet radiation and vision." Arch. Ophth., 50, F48-F63, 1953.

20. Bachem, A.: "Ophtholmic ultraviolet action spectra." Amer. J. Ophth., 41, 969-975, 1956.

21. Birch-Hirschfeld, A.: "Die wirkung der ultravioletten Strahlen auf das Auge." Von Graefe's Archieve, F. Ophth.. 58, 469-562, 1904.

22. Kahnemann, F.: "Experimental studies of retinal lesions caused by short-wave radiations." Ann. ottalm., Parma, 83, 471-481, 1957.

23. Mascart, M.: "Sur la visibilite des rayons ultraviolets." Compt. rend. Acad. Sci., 68, 402-403, 1869.

24. Chardonnet: "Vision des radiations ultraviolettes." Compt. rend. Acad. Sci., 96, 509-511, 1883.

25. Glancy, A. E.: "Limit of visibility in the ultraviolet." Amer. J. Physiol. Optics, 4, 145-151, 1923.

26. Saidman, J. and Dufestel: "Sur la visibilite de la portion initiale due spectre ultraviolet." Compt. rend. Acad. Sci., 182, 1173-1175, 1926.

27. Saidman, J.: "La visibilite de l'ultraviolet jusqu' a la longueur d'onde 3130." Compt. rend. Acad. Sci., 196, 1537-1539, 1933.

Approved for Public Release

Contrails

28. Goodeve, C. F.: "Vision in the ultraviolet." Nature, London, 134, 416-417, 1934.

29. de Groot, W.: "Seeing in the ultraviolet." Nature, London, 134, 494, 1934.

30. Pinegan, N. I.: "Uber die absolute empdindkichkeit des auges im ultravioletten und im sichtbaren spektrum." Compt. rend. Acad. Sci., 30, 206-211, 1941.

31. Pinegan, N. I.: "Absolute photopic sensitivity of the eye in the ultraviolet and in the visible spectrum." Compt. rend. Acad. Sci., 43, 157-158, 1944a.

32. Pinegan, N. I.: "Absolute photopic sensitivity of the eye in the ultraviolet and the visible spectrum." Nature, 154, 770, 1944b.

33. Pinegan, N. I.: "Absolute scotopic sensitivity of the eye in the ultraviolet and in the visible spectrum." Nature, 155, 20-21, 1945.

34. Goodeve, C. F., Tythgoe, R. J. and Schneider, E. E.: "The photosensitivity of visual purple solutions and the scotopic sensitivity of the eye in the ultraviolet." Proc. Roy. Soc., London, S.B. 130, 380-395, 1942.

35. Bachem, A.: "The color of ultraviolet light." Am. J. Psychol., 66, 251-260, 1953.

36. Schneider, E. E.: "Color phenomena in ultraviolet vision." Nature, London, 155, 176, 1945.

37. Friedrich, W. and Schreiber, H.: "Das Sehen des menschlichen Auges im ultraviolett: I. Die extrafovealen Schwellenwerte fur 365 Mu und 546 Mu." Arch. ges. Physiol., 246, 621-632, 1943.

38. Widmark: "Uber die grenze des sichtbaren Spektrums nach der violetten seite." Mitteil Augenblink Stockholm., Jena, 31, 1898.

39. Taylor, H. V.: "Vision in the ultraviolet." Nature, 135, 35, 1935.

40. de Groot, W.: "Vision in the ultraviolet." Nature, 135, 68-69, 1935.

41. Wright, W. D.: "The visual sensitivity of normal and aphakic observers in the ultraviolet." Ann. Psychol., 50, 169-177, 1951.

Approved for Public Release

Contrails

42. Salomon, F.: "L'absorbtion selective de la lumiere par le cristallin." Rev. Opt. (theor. instrumen.), 29, 632-637, 1950.

43. Wawilow, S. I.: "Sensitivity of the retina to the ultraviolet spectrum." Compt. rend. Acad. Sci., 21, 373-375, 1938.

44. Gaydon, A. G.: "Color sensations produced by ultraviolet light." Proc. Phys. Soc., 50, 714-720, 1938.

45. Hosoya, Y.: "Uber die alterverscheidenheit der ultraviolett absorption der menschlichen Augenmedien." Tohoku J. Exper. Med., 13, 510-523, 1929.

46. Wald, G.: "On rhodopsin in solution." J. Gen. Physiol., 21, 795-832, 1938.

47. Schneider, E. E., Goodeve, C. F. and Lythgoe, R. J.: Proc. Roy. Soc., London, S.A., 170, 102-112, 1939.

48. Wald, G.: "The photochemical basis of rod vision." J. Optic. Soc. Amer., 41, 949-956, 1951.

49. Wald, G.: "Human vision and the spectrum." Science, 101, 653-658, 1945.

50. Laurens, H.: "Physiologic effects of ultraviolet radiation." J.A.M.A., III 2385-2392, 1938.

51. Le Grand, Y.: "Sur la fluorescence du cristallin." Compt. rend. Acad. Sci., 207, 1128-1130, 1938.

52. Klang, G.: "Measurement and studies of the fluorescence of the human lens in vivo." Acta Ophth., Supp. 31, 1948.

53. Hecht, S., Hendley, C. D., Ross, S. and Richmond, P.: "The influence of exposure to intense sunlight on subsequent night vision." Bureau Medical Research Project No. X442 (Av-233-W), April 26, 1945.

54. Hecht, S., Hendley, C. D., Ross, S. and Richmond, P.: "The effect of exposure to sunlight on night vision." Amer. J. Ophth., 44, 624, 1948.

55. Clark, B., Johnson, M. L. and Dreher, R. E.: "The effect of sunlight on dark adaptation." Amer. J. Ophth., 29, 828-836, 1946.

56. Peckham, R. H. and Harley, R. D.: "Reduction in visual acuity due to excessive sunlight." Arch. Ophth., 44, 624-627, 1950.

Approved for Public Release

Contrails

57. Whiteside, T. C. D.: "The problems of vision in flight at high altitude." London: Butterworth Scientific Publications, 1957 (ACAR Dograph No. 13).

58. Heine, L.: "Klinische untersuchungen uber die wirkung gewisser glassorten auf den menschlichen lichtsinn und seine storungen." Klin. monatsbl. augenh., 76, 37-45, 1926.

59. Siegfried, W.: "Experimentelle untersuchungen uber den angeblich schadingenden ein glass der ultravioletten strahlen auf die adaptation des auges." Von Graefes Arch. Ophth., 120, 526-539, 1928.

60. Langstroth, G. O., Batho, H. F., Wolfson, J. L., Maclaren, E. H. and McLeod, M. J.: "The effect of exposure to ultraviolet and infrared radiation on the rate of darkness adaptation." Depart. of National Defense of Canada, Directorate of Chemical Warfare and Smoke, Project No. C.E. 128, January 7, 1944.

61. Keil, F. C.: "The effect of aircraft cockpit fluorescent lighting on visual acuity and dark adaptation." U. S. Air Force Sch. Aviat. Med., Project No. 64, Report No. 1, July 1, 1942.

62. Wolf, E.: "Effects of exposure to ultraviolet light on visual thresholds." Proc. Nat. Acad. Sci., 31, 236-241, 1945a.

63. Wolf, E.: "Effects of exposure to ultraviolet light on subsequent dark adaptation." Proc. Nat. Acad. Sci., 31, 349-355, 1945b.

64. Stafford, J.: "Protection from ultraviolet light." Science, 102, 14 (Suppl.), 1945.

65. Wolf, E.: "Effects of exposure to ultraviolet light on human dark adaptation." Proc. Nat. Acad. Sci., 32, 219-226, 1946.

66. Ludvigh, E. and Kinsey, V. E.: "Effect of long ultraviolet radiations on the human eye." Science, 104, 246-247, 1946.

67. Wolf, E.: "Effects of ultraviolet radiations on visual thresholds." Science, 105, 366, 1947.

68. Wolf, E.: "Effects on visual thresholds of exposure to the radiation below 400 angstroms." Tr. Am. Acad. Ophth., 53, 400-413, 1949.

69. Zigler, M. J., Wolf, E. and King, E. S.: "The influence of surrounding brightness and short-wave components of radiation on dark adaptation." J. Optic. Soc. Am., 41, 354-359, 1951.

Approved for Public Release

Contrails

70. Wolf, E. and Zigler, M. J.: "Course of dark adaptation under various conditions of pre-exposure and testing." J. Opt. Soc. Am., 45, 696-702, 1955.

71. Sexton, M., Malone, F. and Farnsworth, D.: "The effect of ultraviolet radiation from fluorescent lights on dark adaptation and visual acuity." Med. Research Lab., Report No. 169, 9, 301-317, 1950.

72. Hulburt, E. O.: "Time of dark adaptation after stimulation by various brightnesses and colors." J. Optic. Soc. Amer., 41, 402-404, 1951.

73. Peckham, R. H.: "A proposed method for predicting light adaptation." J. Optic. Soc. Amer., 42, 65, 1952.

74. Adair, E. R.: "Foveal dark adaptation as a function of the wavelength of preadaptation and test lights." Dissertation Abstr. 15, 2582 - Abstract No. 14, 677, 74 pp., 1955.

75. Moeller, G., Fooks, G., Sperling, H. G., Farnsworth, D. and Wendt, H. W.: (USN Sub Base, New London, Conn.) "Dark adaptation and the near ultraviolet." USN Submar. Med. Res. Lab. Rep., III (8), (No. 268), 12 pp., 1955.

76. Ogilvie, J. C. and Ryan, E. J.: "Threshold sensitivity to light measured with an extraneous ultraviolet source in the visual field." J. Opt. Soc. Amer., 45, 206-209, 1955.

77. Brandenburg, K. C.: "Ultraviolet effects on night vision." Ann. West. Med. Sing., 4, 302, 1950.

78. Harmon, D. B.: "Lighting and the eye." Illum. Engin., 39, 421-500, 1944.

79. Morgan, L. D.: "There is something wrong with our fluorescent lighting applications." Illum. Engin., 41, 275-285, 1945.

80. Luckiesh, M. and Taylor, A. H.: "Radiant energy from fluorescent lamps." Illum. Engin., 40, 77-88, 1945.

81. Sigmund, R.: "Effect of ultraviolet rays on reaction time in man." Strahlentherapie, 101, 623-629, 1956.

82. Dull, B.: "Wetter und gesundheit." Leipzig, 1941.

83. Ronge, H. E.: Acta Physiol. Scand., 15, Suppl. 49, 1948.

84. Seidl, E.: "Ultraviolet irradiation and muscle training." Strahlentherapie, 101, 630-633, 1956.

Approved for Public Release

Contrails

85. Altukhov, G. V.: Journal of Higher Nervous Activity (Inst. I. P. Pavlov), USSR Acad. of Sci., V. VI #3, 353-359, 1956.

86. Furchtgott, E.: "Behavioral effects of ionizing radiations." Psychol. Bull., 53, 321-334, 1956.

87. Kektcheew, K.: "On the changes of thresholds in man in achromatic vision subjected to the action of ultra-short waves, ultraviolet, and x-ray." Probl. Fiziol. Optiki, 1, 77-79, 1941.

88. Lenoir, A.: "Adaptation und rontgenbestrahlung." Radiol. clin., Basel, 13, 264-276, 1944.

89. Richardson, J. J.: "X-rays as an advance in the treatment of impaired hearing." J. Med. Assoc., Georgia, 13, 161-165, 1924.

90. Girden, E.: "Effect of coentgenrays upon hearing in dogs." J. Comp. Physiol. Psychol., 20, 263-290, 1935.

91. Brogden, W. J. and Culler, E.: "Increased acoustic sensitivity in dogs following roeutgen-radiation of the hypoplysis." Amer. J. Physiol., 119, 13-23, 1937.

92. Schreiber, H.: "Zur theorie der lichteinwirkung auf den organismas (The theory of the influence of light on the organism)." Arch. Geschwulstforsch., 6, 220-225 (see Opthal. Lit., 1054, 8(5), Abs 3660), 1954.

Approved for Public Release

Contrails

Additional References

1. Alechinsky, A.: "A propas des phenomenes de choc consecutifs a l'administration d'histidine irradiee." Compt. rend. Soc. Biol., 133, 704-707, 1940.

2. Alechinsky, A.: "The antagonistic biological effects of ultraviolet and infrared rays." Arch. belge. derm. syph., 13, 246-249, 1957.

3. Alechinsky, A.: "Zur Frange einer antagonistischen biologischen Wirkung der ultravioletten und infraroten Strahlen." Strahlentherapie, 102, 149-151, 1957.

4. Alpatov, W. W. and Nastjunkova, O. K.: "The influence of different quantities of ultraviolet radiation on division rate in paramecium." Protoplasm, 18, 281-285, 1933.

5. Anderson, T. F.: "Virus reactions inside of bacterial host cells." J. Bact., 47, 113, 1944.

6. Angus, T. C. and Taylor, H. J.: "Measurements of transmission of radiation through a non-homogeneous medium, viz., the horny layer of the human skin." Proc. Roy. Soc., B, 190, 309-318, 1931.

7. Arnold, H. L., Jr.: "Psoralens and suntan. Augmentation of skin responses to sunlight by psoralen derivatives." Hawaii Med. J., 16, 391-395, 1957.

8. Arnow, L. E.: "The formation of DOPA by the exposure of tyrosine solutions to ultraviolet radiation." J. Biol. Chem., 120, 151-153, 1937.

9. Ashwell, G. and Hickman, J.: "Effect of x-irradiation upon enzyme systems of mouse spleen." Proc. Soc. Exper. Biol. Med., 80, 407-410, 1952.

10. Audiat, J.: "Courbes thermiques d'action du rayonnement ultraviolet sur le nerf isole. Courbe de recuperation." Compt. rend. Soc. Biol., 115, 1526-1528, 1934.

11. Audiat, J.: "Courbes des temperatures d'une action toxique sur le nerf. Ses relations avec les courbes thermiques des rayons ultraviolets." Compt. rend. Soc. Biol., 115, 1528-1532, 1934.

Approved for Public Release

Contrails

12. Audiat, J., Auger, D. and Fessard, A.: "Etude des potentiels d'action des nerfs soumis au rayonnement X. Comparison avec l'action des rayons ultraviolets." Compt. rend. Soc. Biol., 116, 880-883, 1934.

13. Augenstine, L.: "Structural correlation between esterase and protease activities of trypsin." Science, 129, 718-719, 1959.

14. Bachem, A.: "Ultraviolet action spectra." Fed. Proc., 6, 71, 1947.

15. Barner, H. D. and Cohen, S. S.: "Relation of growth to lethal damage induced by ultraviolet irradiation in escherichia coli." J. Bact., 71, 149-157, 1956.

16. Bauwens, P.: "The medical use of light sources." Ann. Phys. Med., 3, 49-54, 1956.

17. Beard, H. H., Boggess, T. S. and von Hamm, E.: "Experimental production of malignant tumors in the albino rat by means of ultraviolet rays." Am. J. Canc., 27, 257-266, 1936.

18. Bechet, P. E.: "Excessive solar and phototherapeutic irradiation, a causative factor in certain diseases of the skin." Arch. Derm. Syph., 29, 221-227, 1934.

19. Bellamy, W. D. and Germain, M. T.: "An attempt to photo-reactivate ultraviolet inactivated streptococci." J. Bact., 70, 351-352, 1955.

20. Bellows, J. G. and Chinn, H.: "Biochemistry of the lens. XV. Studies on the swelling of the isolated lens." Am. J. Ophthal., 24, 979-999, 1941.

21. Berens, C. and McAlpine, P. T.: "Solar keratoconjunctivitis associated with amblyopia. Report of two cases." Am. J. Ophth., 27, 227-231, 1944.

22. Berkley, K. M.: Study on flash burns; the influence of ultraviolet radiation on the production of cutaneous burns. Report on Contract W7401-eng-49, 1 July 1954.

23. Bernhard, F.: "Die Lichtdurchlaessigkeit der menschlichen Haut und ihre Beziehung zur Absorption des Lichtes." Munchen. med. Wechnschur., 71, 427, 1924.

24. Bills, C. E.: "Physiology of the sterols, including vitamin D." Physiol. Rev., 15, 1-97, 1935.

25. Bloom, W., Zirkle, R. E. and Uretz, R. B.: "Irradiation of parts of individual cells. III. Effects of chromosomal and extrachromosomal irradiation on chromosome movements." Ann. New York Acad. Sci., 59, 503-513, 1955.

Approved for Public Release

Contrails

26. Blum, H. F., Butler, E. G. and Schmidt, S. E.: "Regeneration of limb abnormalities after ultraviolet irradiation." J. Cell. Physiol., 52, 177-186, 1958.

27. Blum, H. F. and Mathews, M. R.: "Photorecovery from the effects of ultraviolet radiation in Salamander larvae." J. Cell. Physiol., 39, 57-72, 1952.

28. Blum, H. F., Robinson, J. C. and Loos, G. M.: "The loci of action of ultraviolet and x-radiation and of photorecovery in the egg and sperm of the sea urchin arbacia punctulata." J. Gen. Physiol., 35, 323-342, 1951.

29. Blum, H. F. and Schumacher, I. C.: "Abnormal sensitivity to light in a case of possible Landry's paralysis." Ann. Int. Med., 11, 548-558, 1937.

30. von Borstel, R. C. and Wolff, S.: "Photoreactivation experiments on the nucleus and cytoplasm of the halerobracon egg." Proc. Nat. Acad. Sci., 41, 1004-1009, 1955.

31. Brachet, J.: "The metabolism of nucleic acids during embryonic development." Cold Spring Harb. Symp. Quant. Biol., 12, 18-27, 1948.

32. Brandt, C. L. and Giese, A. C.: "Photoreversal of nuclear and cytoplasmic effects of short ultraviolet radiation on parmacium caudatum." J. Gen. Physiol., 39, 735-751, 1956.

33. Brandt, C. L., Shepard, D. C. and Giese, A. C.: "The effect of nutritional state on photoreversal of ultraviolet injuries in didinium nasutum." J. Gen. Physiol., 38, 295-305, 1955.

34. Broda, E. E.: "The role of the phospholipin in visual purple solutions." Biochem. J., 35, 960-964, 1941.

35. Bruce, V. G. and Maaloe, O.: "The ultraviolet inactivation of synchronously dividing salmonella typhimurium. Post-irradiation effects cn viability." Biochim. et Biophys. Acta, 21, 227-233, 1956.

36. Burge, W. E.: "Analyses of the ash of the normal and the cataractous lens." Arch. Ophthal., 38, 435-450, 1909.

37. Burn, J. H.: "Pigmentation of the skin." Brit. J. Derm., 63, 431-440, 1951.

38. Buschke, W., Friedenwald, J. S. and Fleischmann, W.: "Studies on the mitotic activity of the corneal epithelium. Methods, the effects of colchicine, ether, cocaine, ephedrine." Bull. Johns Hopkins Hosp., 73, 143-168, 1943.

Approved for Public Release

Contrails

39. Buschke, W., Friedenwald, J. S. and Moser, S. G.: "Effects of ultraviolet irradiation on corneal epithelium: mitosis, nuclear fragmentation, post-traumatic cell movements, loss of tissue cohesion." J. Cell. Physiol., 26, 147-164, 1945.

40. Cahn, M. M. and Levy, E. J.: "Ultraviolet light factor in chlorpromazine dermatitis." A.M.A. Arch. Derm., 75, 38-40, 1957.

41. Calkins, G. N.: "Uroleptus Halseyi, N. Sp. I. The effect of ultraviolet rays." Biol. Bull., 57, 59-68, 1929.

42. Carleton, A. A.: "Rythmical periodicity in the mitotic division of animal cells." J. Anat., 68, 251-263, 1933-34.

43. Carlson, J. G.: "Mitotic behavior of induced chromosomal fragments lacking spindle attachments in the neuroblasts of the grasshopper." Proc. Nat. Acad. Sci., 24, 500-507, 1938.

44. Carlson, J. G.: "Protoplasmic viscosity changes in different regions of the grasshopper neuroblast during mitosis." Biol. Bull., 90, 109-121, 1946.

45. Carter, C. E.: "The decomposition of diphosphopyridine-nucleotide (DPN) and adenosinetriphosphate (ATP) by ultraviolet light." J. Am. Chem. Soc., 72, 1835-1838, 1950.

46. Chalkley, H. W.: "The chemistry of cell division. VII. The distribution of sulphlydryl in amoeba proteus in inter-kinesis and mitosis, as shown by the nitroprusside test." Protoplasma, 28, 489-497, 1937.

47. Cheatle, G. L.: "The immediate and remote effects of sunlight on the epidermis." Brit. Med. J., 1, 631, 1925.

48. Claesson, S., Wettermark, G. and Juhlin, L.: "Effect of ultraviolet light on mouse skin over a wide range of intensities." Nature, 180, 992, 1957.

49. Clark, J. H.: "The denaturation of egg albumin by ultraviolet radiation." J. Gen. Physiol., 19, 199-210, 1935.

50. Clark, J. H.: "The effect of ultraviolet radiation on lens protein in the presence of salts and the relation of radiation to industrial and senile cataract." Am. J. Physiol., 113, 538-547, 1935.

51. Clark, J. H.: "Photosensitization by psoralen compounds." Radiation Res., 9 (No. 1), July 1958.

Approved for Public Release

Contrails

52. Claude, A.: "Particulate components of cytoplasm." Cold Spring Harb. Symp. Quant. Biol., 9, 263-271, 1941.

53. Claude, A.: "The constitution of protoplasm." Science, 97, 451-456, 1943.

54. Coblentz, W. W.: "The physical aspects of ultraviolet radiation in vitamin D therapy,: J.A.M.A., 111, 419-423, 1938.

55. Coblentz, W. W., Stair, R. and Hogue, J. M.: "Die spektrale Erythemreaktion der menschlichen Haut auf Ultraviolett-strahlung." Strahlentherapie, 42, 373-378, 1931.

56. Colombi, C.: "Comportamento delle proteine del cristallino dopo irradiazione con R.U.V." Rass. ital. ottal., 19, 449-451, 1950.

57. Cortelyou, J. R., Amundson, L. M. and McWhinnie, M. A.: "Phase contrast study of chromatinic bodies of escherichia coli subsequent to ultraviolet irradiation." J. Bact., 71, 462-473, 1956.

58. Cunningham, I. J.: "Some biochemical and physiological aspects of copper in animal nutrition." Biochem. J., 25, 1267-1294, 1931.

59. Dartnall, H. J. A. and Goodeve, C. F.: "Scotopic luminosity curve and the absorption spectrum of visual purple." Nature, 139, 409-411, 1937.

60. Dekansi, J.: "The effect of protein hydrolysates (leukotaxine) on skin-histamine in cats." J. Physiol., 108, 233-245, 1949.

61. De Serio, N.: "Ultraviolet irradiation of several blood coagulation factors (prothrombin, factor V and factor VII)." Arch. radiol., Nap., 30, 442-452, 1955.

62. De Vecchi: "Contenuto in aminoacidi del cristallino umano catarattaso." Boll. di Oc., 16, 400, 1937.

63. Doniach, I. and Motteram, J. C.: "Sensitization of the skin of mice to light by carcinogenic agents." Nature, 140, 588, 1937.

64. Doniach, I. and Motteram, J. C.: "On the effect of light upon the incidence of tumors in painted mice." Am. J. Canc., 39, 234-240, 1940.

65. Donner, K. O.: "Spectral sensitivity of the pigeon's retinal elements." J. Physiol., 122, 524-537, 1953.

Approved for Public Release

Contrails

66. Donner, K. O. and Granit, R.: "Scotopic dominator and state of visual purple in the retina." Acta Physiol. Scand., 17, 161-169, 1949.

67. Donner, K. O. and Willmer, E. N.: "An analysis of the response from single visual-purple-dependent elements in the retina of the cat." J. Physiol., 111, 160-173, 1950.

68. Duryee, W. R.: "The nature of radiation injury to amphibian cell nuclei." J. Nat. Canc. Inst., 10, 735-795, 1949.

69. Ebbesen, I.: "Tägliche Registrierung der ultraviolett-Strahlung von Sonne und Himmel in Kopenhagen." Strahlen-therapie, 104, 609-617, 1957.

70. Eccles, J. C. and Flynn, A. J.: "Experimental photoretinitis." Med. J. Australia, 1, 339-342, 1944.

71. Edwards, R. G.: "The experimental induction of pseudogamy in early mouse embryos." Experientia, 10, 499-500, 1954.

72. Edwards, R. G.: "The experimental induction of gynogenesis in the mouse. III. Treatment of sperm with trypaflavine, toluidine blue, or nitrogen mustard." Proc. Roy. Soc., B, 149, 117-129, 1958.

73. Ehret, C. F.: "The photoreactivability of sexual activity and rythmicity in paramecium bursaria." Rad. Res., 3, 222-223, 1955.

74. Ellison, S. A., Erlanger, B. F. and Allen, P.: "Chemical reversal of ultraviolet effects on bacteria." J. Bact., 69, 536-540, 1955.

75. Epstein, J. H. and Brunsting, L. A.: "Topical application of chlorpromazine; its effect on the erythema response to ultraviolet light." J. Invest. Dermatol., 30, 91-92, 1958.

76. Erskine, R. C. A.: "Reaction of photosensitized glia cells and neurons in tissue culture to incandescent light and electronic flash." Irish J. Med. Sci., No. 395, 525-526, 1958.

77. Erskine, R. C. A.: "Relative sensitivity of acoustic nerve glia and Schwann cells in vitro to ultraviolet microirradiation." Acta Oto-Laryngol., 50, 278-286, 1959.

78. Evans, T. C.: "Effects of roentgen irradiation at low temperature on the skin of young rats." Am. J. Roentgenol., 45, 888-894, 1941.

Approved for Public Release

Contrails

79. Evans, T. C., Goodrich, J. P. and Slaughter, J. C.: "Radiosensitivity of skin of newborn rats. III. Sensitivity at different temperatures." Proc. Soc. Exp. Biol. Med., 47, 434-437, 1941.

80. Feldgerg, W.: "The action of histamine on the blood vessels of the rabbit." J. Physiol., 63, 211-216, 1927.

81. Findlay, G. M.: "Cutaneous papillomata in the rat following exposure to ultraviolet light." Lancet, 1, 1229-1231, 1930.

82. Fischer, F. P., Vermeulen, D. and Eymers, J. G.: "Uber die zur Schadigung des Auges nötigs Minimalquantität von ultraviolettem und infraroten Licht." Arch. f. Augenheilk., 109, 462-467, 1936.

83. Fitzpatrick, T. B., Lerner, A. B., Calkins, E. and Summerson, W. H.: "Mammalian tyrosinase: Melanin formation by ultraviolet irradiation." Arch. Derm. Syph., 59, 620-625, 1949.

84. Fitzpatrick, T. B. and Lerner, A. B.: "Terminology of pigment cells." Science, 117, 640, 1953.

85. Forbes, G. S. and Heidt, L. J.: "Optimum composition of uranyl oxalate solutions for actinometry." J. Am. Chem. Soc., 56, 2363-2365, 1934.

86. Gates, F. L.: "The temperature coefficient of inactivation of crystalline pepsin by ultraviolet radiation." J. Gen. Physiol., 18, 279-282, 1934.

87. Gaulden, M. E. and Carlson, J. G.: "Cytological effects of cochicine on the grasshopper neuroblast in vitro with special reference to the origin of the spindle." Exp. Cell. Res., 2, 416-433, 1951.

88. Giese, A. C.: "Sublethal effects of long wavelength ultraviolet." Science, 87, 326-327, 1938.

89. Giese, A. C.: "Ultraviolet radiation and cell division. I. Effects of λ2654 and 2804 Å on parmecium caudatum." J. Cell. Physiol., 13, 139-150, 1939.

90. Giese, A. C.: "Comparative sensitivity of sperms and eggs to ultraviolet radiations." Biol. Bull., 91, 81-87, 1946.

91. Giese, A. C.: "Radiations and cell division." Quart. Rev. Biol., 22, 253-282, 1947.

92. Giese, A. C.: "Action of ultraviolet radiation on protoplasm." Physiol. Rev., 30, 431-458, 1950.

Approved for Public Release

Contrails

93. Giese, A. C. and Crossman, E. B.: "The action spectrum of sensitization to heat with ultraviolet light." J. Gen. Physiol., 29, 79-87, 1945.

94. Giese, A. C. and Reed, E. A.: "Ultraviolet radiation and cell division. Variations in resistance to radiation with stock species and nutritional differences in paramecium." J. Cell. Physiol., 15, 395-408, 1940.

95. Giese, A. C., Brandt, C. L. and Wells, P. H.: "Photo-reactivation in colpidium colpoda." Anat. Rec., 111, 579, 1951.

96. Giese, A. C., Iverson, R. M., Shepard, D. C., Jacobson, C. and Brandt, C. L.: "Quantum relations in photoreactivation of colpidium." J. Gen. Physiol., 37, 249-258, 1953.

97. Gillman, T., Penn, J., Bronks, D. and Roux, M.: "Abnormal elastic fibers. Appearance in cutaneous carcinoma, irradiation injuries, and arterial and other degenerative connective tissue lesions in man." A.M.A. Arch. Path., 59, 733-749, 1955.

98. Goldsmith, T. H.: "The visual system of the honeybee." Proc. Nat. Acad. Sci., 44, 123-126, 1958.

99. Goldsmith, T. H. and Ruck, P. R.: "The spectral sensitivities of the dorsal ocelli of cockroaches and honeybees. An electrophysiological study." J. Gen. Physiol., 41, 1171-1185, 1958.

100. Goucher, C. R., Kamei, I. and Kocholaty, W.: "Ultraviolet inactivation and photoreactivation of azobacter." J. Bact., 72, 184-188, 1956.

101. Grady, H. G., Blum, H. F. and Kirby-Smith, J. S.: "Types of tumor induced by ultraviolet radiation and factors influencing their relative incidence." J. Nat. Canc. Inst., 3, 371-378, 1943.

102. Granit, R.: "The colour receptors of the mammalian retina." J. Neurophysiol., 8, 195-210, 1945.

103. Greenstein, J. B. and Algire, G. H.: "Comparative oxidase activity of melanotic and amelanotic melanomas." J. Nat. Canc. Inst., 5, 35-38, 1944.

104. Greenstein, J. B., Werne, J., Eshenbrenner, A. B. and Leuthardt, F. M.: "Chemical studies on human cancer. I. Cytochrome oxidase, cytochrome C, and copper in normal and neoplastic tissues." J. Nat. Canc. Inst., 5, 55-76, 1944.

Approved for Public Release

Contrails

105. Guillaume, A. C.: "Sensibilite et sensibilisation des teguments aux diverses radiations lumineuses: Mode physique et rayons lumineux sur les teguments." Bull. et mem. Soc. med. d. hop. de Paris, 50, 730-732, 1926.

106. Guillaume, A. C.: "Le pigment epidermique la penetration des rayons ultraviolette le mecanisme de l'organisme vis a vis de ces radiation." Bull. Soc. med. d. hop. de Paris, 50, 1133-1135, 1926.

107. Guillaume, A. C.: "Le mecanisme du coup de soleil acridinique." Bull. et mem. Soc. med. d. hop. de Paris, 50, 1426-1428, 1926.

108. Haas, F. L. and Doudney, C. O.: "A relation of nucleic acid synthesis to radiation-induced mutation frequency in bacteria." Proc. Nat. Acad. Sci., 43, 871-883, 1957.

109. Hamano, S.: "Photo-activity of vitamin A, cholesterol, fat and other substances by ultraviolet rays." Japan Med. World., 5, 317-319, 1925.

110. Hartman, P. E., Payne, J. I. and Mudd, S.: "Cytological analysis of ultraviolet irradiated escherichia coli. I. Cytology of lysogenic e. coli and non-lysogenic derivative." J. Bact., 70, 531-539, 1955.

111. Hausmann, W.: "Uber die tierische Lichtempfindlichkeit und über die bedingte Giftigkeit im sinne lichtbiologischen Geschehens." Strahlentherapie, 34, 87-109, 1921.

112. Haxthausen, H.: "Behandlung verschiedener Hautkrankheiten mit Lichtbädern in schwachen, Langsam steigenden Dosen." Strahlentherapie, 34, 40-45, 1929.

113. Haxthausen, H.: "Some experiments with electrophoretic patch tests." J. Invest. Derm., 24, 211-216, 1955.

114. Hayashi, Y.: "Function of aphakic eye." Tohoku J. Exp. Med., 4, 573-600, 1924.

115. Hebrard, H. and Weber, A.: "Pigmented hyperkeratosis and vasocellular epitheliomatosis after exposure to sunlight and ultraviolet rays; case report." Bull. Soc. med. mil. fr., 51, 229-231, 1957.

116. Heilbrunn, L. V. and Wilbur, K. M.: "Stimulation and nuclear breakdown in the nereis egg." Biol. Bull., 73, 557-564, 1937.

117. Heinmets, F.: "Reactivation of ultraviolet inactivated escherichia coli by pyruvate." J. Bact., 66, 455-457, 1953.

Approved for Public Release

Contrails

118. Heinmets, F., Lehman, J. J., Taylor, W. W. and Kathan, R. H.: "The study of factors which influence metabolic reactivation of the ultraviolet inactivated escherichia coli." J. Bact., 67, 511-522, 1954.

119. Hendler, E., Crosbie, R. and Hardy, J. D.: "Measurement of heating of the skin during exposure to infrared radiation." J. Appl. Physiol., 12, 177-185, 1958.

120. Henry, H. and Stacey, M.: "Histochemistry of the gram-staining reaction for microorganisms." Proc. Roy. Soc., B, 133, 391-408, 1946.

121. Henschke, U. and Schulze, R.: "Untersuchungen zum Problem der Ultraviolett-Dasimetrie; uber Pigmentierung durch langwelliges Ultraviolett." Strahlentherapie, 64, 14-42, 1939.

122. Hess, C.: "Versuche über die Einwirkung ultravioletten Lichtes auf die Linse." Arch. f. Augenheilk., 57, 185-196, 1907.

123. Heyndrick, A.: "Reactivity of sulfhydryl and disulfide groups in serum of man." Proc. Soc. Exp. Biol. Med., 96, 508-512, 1957.

124. Hinrichs, M. A.: "Ultraviolet radiation, stimulation and inhibition in lower organisms." Proc. Soc. Exp. Biol. Med., 26, 175-177, 1928.

125. Hinrichs, M. A.: "Production of lenticular apacities by ultraviolet radiation in the presence of certain salts." Proc. Soc. Exp. Biol. Med., 27, 535-538, 1929-30.

126. Hirshfield, H. I.: "Nuclear control of cytoplasmic activities." Ann. New York Acad. Sci., 78, 647-654, 1959.

127. Hirshfield, H. I. and Giese, A. C.: "Ultraviolet radiation effects on growth processes of belpharisma undulans." Exp. Cell. Res., 4, 283-294, 1953.

128. Hirshfield, H. I. and Pecora, P.: "Studies of isolated blepharisma fragments." J. Protazool., 3, 14-16, 1956.

129. Hirshfield, H. I., Zimmerman, A. M. and Marsland, D.: "Effects of ultraviolet irradiation on form and sensitivity to pressure-solation in amoeba proteus." Anat. Rec., 131, 565-566, 1958.

130. vander Hoeve, J.: "Strahlen und auge." Arch. Ophth., 116, 245-248, 1925.

Approved for Public Release

Contrails

131. Hogeboom, G. H. and Adams, M. H.: "Mammalian tyrosinase and DOPA oxidase." J. Biol. Chem., 145, 273-279, 1942.

132. Holden, F. R. and Owings, A. F.: "Protection afforded by clothing against radiation hazards." Arch. Indus. Hyg., 6, 67-73, 1952.

133. Hollaender, A.: "Ultraviolet and related radiation." Radiation Biology, Vol. 2, McGraw-Hill, 1955.

134. Hollaender, A., Greenstein, P. and Jenrette, W. V.: "Effects of ultraviolet radiation on sodium thymonucleate." J. Nat. Canc. Inst., 2, 22-28, 1941.

135. Hollaender, A. and Kimball, R. F.: "Modification of radiation-induced genetic damage." Nature, 177, 726-730, 1956.

136. Hvidberg, E., Kvorning, S. A., Schmidt, A. and Schou, J.: "Reduction of the viscosity of a potassium hyalronate solution by ultraviolet irradiation." Nature, 181, 1338, 1958.

137. Hutton-Rudolph, M.: "Photochemische Versuche au einzelnen." Helv. Physiol. et Pharmacol. Acta, 1, c15-c19, 1943.

138. Jahnke, K. and Heinzler, F.: "Ultraviolet absorption of isolated blood and urinary proteins." Schweiz. Med. Wnschr., 87, 1559-1562, 1957.

139. Jansen, H.: "Untersuchungen ueber die Faehigkeit der bactericiden Lichtstrahlen, durch die Haut zu dringen." Mittlgn. aus Finsens med. Lichtinst., 37, 4-8, 1903-04.

140. Jaquez, J. A., Huss, J., McKeehan, W., Dimitroff, J. M. and Kuppenheim, H. F.: "Spectral reflectance of human skin in the region 0.7 - 2.6 mu." J. Appl. Physiol., 8, 297-299, 1955.

141. Jess, A. and Koschella, S.: "Ueber den Einfluss des ultra-violetten Lichtes auf die Cysteinreakton der Linse." Arch. f. Ophth., 111, 370-373, 1923.

142. Jones, L. A. and Tuttle, C.: "Isolation of desired qualities of radiant energy for biological experimentation." J. Nutrition, 18, 611-618, 1939.

143. Jones, R. F.: "Application of radiant heating in the clinical laboratory." J. Clin. Path., 8, 345-347, 1955.

144. Kahnemann, F.: "Sulla retinite essudativa maculare esterna. Contrilento clinico e considerazioni etiopatogenetiche." Ann. Ottalm. Parma, 81, 125-138, 1955.

Approved for Public Release

Contrails

145. Kanazir, D.: "Metabolisme de l'adenosine triphosphate chez escherichia coli B apres irradiation par les rayons ultra-violets." Arch. Internat. Physiol., 62, 565-566, 1954.

146. Kanazir, D.: "Accumulation d'acide thymidylique chez E. coli B apres irradiation ultraviolet." Biochim. et Biophys. Acta, 13, 589-590, 1954.

147. Kanazir, D. and Errera, M.: "Metabolisme des acides nucleiques chez E. coli B apres irradiation ultraviolette." Biochim. et Biophys. Acta, 14, 62-66, 1954.

148. Kanazir, D. and Errera, M.: "Action des rayons ultraviolette sur l'acide adenosine-triphosphorique des spermatozoides de grenouille." Biochim. et Biophys. Acta, 16, 198-202, 1955.

149. Kanazir, D. and Errera, M.: "Metabolisme anaerobique de l'adenosine triphosphate (ATP) et de l'acide desoxyribo-nucleique (ADN) chez E. coli apres irradiation ultraviolette." Biochim. et Biophys. Acta, 16, 477-481, 1955.

150. Keech, M. K. and Reed, R.: "Electron microscopic study of the effects of prolonged heat and ultraviolet light on human dermis from different age groups." Ann. Rheum. Dis., 16, 198-224, 1957.

151. Keil, F. C.: The effect of aircraft cockpit fluorescent lighting on visual acuity and dark adaptation. Proj. No. 64, Report No. 1, 15 June 1954.

152. Kellenberger, E.: "Les transformations des nucleoides de escherichia coli provoquees par les rayons ultraviolets." Experientia, 8, 263-265, 1952.

153. Kelner, A.: "Action spectra for photoreactivation of ultraviolet-irradiated escherichia coli and streptomyces griseus." J. Gen. Physiol., 34, 835-852, 1951.

154. Kimball, R. F. and Gaither, N.: "The influence of light upon the action of ultraviolet on paramecium aurelia." J. Cell. Physiol., 37, 211-233, 1951.

155. Kleefeld, G.: "Use of the near ultraviolet rays in ophthalmology." Bull. Soc. belge. opht., 113, 293-307, 1956.

156. Klouwen, H. M. and Mighorst, J. C. A.: "The effect of ultraviolet irradiation on oxidative processes in human skin in relation to the appearance of erythema." Acta Physiol. Pharm. Neerl., 5, 353-355, 1957.

157. Klouwen, H. M. and Mighorst, J. C. A.: "Untersuchungen über das Entstehen des Ultraviolett-Erythems. I. Wird das

Approved for Public Release

Contrails

Erythem durch Histamin verursacht?" Strahlentherapie, 104, 618-623, 1957.

158. Kohn-Speyer, A. C.: "Effect of ultraviolet radiation on the incidence of tar cancer in mice." Lancet, 2, 1305-1306, 1929.

159. Krashogorski, N. N.: "New apparatus and method of the measurement of infrared radiations during investigation of the higher nervous activity." Zhurnal vysshei nervnoi deiatel'nosti, Moscow, 7, 619-625, 1957.

160. Krashogorski, N. N.: "Infrakrasnaia radiatsiia detei bol'nykh toksicheskoi dispepsii." Pediatriia, Moskva, 41, 44-49, 1958.

161. Krause, A. C. and Sidwell, A. E., Jr.: "The absorption spectra of visual purple and its decomposition products." Amer. J. Physiol., 121, 215-223, 1938.

162. Krieg, D. R.: A study of gene action in ultraviolet-irradiated bacteriophage, Report on Contract W7401-eng-49, 7 May 1957.

163. Langlo, L.: "The efficiency of local application of chloroquine and mepacrine in preventing the effect of ultraviolet rays on human skin." Acta derm. vener., Stock., 37, 85-87, 1957.

164. Langlo, L. and Mexmand, P. H.: "Ineffectiveness of local application of hydrocortisone in preventing effect of ultraviolet rays on human skin." Acta derm. vener., Stock., 37, 82-84, 1957.

165. Larin, N. M.: "Effect of ultraviolet irradiation on electrophoretic properties of serum proteins." Nature, 181, 65, 1958.

166. Latarjet, R.: "Photo-restauration apres irradiation x chez une bacterie lysogene." Acad. Sci., 232, 1713-1715, 1951.

167. Latarjet, R.: "Spontaneous and induced cell restorations after treatments with ionizing and non-ionizing radiations." Acta Radiol., 41, 84-100, 1954.

168. Laurens, H.: "Studies on the relative physiological value of spectral lights. III. The pupillomotor effects of wave-lengths of equal energy content." Am. J. Physiol., 64, 97-119, 1923.

169. Lehmann, J. F., Brunner, G. D. and Stow, R. W.: "Pain threshold measurements after therapeutic application of

Approved for Public Release

Contrails

ultrasound, microwaves and infrared." Arch. Phys. M., 39, 560-565, 1958.

170. Lerner, A. B. and Fitzpatrick, T. B.: "Biochemistry of melanin formation." Physiol. Rev., 30, 91-126, 1950.

171. Lerner, A. B., Fitzpatrick, T. B., Calkins, E. and Summerson, W. H.: "Enzymatic oxidation of tyrosine and dihydroxyphenylalanine by melanoma extracts." Fed. Proc., 7, 167, 1948.

172. Lerner, A. B., Fitzpatrick, T. B., Calkins, E. and Summerson, W. H.: "Mammalian tyrosinase: Preparation and properties." J. Biol. Chem., 178, 185-195, 1949.

173. Lerner, A. B., Fitzpatrick, T. B., Calkins, E. and Summerson, W. H.: "Mammalian tyrosinase: Action on substances structurally related to tyrosine." J. Biol. Chem., 191, 799-806, 1951.

174. Levy, E. J., Cahn, M. M. and Shaffer, B.: "Polymorphous light eruptions; some unusual reactions in ultraviolet light test sites." J. Invest. Derm., 28, 147-153, 1957.

175. Lewis, M. R.: "The photosensitivity of chick-embryo cells growing in media containing certain carcinogenic substances." Am. J. Cancer, 25, 305-309, 1935.

176. Lewis, S. J.: "The ultraviolet absorption spectra of blood sera." Proc. Roy. Soc., B, 89, 327-335, 1916.

177. Lewis, T. and Grant, R. T.: "Vascular reactions of the skin to injury. Part II. The liberation of a histamine-like substance in injured skin; the underlying cause of factitious urticaria and of wheals produced by burning; and observations upon the nervous control of certain skin reactions." Heart, 11, 209-265, 1924.

178. Lindegren, C. C.: "The origin of volutin on the chromosomes, its transfer to the nucleolus, and suggestions concerning the significance of this phenomenon." Proc. Nat. Acad. Sci., 34, 187-193, 1948.

179. Litwer, G.: "Die Einfluss von teringen Dosen der Ultraviolettstrahlen auf die Stabilitat des Sporulation-szyklus bei Kaninchen Coccidien." Arch. Prot., 85, 395-411, 1935.

180. Lomholt, S.: "Zur Bestimmung des Wirksamen Strahlengebietes bie der Finsenbehandlung von Lupus vulgaris mit konzen-triertem Kohlenhogenlicht." Acta Radiol., 17, 311-344, 1936.

Approved for Public Release

Contrails

181. Loofbourow, J. R., Cook, E. S. and Stimson, M. M.: "Chemical nature of proliferation-promoting factors from injured cells." Nature, 142, 573-574, 1938.

182. Luckiesh, M., Holladay, L. L. and Taylor, A. H.: "Reaction of untanned human skin to ultraviolet radiation." J. Opt. Soc. Am., 20, 423-432, 1930.

183. Luckiesh, M. and Taylor, A. H.: "Radiant energy from fluorescent lamps." Illum. Engin., 40, 77-88, 1945.

184. Ludford, R. J., Smiles, J. and Welch, F. V.: "Ultraviolet microscopy of living malignant cells." Nature, 162, 650-651, 1948.

185. Ludvigh, E.: "Determination and significance of the scotopic retinal visibility curve." A.M.A. Arch. Ophthal., 20, 713-725, 1938.

186. Luthy, H.: "Absorption Spectrometrie markloser und markhaltiger Nervenfasern im naturlichen und polarisierten ultravioletten Licht." Pflügers Arch., 253, 477-502, 1951.

187. Lutz, W.: "Zur Kenntnis der biologischen Wirkung der Strahlen auf die Haut, mit spezieller Berücksichtigung Pigmentbildung." Arch. für Dermatol. Syph., 124, 233-296, 1917.

188. Macht, D. I., Bell, F. K. and Elvers, C. F.: "Penetration of ultraviolet rays through animal tissues." Proc. Soc. Exp. Biol. Med., 23, 210-211, 1925.

189. Markert, C. L.: "Lethal and mutagenic effects of ultraviolet radiation on glomerella conidia." Exp. Cell. Res., 5, 427-435, 1953.

190. McLaren, A. D.: "Relations among cystine content, molecular weights and ultraviolet light sensitivity of proteins." Biochim. Biophys. Acta, 18, 601-602, 1955.

191. Mefferd, R. B. and Matney, T. S.: "Protection of escherichia coli against ultraviolet radiation by pretreatment with carbon monoxide." Science, 115, 116-117, 1952.

192. Meyer, H. U.: "Crossing-over in the germ line of drosophila melanogaster males following irradiation of the embryonic pale cells with ultraviolet." Genetics, 39, 982, 1954.

193. Meyer, J.: "La sensitometrie actinique en dermatologie." Ann. derm. Syph., Paris, 83, 45-55, 1956.

Approved for Public Release

Contrails

194. Meyer, J.: "Le bronzage par les sources de lumiere artificielle." Concours Med., 80, 2563-2564, 1958.

195. Miescher, G.: "Untersuchungen uber die Bedentung des Pigments fur den ultraviolette-Lichtschutz der Haut." Strahlentherapie, 45, 201-216, 1932.

196. Miescher, G.: "Sind Ultraviolett-und Sonnenbestrahlung gefährlich?" Strahlentherapie, 60, 134-141, 1937.

197. Milano, A.: "Comportamento del contenuto in aminoacidi del cristallino dopo radiazioni con R.U.V." Ann. di Ott. e. Clin. Oc., 67, 541-549, 1939.

198. Miller, E. C.: "Studies on the formation of protein-bound derivatives of 3,4-benzpyrene in the epidermal fraction of mouse skin." Canc. Res., 11, 100-108, 1951.

199. Miller, E. C., Miller, J. A., Sapp, R. W. and Weber, G. M.: "Studies on the protein-bound aminoazo dyes formed in vivo from 4-dimethylaminoazobenzene and its C-monomethyl derivatives." Canc. Res., 9, 336-343, 1949.

200. Minton, J.: "Occupational diseases of the lens and retina." Brit. M. J., 1, 392-394, 1949.

201. Montgomery, P. O'B. and Bonner, W. A.: "A new technique for ultraviolet microbeam irradiation of living cells." A.M.A. Arch. Path., 66, 418-421, 1958.

202. Montgomery, P. O'B. and Bonner, W. A.: "Ultraviolet motion picture studies of effects of ribonuclease and deoxyribo-nuclease on living HeLa cells." Proc. Soc. Exp. Biol. Med., 99, 309-311, 1958.

203. Moraes, W. R. De: "Ultraviolet ray therapy of zona ophthalmica." Rev. Brasil. Med., 14, 830-831, 1957.

204. Morris, G. E.: "Toxic Hazards. Ultraviolet radiation." New Engl. J. Med., 258, 401-402, 1958.

205. Morris, G. E.: "Toxic Hazards. Infrared radiation." New Engl. J. Med., 258, 1272-1273, 1958.

206. Morton, J. J., Luce-Clausen, E. M. and Mahoney, E. B.: "The effect of visible light on the development of tumors induced by benzpyrene in the skin of mice." Am. J. Roentgenol. Radium Ther., 43, 896-898, 1940.

207. Morton, J. J., Luce-Clausen, E. M. and Mahoney, E. B.: "Visible light and skin tumors induced with benzpyrene in mice." Canc. Res., 2, 256-260, 1942.

Approved for Public Release

Contrails

208. Morton, J. J., Mider, G. B., Luce-Clausen, E. M. and Mahoney, E. B.: "The effect of visible light on the development in mice of skin tumors and leukemia induced by carcinogens." Canc. Res., 11, 559-561, 1951.

209. Moser, F.: "Studies on a cortical layer response to stimulating agents in the arbacia egg. II. Response to chemical and physical agents." J. Exp. Zool., 80, 447-472, 1939.

210. Mottram, J. C. and Doniach, I.: "The photodynamic action of carcinogenic agents." Lancet, 1, 1156-1159, 1938.

211. Nikolova, L.: "Considerations on ultraviolet erythemas as an index of reactivity of the organism." Khirurgiia, Sofia, 9, 903-909, 1956.

212. Nilzen, A.: "Action of histamine antagonists on histamine wheal and eczema test (patch test)." Acta Dermat. Vener., 30, 1-8, 1950.

213. Norman, A.: "The nuclear role in the ultraviolet inactivation of neurospora conidia." J. Cell. Physiol., 44, 1-10, 1954.

214. Norman, A. and Rowen, J. W.: "Effects of irradiation in vivo on bacterial deoxyribonucleic acid." Biochim. et Biophys.

215. Nowak, A. and Szlenkier, E.: "Histamine level and activity in blood of persons irradiated with ultraviolet rays." Gruzlica, Warsaw, 25, 31-42, 1957.

216. Nutting, P. G.: "The reactions of the retina to light." Am. J. Physiol. Optics, 1, 142-170, 1920.

217. Ogilvie, J. C. and Ryan, J. E.: "Threshold sensitivity to light measured with an extraneous ultraviolet source in the visual field." J. Opt. Soc. Am., 45, 206-209, 1955.

218. Oksala, A.: "Den ultravioletta strälingen och ägat." Nord. Med., 50, 1561-1563, 1953.

219. O'Neal, M. A. and Griffin, A. C.: "The effect of oxypsoralen upon ultraviolet carcinogenesis in albino mice." Canc. Res., 17, 911-916, 1957.

220. Peckham, R. H.: "A proposed method for predicting light adaptation." J. Opt. Soc. Am., 42, 65, 1952.

221. Percival, G. H. and Scott, C. M.: "A study of the skin vessels in some forms of inflammation of the skin." J. Pharm., 41, 147-163, 1931.

Approved for Public Release

Contrails

222. Phillips, C.: "Skin cancer in southwest United States. Study No. 1. A statistical survey of 1482 cancers in Texas." Third Internat. Congr. on Cancer, Atlantic City, p. 32, 1939.

223. Powers, E. L. and Ehret, C. F.: "Radiation effects on cells: Genetics or physiology?" Proc. Internat. Conf. Peaceful Uses Atomic Energy, 11, 266-272, Geneva, 1955; U. N., 1956.

224. "Contribution a l'etude de l'action genetique des rayons ultraviolets sur la drosophile." C. r. Acad. Sci., 246, 986-989, 1958.

225. Pressman, B. and Lange, J.: "In situ interconversions of intramitochondrial nucleotides." Fed. Proc., 16, 235, 1957.

226. Rascanu, V., Kapri, M. and Popvici, G.: "Action des rayons x sur la fonction des centres moteurs corticaux." C. r. Soc. Biol., 129, 710-712, 1938.

227. Raut, C.: "Heritable non-genic changes induced in yeast by ultraviolet light." J. Cell. Physiol., 44, 463-475, 1954.

228. Reddi, K. K.: "Ultraviolet absorption studies on tobacco mosaic virus." Biochim. et Biophys. Acta, 24, 238-241, 1957.

229. Rentschler, H. C. and Giese, A. C.: "Injury and death resulting from microsecond flashes of ultraviolet light." J. Cell. Physiol., 17, 395-397, 1941.

230. Roaf, H. E.: "The absorption of light by the coloured globules in the retina of the domestic hen." Proc. Roy. Soc., B, 105, 371-374, 1929.

231. Roffo, A. H.: "Cancer y sol; carcinomas y sarcomas producidos por la accion del sol total." Prensa med. argent., 21, 1755-1777, 1934.

232. Roffo, A. H.: "Die Wirkung der Sonnenstrahlen in toto auf die Entwicklung der Krebskrankheit." Experimentelle Ergebnisse, Centralbl. f. allg. Path. u. path. Anat., 62, 324-334, 1935.

233. Rogers, S.: "The in vitro initiation of pulmonary adenomas in fetal mouse lung by a single exposure to ultraviolet irradiation of wavelength 2537 Å." J. Nat. Canc. Inst., 15, 1001-1004, 1955.

234. Rohrschneider, W.: "Linsenschädigung durch ultraviolette Strahlen im Tierversuch." (Graefe's) Arch. f. Ophthal., 135, 282-292, 1936.

Approved for Public Release

Contrails

235. Ronge, H. E.: "Ultraviolet irradiation with artificial illumination." Acta Physiol. Scand., 15 (Suppl. 49), 1-191, 1948.

236. Ross, A. A. C.: "Activity of the anti-histamine drugs. A method of assessment in man." Lancet, 2, 62-63, 1951.

237. Rothman, S.: "In vitro studies on pigmentation. I. The oxidation of tyrosine by ultraviolet irradiation. II. Influence of ascorbic acid on oxidation of tyrosine by ultraviolet irradiation." J. Invest. Derm., 5, 61-75, 1942.

238. Rottier, P. B.: "The erythematogenous action of ultraviolet light on human skin. I. The measurements of the spectral response with continuous and intermittent light." J. Clin. Invest., 32, 681-689, 1953.

239. Rusch, H. P. and Kline, B. E.: "The influence of a rest period on the carcinogenicity of ultraviolet irradiation applied in interrupted doses." Canc. Res., 6, 486, 1946.

240. Saidman, J.: "Sur la visibilite de l'ultraviolet jusqu'a la longueur d'onde." C. r. Acad. Sci., 196, 1537, 1933.

241. Sarachek, A.: "Ultraviolet inactivation of saccharomyces during the budding cycle." Exp. Cell. Res., 6, 45-55, 1954.

242. Sax, K.: "The time factor in x-ray production of chromosome aberrations." Proc. Nat. Acad. Sci., 25, 225-233, 1939.

243. Schanz, F. and Stockhausen, K.: "Zur Ätiologie des Glasmacherstars." Arch. f. Ophthal., 73, 553-560, 1909-10.

244. Schechtman, A. M. and Baronofsky, I. D.: "Analysis of experimental production of bifid tails by ultraviolet irradiation." Proc. Soc. Exp. Biol. Med., 39, 209-210, 1938.

245. Schoenborn, H. W.: "Protection against lethal damage induced by ultraviolet radiation." J. Protozool., 3, 97-99, 1956.

246. Schultz, F.: "Neue Gesichtspunkte in den prinzipiellen Fragen der Lichttherapie." Dermat. Ztschr., 17, 319, 1910.

247. Schultze, W. and Rothman, S.: "Ueber die Absorption des entzuendungserregenden ultravioletten Lichtes in menschlichen Haut." Strahlentherapie, 22, 736, 1926.

248. Scott, F. :. and Molhuysen-v.d. Walle, S. M. C.: "Light dermatoses." Dermatologica, 116, 96-104, 1958.

249. Seelig, M. G. and Cooper, Z. K.: "Light and tar cancer. An experimental study, with a critical review of the

Approved for Public Release

Contrails

literature on light as a carcinogenic factor." Surg., Gyn., Obst., 56, 752-761, 1933.

250. Setlow, R. and Doyle, B.: "The action of monochromatic ultraviolet light on proteins." Biochim. et Biophys. Acta, 24, 27-41, 1957.

251. Siliato, F.: "Nuovi orientamenti sulla interpretazione patogenetica della retinite da abbagliamento." Ann. Ottalm., Parma, 83, 199-204, 1957.

252. Smith, F. C.: "The ultraviolet absorption spectra of certain aromatic amino-acids, and of the serum proteins." Proc. Roy. Soc., B, 104, 198-205, 1929.

253. Stadler, L. J.: "The comparison of ultraviolet and x-ray effects on mutation." Cold Spring Harb. Symp. Quant. Biol., 9, 168-178, 1941.

254. Stavely, H. E. and Bergmann, W.: "The photoactivity of irradiated cholesterol, a pseudo-photographic effect." Am. J. Canc., 30, 749-752, 1937.

255. Stein, G.: "Ultraviolet absorption investigation of melanins." Nature, 175, 472, 1955.

256. Stein, L.: "Ophthalmoulminescence. Definitions and descriptions of effects." Am. J. Ophthal., 44, 360-372, 1957.

257. Sykowski, T.: "Dendritic type of keratitis produced by a welding arc." Am. J. Ophth., 34, 99-100, 1951.

258. Tamura, S., Ikui, H., Nakano, K., Hiwatashi, R. and Oshio, S.: "Ocular lesions from the atomic bomb." Ganka Rinsho Iho, 40, 90, 1946.

259. Tanaka, Y.: "Staphyloma corneae, especially keloid-like thickening of the cornea by the atom bomb." Niigata Igakkai Zasshi, 61, 1947.

260. Tansley, K., Spear, F. G. and Glücksmann, A.: "The effect of gamma rays on cell division in the developing rat retina." Brit. J. Ophthal., 21, 273-298, 1937.

261. Tarras-Wahlberg, B.: "Uber den Histamingehalt der haut nach ultraviolettbestrahlung." Klin. Wnschr., 16, 958-960, 1937.

262. Taussig, J., Cooper, Z. K. and Seeling, M. G.: "The effect of light on benzpyrene cancer in mice." Surg., Gyn., Obst., 66, 989-993, 1938.

Approved for Public Release

Confidential

263. Thomson, M. L.: "The cause of changes in sweating rate after ultraviolet radiation." J. Physiol., 112, 31-42, 1951.

264. Thomson, M. L.: "Relative efficiency of pigment and horny layer thickness in protecting the skin of Europeans and Africans against solar ultraviolet radiation." J. Physiol., 127, 236-246, 1955.

265. Tomizawa, J. I. and Sunakawa, S.: "The effect of chloramphenicol on deoxyribonucleic acid synthesis and the development of resistance to ultraviolet irradiation in E. coli infected with bacteriophage T2." J. Gen. Physiol., 39, 553-565, 1956.

266. Tost, M.: "Zur Begutachtung von Fundusveränderungen bei der Ophthalmia electria." Ztschr. f. arztl. fortbild., 52, 560-565, 1958.

267. Tronnier, H.: "Course of erythema in human skin and different erythema-pigment distribution in various ultraviolet radiators." Strahlentherapie, 101, 641-646, 1956.

268. Tschachotin, S.: "Die mikroskopische Strahlenstichmethode, line Zelloperationsmethode." (Vorlaufige Mitteilung.), Biol. Centralbl., 32, 623-630, 1912.

269. Tschachotin, S.: "Das zytologische Mikroexperiment. (Untersuchungen an isolierten Zellen mit der Mikrostrahlstichmethode)." Arch. f. exp. Zellforsch., 19, 498-506, 1937.

270. Tullis, J. L.: Mutations induced in lower forms of life by ultraviolet rays and chemical substances. Technical Report No. ONRL 18-45, 15 February 1954.

271. Uesaki, H.: "Influence of ultraviolet rays on vitamin B_2 content inthe retinochoroidal tissue. Parts I and II." Acta Soc. Ophth. Japan, 59, 661-664; 1291-1298, 1955. (Abstracted in Am. J. Ophth., 41, 569, 1956.)

272. Uretz, R. B.: "Additivity of x-rays and ultraviolet light in the inactivation of haploid and diploid yeast." Rad. Res., 1, 564-565, 1954.

273. Uretz, R. B., Bloom, W. and Zirkle, R. E.: "Irradiation of parts of individual cells. II. Effects of an ultraviolet microbeam focused on parts of chromosomes." Science, 120, 197-199, 1954.

274. Vles, F., de Coulon, A. and Ugo, A.: "Sur les facteurs de l'evolution des cancers de gandron chez la Souris." Compt. rend. Acad. Sci., 193, 893-896, 1931.

Approved for Public Release

Contrails

275. Wainwright, S. D. and Nevill, A.: "Some effects of post-irradiation treatment with metabolic inhibitors and nutrients upon ultraviolet irradiated spores of streptomyces T12." Can. J. Microbiol., 1, 416-426, 1955.

276. Wainwright, S. D. and Nevill, A.: "Relationships between postradiation treatments with iodoacetate and light." Can. J. Microbiol., 1, 427-439, 1955.

277. Wainwright, S. D. and Nevill, A.: "Modification of the biological effects of ultraviolet irradiation by post-irradiation treatment with iodoacetate and peptone." J. Gen. Microbiol., 12, 1-12, 1955.

278. Wald, G.: "The spectral sensitivity of the human eye. I. A spectral adaptometer." J. Opt. Soc. Am., 35, 187-196, 1945.

279. Wald, G. and Griffin, D. R.: "The change in refractive power of the human eye in dim and bright light." J. Opt. Soc. Am., 37, 321-336, 1947.

280. Walker, J.: "The effect of ultraviolet irradiation on the spores of trichophyton sulferium." A.M.A. Arch. Derm., 78, 153-156, 1958.

281. Waller, M. D.: "The measurement of actinic erythema produced by ultraviolet radiation with special reference to the latent time." Proc. Roy. Soc., B, 108, 258-263, 1931.

282. Waller, M. D.: "The relation between energy doses of ultraviolet radiation and actinic erythema produced." Proc. Roy. Soc., B, 108, 264-270, 1931.

283. Walther, J. B. and Dodt, E.: "Elektrophysiologische Untersuchungen über die Ultraviolettempfindlichkeit von Insekterraugen." Experienta, 13, 333-337, 1957.

284. Walther, J. B. and Dodt, E.: "Die Spektralsensitivitat von Insekten-Komplexaugen im Ultraviolett bis 290 mu." Ztschr. f. Naturforsch., 14, 273-278, 1959.

285. Warren, S.: "Histopathology of radiation lesions." Physiol. Rev., 24, 225-238, 1944.

286. Wells, P. H.: "The influence of visible light on recovery of cells from ultraviolet radiation injury." Turtox News, 28, July 1950.

287. Wells, P. H. and Hamilton, T. J.: "Photoreversal of lethal and molt retarding effects of ultraviolet radiation on milkweed bug nymphs." Anat. Rec., 117, 644, 1953.

Approved for Public Release

Contrails

288. Wennig, F.: "Changes in blood elements caused by ultraviolet irradiation in oxygen atmosphere." Wien. Med. Wnschr., 106, 1067-1069, 1956.

289. Whitaker, D. M.: "Counteracting the retarding and inhibitory effects of strong ultraviolet on fucus eggs by white light." J. Gen. Physiol., 25, 391-397, 1942.

290. Whitehead, H. A.: "A factor released by irradiated cells that induces recovery of viability of ultraviolet irradiated bacteria." Can. J. Microbiol., 1, 266-270, 1955.

291. Widmark, E. J.: "Ueber den Einfluss des Lichtes auf die vorderen Medien des Auges." Skand. Arch. f. Physiol., 1, 264-330, 1889.

292. Witken, E. M.: "Time, temperature and protein synthesis: A study of ultraviolet induced mutation in bacteria." Sympos. Quant. Biol., 21, 123-140, 1956.

293. Wolf, E.: "Effects of exposure to ultraviolet light on human dark adaptation." Proc. Nat. Acad. Sci., 32, 219-226, 1946.

294. Woodside, E. E. and Kocholaty, W.: A comparison of ultraviolet sensitivity and photoreactivation phenomena in radiation resistant and sensitive strains of escherichia coli. AMRL Proj. No. 6-59-08-014; Report No. 142, 2 July 1945.

295. Woodard, V. W. and Clark, C. M.: "Genetic and non-genetic effects of radiations in neurospora." Science, 121, 641-642, 1955.

296. Wright, G. H.: "The effects of skin-blackening on warmth sensations and thresholds." Clin. Sci., 17, 43-53, 1958.

297. Zigler, M. J., Wolf, E. and King, E. S.: "The influence of surround brightness and short-wave components of radiation on dark adaptation." J. Opt. Soc. Am., 41, 354-359, 1951.

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