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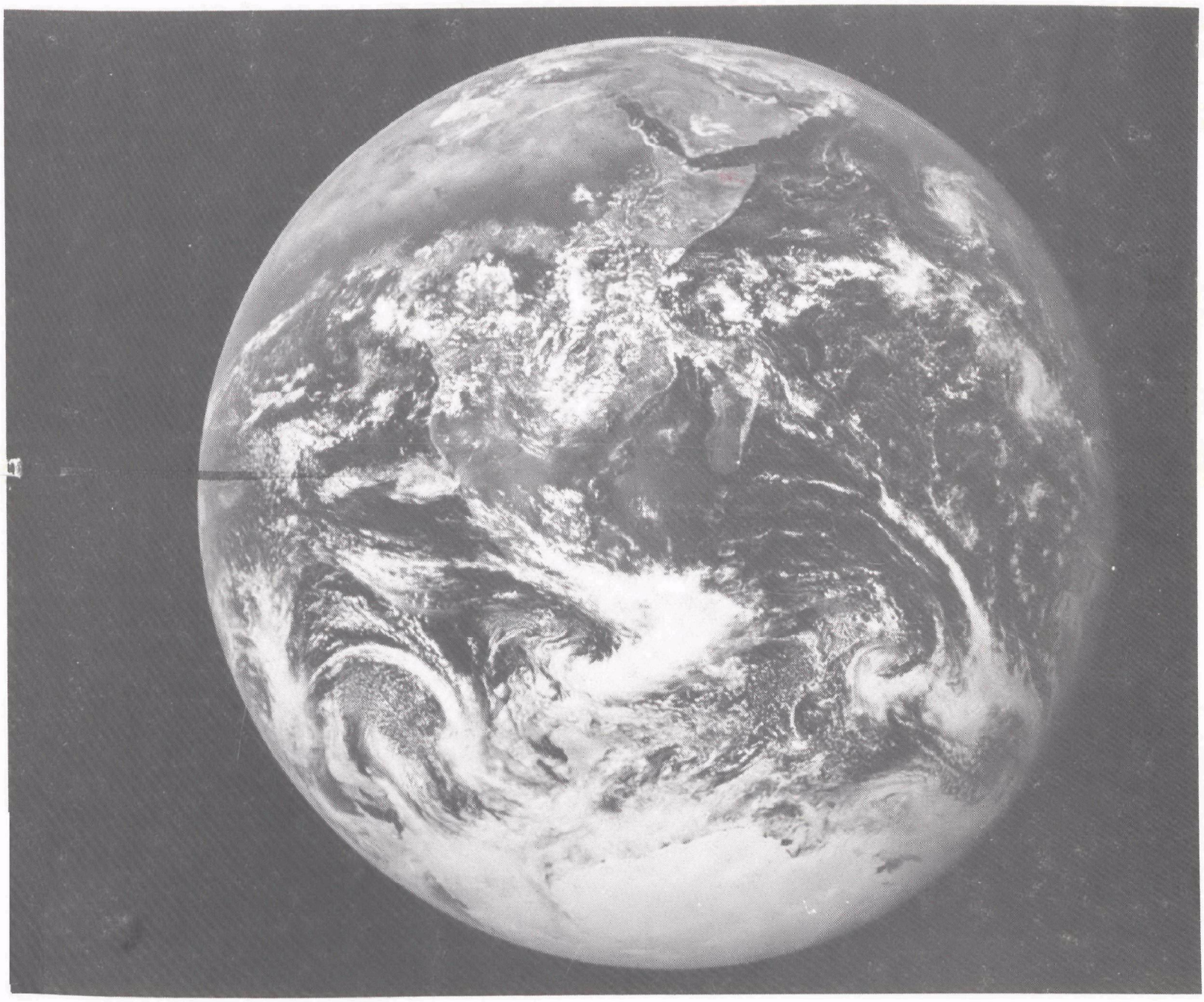
EFFECTS OF CHANGES IN STRATOSPHERIC OZONE AND GLOBAL CLIMATE

Volume 2: Stratospheric Ozone



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EFFECTS OF CHANGES IN STRATOSPHERIC OZONE AND GLOBAL CLIMATE

Volume 2: Stratospheric Ozone

Edited by
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This report represents the proceedings of the INTERNATIONAL CONFERENCE ON HEALTH AND ENVIRONMENTAL EFFECTS OF OZONE MODIFICATION AND CLIMATE CHANGE sponsored by the United Nations Environment Programme and the U.S. Environmental Protection Agency. The purpose of the conference was to make available the widest possible set of views. Accordingly, the views expressed herein are solely those of the authors and do not represent official positions of either agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

This document is part of a four volume report that examines the possible consequences of projected changes in stratospheric ozone and global climate resulting from emissions of chlorofluorocarbons, carbon dioxide, methane, and other gases released by human activities. In June 1986, the United Nations Environment Programme and the U. S. Environmental Protection Agency sponsored an International Conference on the Health and Environmental Effects of Ozone Modification and Climate Change, which was attended by scientists and officials, representing twenty-one countries from all areas of the world.

This volume examines the possible effects of ozone depletion. Volume 1 of the proceedings provides an overview of the issues as well as the introductory remarks and reactions from top officials of the United Nations and the United States. Volumes 3 and 4 focus on the effects of the change in climate and rise in sea level that might result from a global warming.

This report does not present the official views of either the U.S. Environmental Protection Agency or the United Nations Environment Programme.

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INTRODUCTION

Overview of the Effects of Changing the Atmosphere

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INTRODUCTION

Society is conducting a global experiment on the earth's atmosphere. Human activities are increasing the worldwide atmospheric concentrations of chlorofluorocarbons, carbon dioxide, methane, and several other gases. A growing body of scientific evidence suggests that if these trends continue, stratospheric ozone may decline and global temperature may rise. Because the ozone layer shields the earth's surface from damaging ultraviolet radiation (UV) future depletion could increase the incidence of skin cancer and other diseases, reduce crop yields, damage materials, and place additional stress on aquatic plants and animals. A global warming from the "greenhouse effect" could also threaten human health, crop yields, property, fish, and wildlife. Precipitation and storm patterns could change, and the level of the oceans could eventually rise.

To improve the world's understanding of these and other potential implications of global atmospheric changes, the United Nations Environment Programme (UNEP) and the U.S. Environmental Protection Agency (EPA) sponsored an International Conference on the Health and Environmental Effects of Ozone Modification and Climate Change during the week of June 16-20, 1986. The conference brought together over three hundred researchers and policy makers from approximately twenty nations. This four-volume report presents the seventy-three papers that were delivered at the conference by over eighty speakers, including two U.S. Senators, top officials from UNEP and EPA, some of the leading scientists investigating the implications of atmospheric change, and representatives from industry and environmental groups. Volume 1 presents a series of overview papers describing each of the major areas of research on the effects of atmospheric change, as well as policy assessments of these issues by well-known leaders in government, industry, and the environmental community. Volumes 2, 3, and 4 present the more specialized papers on the impacts of ozone modification, climate change, and sea level rise, respectively, and provide some of the latest research in these areas. This paper summarizes the entire four-volume report.

OZONE MODIFICATION

Atmospheric Processes

The ozone in the upper part of the atmosphere--known as the stratosphere--is created by ultraviolet radiation. Oxygen (O_2) is continuously converted to ozone (O_3) and back to O_2 by numerous photochemical reactions that take place in the stratosphere, as Stordal and Isaksen (Volume 1) describe. Chlorofluorocarbons and other gases released by human activities could alter the current balance of creative and destructive processes. Because CFCs are very stable compounds, they do not break up in the lower atmosphere (known as the troposphere). Instead, they slowly migrate to the stratosphere, where ultraviolet radiation breaks them down, releasing chlorine.

Chlorine acts as a catalyst to destroy ozone; it promotes reactions that destroy ozone without being consumed. A chlorine (Cl) atom reacts with ozone (O_3) to form ClO and O_2. The ClO later reacts with another O_3 to form two molecules of O_2, which releases the chlorine atom. Thus, two molecules of ozone are converted to three molecules of ordinary oxygen, and the chlorine is once again free to start the process. A single chlorine atom can destroy thousands of ozone molecules. Eventually, it returns to the troposphere, where it is rained out as hydrochloric acid.

Stordal and Isaksen point out that CFCs are not the only gas released by human activities that might alter the ozone balance. Increasing concentrations of methane in the troposphere increase the water vapor in the stratosphere, which helps create ozone. Carbon dioxide and other greenhouse gases (discussed below) warm the earth's surface but cool the upper atmosphere; cooler stratospheric temperatures slow the process of ozone depletion. Nitrous oxide (N_2O) reacts with both chlorine and ozone.

Stordal and Isakson present results of possible ozone depletion over time, using their two-dimensional atmospheric-chemistry model. Unlike one-dimensional models which provide changes in ozone in the global average, this model calculates changes for specific latitutdes and seasons. The results show that if concentrations of the relevant trace gases grow at recent levels, global average ozone depletion by 2030 would be 6.5 percent. However, countries in the higher latitudes (60°N) would experience 16 percent depletion during spring. Even in the case of constant CFC emissions, where global average depletion would be 2 percent by 2030, average depletion would be 8 percent in the high northern latitudes.

Watson (Volume 1) presents evidence that ozone has been changing recently more than atmospheric models had predicted. As Plate 1 shows, the ozone over Antarctica during the month of October appears to have declined over 40 percent in the last six to eight years. Watson also discusses observations from ozone monitors that suggest a 2 to 3 percent worldwide reduction in ozone in the upper portion of the stratosphere (thirty to forty kilometers above the surface), which is consistent with model predictions. Finally, he presents preliminary data showing a small decrease since 1978 in the total (column) ozone worldwide. However, he strongly emphasizes that the data have not yet been fully reviewed and that it is not possible to conclusively attribute observed ozone depletion to the gases released by human activities. While

there are several hypotheses to explain why ozone concentrations have declined, none have been adequately established; nor did any of the atmospheric models predict the measured loss of ozone over Antarctica.

Ultraviolet Radiation

Many of the chemical reactions investigated by atmospheric scientists take place only in the stratosphere because they are caused by types of radiation only found in the upper atmosphere. As Frederick (Volume 1) explains, the sun emits radiation over a broad range of wavelengths, to which the human eye responds in the region from approximately 400 to 700 nanometers (nm). Wavelengths from 320 to 400 nm are known as UV-A; wavelengths from 280 to 320 nm are called UV-B, and wavelengths from 200 to 280 nm are known as UV-C.

Frederick explains why attention has primarily focused on the UV-B part of the spectrum. The atmosphere absorbs virtually all UV-C, and is expected to continue to do so under all foreseeable circumstances. On the other hand, UV-A is not absorbed by ozone.[1]. By contrast, UV-B is partially absorbed by ozone, and future depletion would reduce the effectiveness of this shield.

We now examine the potential implications of such changes on human health, plants, aquatic organisms, materials, and air pollution.

Effects on Human Health

The evidence suggests that solar ultraviolet radiation induces skin cancer, cataracts, suppression of the human immune response system, and (indirectly through immunosuppression) the development of some cutaneous infections, such as herpes. Emmett (Volume 1) discusses the absorption of UV radiation by human tissue and the mechanisms by which damage and repair may occur.

Emmett also examines UV radiation as the cause of aging of the skin and both basal and squamous skin cancers. In reviewing the role of UV radiation in melanoma (the most frequently fatal skin cancer), he states that some evidence suggests this link, but that currently there is no acceptable animal model that can be used to explore or validate this relationship. He concludes that future studies must focus on three major factors--exposure to solar radiation, individual susceptibility, and personal behavior. Waxler (Volume 1) presents evidence of a link between UV-B exposure and cataracts.

Volume 2 presents specific research results and provides more detail on many of the aspects covered in this volume. Scotto presents epidemiological evidence linking solar radiation with skin cancers, other than melanoma. His analysis suggests that Caucasians in the United States have a 12 to 30 percent chance of developing these cancers within their lifetimes, even without ozone depletion. Armstrong examines the role of UV-B exposure to melanoma in a study of 511 matched melanoma patients and control subjects in Western Australia. He shows that "intermittent exposure" to sunlight was closely associated with this type of cancer.

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[1] However, O_2 and N_2 reflect some UV-A back to space.

In a paper examining nonmelanoma skin cancer in Kuwait, Kollias and Baqer (Volume 2) show that despite the presence of protective pigmentation, 75 percent of cancers occur on the 10 percent of the skin exposed to sunlight. A second paper on skin cancer presents experimental evidence suggesting that the mechanism by which skin cancer could occur involves disruption of the cytoskeleton from exposure to UV-A and UV-B light (Zamansky and Chow, Volume 2).

The pathways by which suppression of the immune response might be triggered are explored in papers by DeFabo and Noonan, Daynes et al., and Elmets et al (all Volume 2). Davies and Forbes (Volume 2) show that mice exposed to UV-B radiation had a decrease in lifetime that was proportional to the quantity of radiation and not directly related to the incidence of skin cancer.

Possible implications of immune suppression of diseases and the mechanisms by which it occurs are still uncertain. However, several papers in Volume 2 suggest that in addition to skin cancer and contact hypersensitivity, diseases influenced by UV-B induced immune suppression include leishmaniasis and herpes infections. Fisher et. al (Volume 2) show that at least one sunscreen effectively protects mice exposed to UV-B radiation from sunburn; but it does not stop the immune suppression from interfering with a contact hypersensitivity (allergic) reaction.

Effects on Plants

The effects of increased exposure to UV-B radiation on plants has been a primary area of research for nearly a decade. Teramura (Volume 1) reports that of the two hundred plants tested for their sensitivity to UV-B radiation, over two-thirds reacted adversely; peas, beans, squash, melons, and cabbage appear to be the most sensitive. Given the complexities in this area of research, he warns that these results may be misleading. For example, most experiments have used growth chambers. Studies of plants in the field have shown them to be less sensitive to UV-B. Moreover, different cultivars of the same plant have shown very different degrees of sensitivity to UV-B radiation. Finally, Teramura suggests that potential effects from multiple stresses (e.g., UV-B exposure plus water stress or mineral deficiency) could substantially alter a plant's response to changes in UV-B alone.

In Volume 2, Teramura draws extensively from the results of his five years of field tests on soybeans. His data show that a 25 percent depletion in ozone could result in a 20 to 25 percent reduction in soybean yield and adverse impacts on the quality of that yield. Because soybeans are the fifth largest crop in the world, a reduction in yields could have serious consequences for world food supplies. However, some soybean cultivars appear to be less susceptible to UV-B radiation, which suggests that selective crop breeding might reduce future losses, if it does not increase vulnerability to other environmental stresses.

Bjorn (Volume 2) examines the mechanisms by which plant damage occurs. His research relates specific wavelengths with those aspects of plant growth that might be susceptible, including the destruction of chloroplast, DNA, or enzymes necessary for photosynthesis.

Aquatic Organisms

Aquatic plants would also be adversely affected by increased ultraviolet radiation. Worrest (Volume 1) points out that most of these plants, which are drifters (phytoplankton), spend much of their time near the surface of the water (the euphotic zone) and are therefore exposed to ultraviolet radiation. A reduction in their productivities would be important because these plants directly and indirectly provide the food for almost all fish. Furthermore, the larvae of many fish found in the euphotic zone would be directly affected, including crabs, shrimp, and anchovies. Worrest points out that fish account for 18 percent of the animal protein that people around the world consume, and 40 percent of the protein consumed in Asia.

An important question is the extent to which current UV-B levels are a constraint on aquatic organisms. Calkins and Blakefield (Volume 2) conclude that some species are already exposed to as much UV-B as they can tolerate. Thomson (Volume 2) shows that a 10 percent decrease in ozone could increase the number of abnormal larvae as much as 18 percent. In a study of anchovies, a 20 percent increase in UV-B radiation over a 15-day period caused the loss of all the larvae within a 10-meter mixed layer in April and August.

Many other factors could affect the magnitude of the impacts on specific species, ecosystems, and the food chain. An important mechanism by which species could adapt to higher UV-B incidence would be to reduce their exposure by moving further away from the water's surface during certain times of the day or year when exposure is greatest. Haeder (Volume 2) suggests, however, that for certain species such avoidance may be impaired by UV-B radiation.

Even for those organisms that could move to avoid exposure, unwanted consequences may result. Calkins and Blakefield present model results showing that movement by phytoplankton away from sunlight to reduce exposure to a 10 percent increase in UV-B would result in a 2.5 to 5 percent decrease in exposure to the photosynthetically active radiation on which their growth depends. Increased movement requires additional energy consumption, while changes in location may affect the availability of food for zooplankton, which could cause other changes in shifts in the aquatic food chain.

To a certain extent, losses within a particular species of plankton may be compensated by gains in other species. Although it is possible that no net change in productivity will occur, questions arise concerning the ecological impacts on species diversity and community composition (Kelly, Volume 1). Reductions in diversity may make populations more susceptible to changes in water temperatures, nutrient availability, diseases, or pollution. Changes in community composition could alter the protein content, dry weight, or overall food value of the initial stages of the aquatic food chain.

Polymer Degradation and Urban Smog

Current sunlight can cause paints to fade, transparent window glazing to yellow, and polymer automobile roofs to become chalky. These changes are likely to occur more in places closer to the equator where UV-B radiation is greater. They are all examples of degradation that could accelerate if depletion of the ozone layer occurs. Andrady and Horst (Volume 2) present a case study of the potential magnitude of loss due to increased exposure to

UV-B radiation on polyvinyl chloride (PVC). This chemical is used in outdoor applications where exposure to solar radiation occurs over a prolonged period. It is also used in the construction industry in siding and window frames and as a roofing membrane.

To analyze the potential economic impact of future ozone depletion on PVC, the authors assumed that the future service life of polymers would be maintained by increasing the quantity of light stabilizers (titanuim dioxide) used in the product. As a result, the costs associated with increased UV-B radiation would be roughly equal to the costs of increased stablizers. Preliminary results show that for a 26 percent depletion by 2075, the undiscounted costs would be $4.7 billion (1984 dollars).

Increased penetration of UV-B radiation to the earth's surface could play an important role in the formation of ground level oxidants (smog). UV-B affects smog formation through the photolysis of formaldehyde, from which radicals are the main source for deriving chain reactions that generate photochemical smog. Whitten and Gery (Volume 2) analyze the relationship between UV-B, smog, and warmer temperatures. The results of this preliminary study of Nashville, Philadelphia, and Los Angeles show that large depletions in stratospheric ozone and increases in temperature would increase smog by as much as 50 percent. In addition, because oxidants would form earlier in the day and closer to population centers (where emissions occur), risks from exposure could increase by an even higher percentage increase. Whitten and Gery also report a sensitive relationship between UV-B and hydrogen peroxide, an oxidant and precursor to acid rain.

CLIMATE CHANGE

The Greenhouse Effect

Concern about a possible global warming focuses largely on the same gases that may modify the stratospheric ozone: carbon dioxide, methane, CFCs, and nitrous oxide. The report of a recent conference convened by UNEP, the World Meteorological Organization, and the International Council of Scientific Unions concluded that if current trends in the emissions of these gases continue, the earth could warm a few degrees (C) in the next fifty years (Villach 1985). In the next century, the planet could warm as much as five degrees (NAS 1983), which would leave the planet warmer than at any time in the last two million years.

A planet's temperature is determined primarily by the amount of sunlight it receives, the amount of sunlight it reflects, and the extent to which the atmosphere retains heat. When sunlight strikes the earth, it warms the surface, which then reradiates the heat as infrared radiation. However, water vapor, CO_2, and other gases in the atmosphere absorb some of the energy rather than allowing it to pass undeterred through the atmosphere to space. Because the atmosphere traps heat and warms the earth in a manner somewhat analogous to the glass panels of a greenhouse, this phenomenon is commonly known as the "greenhouse effect." Without the greenhouse effect of the gases that occur naturally in the atmosphere, the earth would be approximately 33°C colder than it is currently (Hansen et al. 1984).

In recent decades, the concentrations of greenhouse gases have been increasing. Since the beginning of the industrial revolution, the combustion of fossil fuels, deforestation, and a few other activities have released enough CO_2 to raise atmospheric concentrations by 20 percent; concentrations have risen 8 percent since 1958 (Keeling, Bacastow, and Whorf 1982). More recently, Ramanathan et al. (1985) examined the greenhouse gases other than CO_2 (such as methane, CFCs, and nitrous oxide), and concluded that these other gases are likely to double the warming caused by CO_2 alone. Using these results, the Villach Conference estimated that an "effective doubling" of CO_2 is likely by 2030.[2]

Hansen et al. (Volume 1) and Manabe & Wetherald (Volume 1) present the results that their climate models predict for an effective doubling of CO_2. Both models consider a number of "climatic feedbacks" that could alter the warming that would directly result from CO_2 and other gases released by human activities. Warmer temperatures would allow the atmosphere to retain more water vapor, which is also a greenhouse gas, thereby resulting in additional warming. Ice and snow cover would retreat, causing sunlight that is now reflected by these bright surfaces to be absorbed instead, causing additional warming. Finally, a change in cloud cover might result, which could increase or decrease the projected warming. Although the two models differ in many ways, both conclude that an effective doubling of greenhouse gases would warm the earth's surface between two and four degrees (C).

Hansen et al. project the doubling to occur between 2020 and 2060. They also provide estimates of the implications of temperature changes for Washington, D.C., and seven other U.S. cities for the middle of the next century. For example, Washington would have 12 and 85 days per year above 38°C (100°F) and 32°C (90°F), respectively, compared with 1 and 35 days above those levels today. While evenings in which the thermometer fails to go below 27°C (80°F) occur less than once per year today in that city, they project that such evenings would occur 19 times per year. (See Plates 2 and 3 for worldwide maps of historical and projected temperature changes.)

Water Resources

Manabe and Wetherald (Volume 1) focus on the potential changes in precipitation patterns that might result from the greenhouse warming. They project substantial increases in summer dryness at the middle latitudes that currently support most of the world's agriculture. Their model also projects increased rainfall for late winter.

Beran (Volume 1) reviews the literature on the hydrological and water resource impacts of climate change. He expresses some surprise that only twenty-one papers could be found that address future water resource impacts. One of the problems, he notes, is that there is a better scientific understanding of how global average temperatures and rainfall might change, than for the changes that specific regions may experience. Nevertheless, he

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[2] Studies on the greenhouse effect generally discuss the impacts of a carbon dioxide doubling. By "effective doubling" we refer to any combination of increases in concentrations of the various gases that causes a warming equal to the warming of a doubling of carbon dioxide alone.

demonstrates that useful information can be extracted by studying the implications of particular scenarios.

Nicholson (Volume 3) shows how historical changes in water availability have caused problems for society in the past. The best lesson of climatic history, she writes, "is that agricultural and economic systems must be flexible enough to adapt to changing conditions and, in the face of potential water scarcity, systems must be designed that require minimum use of resources." Wilhite (Volume 3) examines drought policies in Australia and the United States, concluding that the lack of national drought plans could substantially impair the ability of these two nations to successfully adapt to hydrologic changes resulting from the greenhouse warming.

Cohen (Volume 3) examines the potential implications of the global warming for water levels in the Great Lakes that separate Canada from the United States. Using results from the models of both Hansen et al. and Manabe & Wetherald, he concludes that lake levels could drop 10 to 30 centimeters. This drop would significantly reduce the capacity of ocean-going vessels that enter the Great Lakes. On the other hand, such a drop might be viewed as a benefit by the owners of critically eroding property whose homes are currently threatened by historically high lake levels. Street-Perrott et al. (Volume 3) discuss the historic impacts of changes in climate on the levels of lakes in North America, South America, Australia, and Africa.

Gleick (Volume 3) uses scenarios from the Hansen et al. and Manabe & Wetherald models (as well as a third developed by the National Center for Atmospheric Research) to drive a water-balance model of the Sacramento Basin in California. He finds that reductions in runoff could occur even in months where precipitation increases substantially, because of the increased rates of evaporation that take place at higher temperatures. He also points out that the models predict that changes in monthly runoff patterns will be far more dramatic than changes in annual averages. For seven of ten scenarios, soil moisture would be reduced every month of the year; for the other three cases, slight increases in moisture are projected for winter months. Mather (Volume 3) conducts a detailed analysis for southern Texas and northern Mexico; examines in less detail twelve regions around the world; and projects shifts in global vegetation zones.

Agriculture and Forestry

The greenhouse warming could affect agriculture by altering water availability, length of growing season, and the number of extremely hot days. Increased CO_2 concentrations could also have two direct impacts unrelated to climate change: At least the laboratory, plants grow faster (the CO_2 fertilization effect) and retain moisture more efficiently. The extent to which these beneficial effects offset the impacts of climate change will depend on the extent to which global warming is caused by CO_2 as opposed to other greenhouse gases, which do not have these positive impacts.

Parry (Volume 1) provides an overview of the potential impacts of climate change on agriculture and forestry. He points out that commercial farmers plan according to the average year, while family and subsistence farmers must ensure that even in the worst years they can make ends meet. Thus, the commercial farmer would be concerned about the impact of future climate change

on average conditions and average yields, while farmers at the margin would be most concerned with changes in the probability of (for example) a severe drought that causes complete crop failure. Parry notes that the probability of two or more anomalous years in a row could create disproportionately greater problems for agriculture. For example, a persistent drought in the U.S. Great Plains from 1932 to 1937 contributed to about two hundred thousand farm bankruptcies.

Parry discusses a number of historical changes in climate. The Little Ice Age in western Europe (1500-1800 A.D.) resulted in the abandonment of about half the farms in Norway, an end to cultivation of cereals in Iceland, and some farmland in Scotland being permanently covered with snow. Concerning the late medieval cooling (1250-1500) he writes: "The failure to adapt to the changing circumstances is believed to explain much of the Norse decline. The Norse continued to emphasize stock-raising in the face of reduced capacity of the already limited pastures. The option of exploiting the rich seas around them, as the Inuit (Eskimos) successfully did, was not taken up . . . This is an extreme example of how governments can fail to identify and implement appropriate policies of response." It also suggests that effective responses can reduce damages from climate change.

The paper reviews a number of studies that project impacts of climate change on agriculture. "Warming appears to be detrimental to cereals in the core wheat-growing areas of North America and Europe." If no precipitation changes take place, a one-degree warming would decrease yields 1 to 9 percent while a two-degree (C) warming would decrease yields 3 to 17 percent. Parry also discusses how particular crop zones might shift. A doubling of CO_2 would substantially expand the wheat-growing area in Canada due to higher winter temperatures and increased rainfall. In Mexico, however, temperature stresses would increase, thereby reducing yields.

A number of studies have been conducted using the models of Hansen et al., Manabe & Wetherald, and others. Although these projections cannot be viewed as reliable forecasts, they do provide consistent scenarios that can be useful for examining vulnerability to climate change. Parry indicates that investigations of Canada, Finland, and the northern USSR using the model by Hansen et al. show reduced yields of spring-sown crops such as wheat, barley, and oats, due to increased moisture stress early in the growing period. However, switching to winter wheat or winter rye might reduce this stress. Parry goes on to outline numerous measures by which farmers might adapt to projected climate change.

Waggoner (Volume 3) points out that the global warming would not affect plants uniformly. Some are more drought-resistant than others, and some respond to higher CO_2 concentrations more vigorously than others. C_3 plants, such as wheat, respond to increased CO_2 more than C_4 plants such as maize. Thus, the CO_2 fertilization effect would not help the farmer growing C_4 crops accompanied by C_3 weeds. Waggoner also examines the impact of future climate change on average crop yields and pests, and the probability of successive drought years. He concludes that although projections of future changes are useful, historical evidence suggests that surprises may be in store, and that "agricultural scientists will be expected to aid rather than watch mankind's adaptation to an inexorable increase in CO_2 and its greenhouse effect."

The impact of future climate change on yields for spring wheat in Saskatchewan, Canada, is the subject of the paper by Stewart (Volume 3). Using the output from the Hansen et al. model (Volume 1), which projects that the effective doubling of carbon dioxide would increase average annual temperatures in that region by 4.7°C, he estimates that the growing season would start two or three weeks earlier and end three or four weeks later. Although average precipitation during the growing season would increase, he also finds that the area would become more prone to drought. The impact of climatic change would be to reduce yields 16 to 26 percent. Stewart estimates that the fertilization effect of a CO_2 doubling would reduce the losses to 6 to 15 percent. Cooter (Volume 3) examines the economic impact of projected climate change on the economy of Oklahoma, concluding that the Gross State Product would decline 75 to 300 million dollars. (The state's gross product in 1985 was approximately 50 billion dollars.)

Fritts (Volume 3) examines tree rings to assess how past changes in climate have affected forests, and concludes that tree rings are useful for estimating past changes in climate. Solomon and West (Volume 3) discuss the results of their efforts to model the future impacts. Considering the impact of climate change caused by doubled CO_2 without the fertilization effect, they find that "biomass (for boreal forests) declines for 50-75 years as warming kills off large boreal forest species, before new northern hardwoods can grow into the plot."

"Warming at the transition site causes an almost immediate response in declining biomass from dieback of mature trees, and in decline of tree mass as large trees die and are temporarily replaced by small young trees," they write. "The deciduous forest site . . . results in permanent loss of dense forest. One might expect the eventual appearance of subtropical forests similar to those in Florida today, but the real difficulty is the moisture balance (which is) more similar to those of treeless Texas today, than to those of southern Florida." Solomon and West go on to show how the fertilization effect from increased concentrations of CO_2 could offset part but not all of the drop in forest productivity.

Sea Level Rise

One of the most widely recognized consequences of a global warming would be a rise in sea level. As Titus (Volume 1) notes, global temperatures and sea level have fluctuated over periods of one hundred thousand years, with temperatures during ice ages being three to five degrees (C) lower and sea level over one hundred meters lower than today. By contrast, the last interglacial period (one hundred thousand years ago) was one or two degrees warmer than today, and sea level was five to seven meters higher.

The projected global warming could raise sea level by heating and thereby expanding ocean water, melting mountain glaciers, and by causing polar glaciers in Greenland and Antarctica to melt and possibly slide into the oceans. Thomas (Volume 4) presents new calculations of the possible contribution of Antarctica and combines them with previous estimates for the other sources, projecting that a worldwide rise in sea level of 90 to 170 centimeters by the year 2100 with 110 centimeters most likely. However, he also estimates that if the global warming is substantially delayed, the rise in sea level could be cut in half. Such a delay might result either from

actions to curtail emissions or from the thermal lag induced by the oceans' ability to absorb heat.

On the other hand, Thomas also estimates that if a warming of four degrees results from a CO_2 doubling (which the model of Hansen et al. projects) and concentrations continue to grow after 2050, the rise could be as great as 2.3 meters. He also notes that an irreversible deglaciation of the West Antarctic Ice Sheet might begin in the next century, which would raise sea level another six meters in the following centuries.

Titus (Volume 1) notes that these projections imply that sea level could rise 30 centimeters by 2025, in addition to local subsidence trends that have been important in Taipei, Taiwan; Venice, Italy; the Nile Delta, Egypt; and most of the Atlantic and Gulf Coasts of the United States. The projected rise in sea level would inundate low-lying areas, destroy coastal marshes and swamps, erode shorelines, exacerbate coastal flooding, and increase the salinity of rivers, bays, and aquifers.

Bruun (Volume 4) argues that with a combination of coastal engineering and sound planning, society can meet the challenge of a rising sea. He discusses a number of engineering options, including dikes (levees) and seawalls, and adding sand to recreational beaches that are eroding, with a section on the battle that the Dutch have fought with the sea for over one thousand years. Goemans (Volume 4) describes the current approach of the Dutch for defending the shoreline, and estimates that the cost of raising their dikes for a one meter rise in sea level would be 10 billion guilders, which is less than 0.05 percent of their Gross National Product for a single year.

Goemans concludes that there is no need to anticipate such a rise because they could keep up with it. However, he is more concerned by the two-meter scenario: "Almost immediately after detection, actions would be required. It is not at all certain that decision-makers act that fast. . . . The present flood protection strategy came about only after the tragic disaster of 1953. When nobody can remember a specific disaster, it is extremely difficult to obtain consensus on countermeasures." For his own country, Goemans sees one positive impact: Referring to the unique experience of Dutch engineering firms in the battle with the sea, he suggests that "a rising sea may provide a new global market for this expertise." But he predicts that "the question of compensation payments may come up," for the poorer countries who did not cause climate change but must face its consequences.

Broadus et al. (Volume 4) examine two such countries in detail: Egypt and Bangladesh. The inhabited areas of both countries are river deltas, where low-lying land has been created by the sediment washing down major rivers. In the case of Egypt, the damming of the Nile has interrupted the sediment, and as the delta sinks, land is lost to the Mediterranean Sea. Broadus et al. estimate that a 50-centimeter rise in global sea level, when combined with subsidence and the loss of sediment, would result in the loss of 0.3 to 0.4 percent of the nation's land area; a 200-centimeter rise would flood 0.7 percent. However, because Egypt's population is concentrated in the low-lying areas, 16 and 21 percent of the nation's population currently reside in the areas that would be lost in the two scenarios.

The situation would be even more severe in Bangladesh. As Plate 4 shows, this nation, which is already overcrowded, would lose 12 to 28 percent of its total area, which currently houses 9 to 27 percent of its population. Moreover, floods could penetrate farther inland, which could leave the nation more vulnerable to the type of tropical storm that killed 300,000 people in the early 1970s, especially if the frequency of tropical storms doubled due to warmer water temperatures, which deSylva (Volume 4) projects. Broadus et al. conclude that the vulnerability of Bangladesh to a rise in sea level will depend in large measure on whether future water projects disrupt land-creating sediment washing down the Ganges.

Bird (Volume 4) examines the implications of sea level rise for other African and Asian nations, as well as Australia. While holding back the sea may be viable in Australia, he shows areas in New Guinea where people live in small cottages on the water's edge on a barrier island that almost certainly would be unable to justify construction of a dike. He also points to the Philippines, where many people have literally "taken to the water," living in small boats and maintaining fishing nets in their own plots of bay instead of land. Current wetlands, he suggests, may convert to these shallow bays, with people converting to a more water-based economy.

Leatherman (Volume 4) examines the implications of sea level rise for South America. He notes that such popular resorts as Copacabana Beach, Brazil; Punta del Este, Uruguay; and Mar del Plata, Argentina, are already suffering serious erosion. He concludes that because of the economic importance of resorts, governments will allocate the necessary funds to maintain their viability. However, he predicts that "coastal wetlands will receive benign neglect" and be lost.

Park et al. (Volume 4) focus on the expected drowning of coastal wetlands in the United States. Using a computer model of over 50 sites, they project that 40-75 percent of existing U.S. coastal wetlands could be lost by 2100. Although these losses could be reduced to 20-55 percent if new wetlands form inland as sea level rises, the necessary wetland creation would require existing developed areas to be vacated as sea level rises, even though property owners would frequently prefer to construct bulkheads to protect their property. Because coastal wetlands are important for many commerically important seafood species, as well as birds and furbearing animals, Park et al. conclude that even a one-meter rise in sea level would have major impacts on the coastal environment.

DeSylva (Volume 4) also examines the environmental implications of sea level rise, noting that in addition to wetlands being flooded, estuarine salinity would increase. Because 66 to 90 percent of U.S. fisheries depend on estuaries, he writes that these impacts could be important. He also suggests that coral reefs could become vulnerable because of sea level rise, increased temperatures, and the decrease in the pH (increased acidity) of the ocean.

Kuo (Volume 4) examines the implications of sea level rise for flooding in Taipei, Taiwan, and coastal drainage in general. Although Taipei is upstream from the sea, Kuo concludes that projected sea level rise would cause serious problems, especially because Taiwan is also sinking. He recommends that engineers around the world take "future sea level rise into consideration . . . to avoid designing a system that may become prematurely obsolete."

Gibbs (Volume 4) estimates that sea level rise could result in economic damages in Charleston, South Carolina, equal to as much as 25 percent of the annual product of the community. Anticipatory measures, however, could reduce these impacts by half. Gibbs finds that in some areas actions should be taken today, in spite of the current uncertainty regarding future rates of sea level rise, while for other areas it would be more prudent to wait until uncertainties are resolved.

Ken Smith, a realtor from coastal New Jersey, reacts to the other papers presented in Volume 4. He argues that the issue of sea level rise should be taken seriously today, but laments the fact that many of his fellow realtors make comments such as "What do you care? You won't be around to see it!" and the scientific community is "a bunch of eggheads who don't want us (to build on the coast) anyway." Smith suggests that part of the resistance to taking the issue seriously is that there are a number of "naturalists" who oppose building near the shore, and "most of the discussion seems to come from the 'naturalist' camp." Nevertheless, Smith argues that "the solutions--if there are any--should be contemplated now as part of a concerted global effort. This is a beautiful world, and we are its stewards."

Human Health and Ecological Impacts

Climate and weather have important impacts on human health. A global warming would increase the stresses due to heat, decrease those due to cold, and possibly enable some disease that require warm year-round temperatures to survive at higher latitudes. Kalkstein et al. (Volume 3) present a preliminary statistical assessment of the relationship of mortality rates to fluctuations in temperature in New York City. They find that a two to four degree (C) warming would substantially increase mortality rates in New York City, if nothing else changed. However, they caution that if New Yorkers are able to acclimatize to temperatures as well as people who currently live in U.S. cities to the south, fewer deaths would occur. Kalkstein et al. write that knowledgeable observers disagree about whether and how rapidly people adapt to higher temperatures; some people undoubtedly adjust more readily than others.

Although people may be able to adapt to changes in climate, other species on the planet would also be affected and may not be as able to control their habitats. Peters and Darling (Volume 3) examine the possibility that changes in climate would place multiple stresses on some species which would become extinct, resulting in a significant decline in biodiversity. (Mass extinctions appear to have accompanied rapid changes in temperatures in the past.)

Throughout the world reserves have been set aside where targeted species can remain relatively free of human intrusion. Peters and Darling ask: Will these reserves continue to serve the same function if the climate changes? In some cases, it will depend on whether the reserve's boundaries encompass areas to which plants and animals could migrate. Some species may be able to migrate "up the mountain" to find cooler temperatures; coastal wetlands could migrate inland. A northerly migration of terrestrial species would be possible in the undeveloped arctic regions of Alaska, Canada, and the Soviet Union; but human development would block migration of larger animals in many areas.

POLICY RESPONSES

Papers by UNEP Deputy Director Genady Golubev and EPA Administrator Lee Thomas (both in Volume 1) provide official views on the nature of the effects from projected changes in the atmosphere and the role of their institutions in addressing those changes. Golubev notes that while "the global issues are complex, uncertainty exceeds understanding, and patience is prudence," there is an other side to the story: "Our legacy to the future is an environment less benign than that inherited from our forbearers. The risks are sufficient to generate a collective concern that forebodes too much to wait out the quantifications of scientific research. Advocating patience is an invitation to be a spectator to our own destruction."

Golubev also points out that UNEP has worked for the achievement of the Vienna Convention for the Protection of the Ozone Layer, in which many nations have agreed to act in concert to address an environmental issue whose impacts have not yet been detected. Yet he notes that the agreement is for cooperation in research and does not yet bind nations to observe limits in production and emissions of gases that could deplete stratospheric ozone.

Thomas points out that both the potential depletion of ozone and the global warming from the greenhouse effect are examples of environmental problems that involve the "global commons." Because all nations contribute to the problem and experience the consequences, only an international agreement is likely to be effective. He urges scientists around the world to discuss this issue with their colleagues and key officials.

Richard Benedick, Deputy Assistant Secretary in the U.S. Department of State (Volume 1), describes the emerging international process addressing the ozone issue. Although the process for addressing climate change has not yet proceeded as far, he writes, "from my perspective as a career diplomat, it appears that the greenhouse effect has all the markings of becoming a high visibility foreign policy issue. . . . How we address this issue internationally depends to a great extent on our success or failure in dealing with the ozone depletion issue."

J.P. Bruce (Volume 1) of Environment Canada presents the issue of atmospheric change in the context of air pollution in general. He writes that ozone modification and climate change are "urgent issues," especially because important long-term decisions are being made today whose outcomes could be strongly affected by changes in climate and the ozone layer. Bruce recommends that emissions of CFCs be reduced, and concludes that "a new approach, a new ethic towards discharging wastes and chemical materials into the air we all breathe must soon be adopted on a international scale."

Two U.S. Senators also provide their reactions. John Chafee from Rhode Island (Volume 1) describes hearings that his Subcommittee on Environmental Pollution held June 10-11, 1986. "Why are policy makers demanding action before the scientists have resolved all of the questions and uncertainties?" he asks. "We are doing so because there is a very real possibility that society--through ignorance or indifference, or both--is irreversibly altering the ability of our atmosphere to perform basic life support functions for the planet." Albert Gore, Jr. from Tennessee, who has chaired three congressional hearings on the greenhouse effect, explains why he has introduced a bill in

the U.S. Senate to establish an International Year of the Greenhouse Effect. "The legislations would coordinate and promote domestic and international research efforts on both the scientific and policy aspects of this problem, identify strategies to reduce the increase of carbon dioxide and trace gases, investigate ways to minimize the impact of the greenhouse effect, and establish long-term research plans." Senator Gore closes by quoting Sherwood Rowland (discussed below): "What's the use of having developed a science well enough to make predictions, if in the end all we're willing to do is stand around and wait for them to come true?" Both Senators call for immediate action to reduce global use of CFCs.

John S. Hoffman (Volume 1) emphasizes the inertia of the atmosphere and oceans. Because there are time lags between changes in emission rates, atmospheric concentrations, and changes in ozone and global warming temperatures, the types of management strategies must be different from those that are appropriate for controlling, for example, particulate pollution, where the problem goes away as soon as emissions are halted. CFC emissions would have to be cut 80 percent simply to keep concentrations from increasing. Although constant concentrations would prevent ozone depletion from worsening, Hoffman points out that even if we hold the concentrations of greenhouse gases constant once the earth has warmed one degree, the planet would warm another degree as the oceans come into equilibrium. Thus it might be impossible to prevent a substantial warming if we wait until a small warming has taken place.[3]

The final section of this volume presents the papers from the final day of the conference. Peter Usher of UNEP recounts the evolution of the ozone issue. Following Rowland and Molina's hypothesis that chlorofluorocarbons could cause a depletion of stratospheric ozone in 1974, UNEP held a conference in 1977 that led to a world plan of action to assess the issue and quantify risks. Since that time, UNEP has held numerous coordinating meetings leading up the the Vienna Convention. However, Usher suggests that motivating international effort on the greenhouse effect will be more difficult: "Prohibition of nonessential emissions of relatively small amounts (to control ozone depletion) is one thing, limiting emissions of carbon dioxide from coal- and oil-burning is quite another."

Dudek and Oppenheimer of the Environmental Defense Fund (U.S.) analyze some of the costs and benefits of controlling emissions of CFCs. They estimate that by holding emissions constant, 1.65 million cases of nonmelanoma skin cancers could be prevented worldwide, and that the cost of these controls would be 196 to 455 million dollars, depending on the availability of alternative chemicals.

Two former high-ranking environmental officials in the United States argue that we should be doing more to address these problems. John Topping recommends that CFCs in aerosol spray cans, egg cartons, fast-food containers, and other nonessential uses be phased out, and that people recognize that

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[3] Titus (Volume 1) and Thomas (Volume 4) also explore inertia, noting that even if temperatures remained constant after warming somewhat, sea level would rise at an accelerated rate as the oceans, mountain glaciers, and ice sheets came into equilibrium with the new temperature.

along with energy conservation, nuclear power is the most likely alternative to fossil fuels over the next generation or two. He also recommends that society take steps to minimize the impacts of climate change and sea level rise, for example, by requiring environmental impact statements to consider the likely impacts.

Gus Speth, president of the World Resources Institute, recommends international efforts to stop tropical deforestation; a production cap for chlorofluorocarbons; increased energy conservation; advanced technologies for producing electricity from natural gas; and tighter regulations to limit carbon monoxide from automobiles, which would indirectly limit increases in atmospheric methane. He agrees with Topping that environmental impact statements for projects that could contribute to or be affected by climate change or ozone modification should consider these impacts.

Doniger and Wirth, from the Natural Resources Defense Council (U.S.), argue that the current uncertainties are no longer a reason to wait for additional information: "With the stakes so high, uncertainty is an even more powerful argument for taking early action." These authors conclude that sharp reductions in CFCs are necessary, pointing out that even with a production cap, atmospheric concentrations of these gases will continue to grow. Therefore, Doniger and Wirth propose an 80 percent cut in production over the next five years for CFCs 11 and 12, the halons, and perhaps some other compounds, with a complete phaseout in the next decade.

Richard Barnett of the Alliance for a Responsible CFC Policy (which represents CFC using industries) agrees that we should not delay all action until the effects of ozone depletion and climate change are felt; but he "would hardly characterize the activities over the last twelve years as 'wait and see' . . . The science, as we currently understand it, however, tells us that there is additional time in which to solidify international consensus. This must be done through discussion and negotiation, not through unilateral regulation."

Barnett adds that industry should "take precautionary measures while research and negotiations continue at the international level. We will continue to examine and adopt such prudent precautionary measures as recapturing, recycling, and recovery techniques to control CFC emissions; transition to existing alternative CFCs that are considered to be more environmentally acceptable; practices to replace existing systems at the expiration of their useful lives to equipment using other CFC formulations; practices in the field to prevent emissions where possible; encouragement of CFC users to look for processes or substances that are as efficient, safe, and productive--or better--than what is presently available."

Barnett concludes that "these environmental concerns are serious, but their successful resolution will require greater global cooperation in conducting the necessary research and monitoring, and in developing coordinated, effective, and equitable policy decisions for all nations."

We hope that this paper has provided the reader with a "road map" through the papers of this four-volume report on the potential effects of changing the atmosphere. But we have barely scratched the surface of each, just as the existing research has barely scratched the surface in discovering and

demonstrating the possible risks of ozone modification and climate change. A continual evolution of our understanding will be necessary for our knowledge to stay ahead of the global experiment that society is conducting.

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HUMAN HEALTH

UVR Dose-Dependent Life Shortening in Mice

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The type of ultraviolet referred to as "UV-B" has properties and effects of considerable significance. It is absorbed strongly by proteins, nucleic acids, and other biological molecules, and also by ozone and other environmental materials. Absorption by atmospheric ozone largely determines how much of the sun's UV-B emission reaches the earth's surface. The fraction that does reach the surface is strongly absorbed by exposed tissue and delivers sufficient energy (about 4 eV per photon) to produce direct or indirect chemical reactions, some of which are deleterious.

Because of this strong absorption, most of the direct effects of UV-B are limited to superficial tissues a few tens of microns thick: in animals this usually means the cutis or skin, and specialized external tissues such as the eye. Many animals have superficial nonliving products such as scales, hair, or feathers that absorb most or all of the UV-B. In others, such as our species, constitutive absorbers, such as stratum corneum and melanin, can be enhanced in response to damage, reducing the effects of subsequent exposure. Most biological consequences of UV-B, both damage and secondary responses, are not only superficial, but are limited to areas not protected by natural absorbers or artificial coverings such as clothing. Characteristic UV-B induced effects in humans, including acute (sunburn), subacute (pigmentation) and chronic (skin cancer, "aging"), almost invariably appear in the most exposed areas such as face and hands.

Not all effects of UV-B are so highly localized, and a few appear to be beneficial. Exposure to UV-B can prevent the bone disease rickets: the mechanism involves production, in the exposed skin, of a form of vitamin D from an inactive precursor, followed by redistribution of this essential vitamin to the rest of the body. There are also reports, less easily measured or explained, of beneficial physiologic (work capacity) and psychologic effects of sunlight, at least some of which appear to be related to UV-B.

Another category of systemic effects was recognized with the observation that specific immune responses could be inhibited by UV-B. One such response, the ability to reject certain transplanted tumors, was reduced or eliminated on a system-wide, apparently permanent basis by localized UV-B irradiation. In another response, the development of contact hypersensitivity, suppression by UV-B is transient and the degree of systemic involvement is less clearly established.

We recently described an effect apparently attributable to UV-B that is unarguably systemic. Mice that had been exposed chronically to "sun-like" radiation exhibited an increase in mortality that was proportional to the quantity of radiation delivered. Since the effect was observed in experiments designed to study UV-B induced skin cancer, many of the mice had cancers at the time of their death. However, the severity of the carcinogenic response was not sufficient to account for the difference in survival, especially considering that such tumors are relatively slow growing and rarely metastasize. The amount of UV-B delivered was less, in most cases, than that required to produce any obvious acute or chronic damage, although some skin thickening undoubtedly occurred.

Survival curves in healthy, genetically uniform laboratory mice usually exhibit two distinct phases. Initially deaths are random and usually infrequent, resulting in a slow, roughly linear decline that is unrelated to specific treatment conditions. These deaths usually result from injuries or other types of errors. Subsequently, survival declines rapidly; in a particular strain, under uniform conditions, the time of this terminal phase is quite reproducible.

In the strain of mice with which we have the most experience, the approximate median age at death is about 90 weeks. Daily exposure to an amount of radiation corresponding to about two minutes at midday summer sunshine produces a detectable reduction in this value, and doubling the daily dose reduced median survival to about 50 weeks. Higher doses, some of which produced chronic irritation, produced further but less dramatic decreases in life span. In all cases it is the second, precipitous stage of the survival curves that is affected. Comparison of light sources delivering different qualities of radiation indicates that most or all of the effect is attributable to the UV-B portion of the spectrum.

To our knowledge, no studies have been designed specifically to examine the effects of chronic UV irradiation on the survival of mammals. Available data come from experiments designed to study photocarcinogenesis, and most have been conducted with mice. Where mortality data are published, similar effects on survival can be seen in other mice, both haired and hairless. Specific studies with Drosophila also show dose-dependent life shortening in response to UV-B irradiation. Thus the data, though not extensive, appear to be consistent, and suggest that UV-B can produce cumulative systemic changes that reduce the potential life span in some species.

It is not clear whether this phenomenon has ecologic significance. The effect in laboratory mice is to accelerate apparently spontaneous death with no obvious effect on intermediate health. Animal survival in nature is often limited by specific processes such as disease or predation, and this more closely resembles the first phase of the survival curve. Clearly, however,

accelerated death must result from significant systemic damage. Whether such damage could modify health or life span of other species, including ours, is open to speculation.

An Estimation of Sunspot Induced Ozone Changes From a Sunburn Ultraviolet Meter

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A sunburn ultraviolet meter recording continuously since 1973 has provided data for determining ozone variation over an 11-year sunspot cycle (Berger and Urback 1982). This meter is part of a network of similar meters whose purpose is to determine the normal levels of biologically effective short ultraviolet radiation reaching the biosphere and of long-term trends. These purposes have been the stated goals of all agencies, both domestic and international, charged with the responsibility for studying the ozone layer and protecting it insofar as anthropogenic activities have an effect.

A ground-based monitor of short UV provides data important not only in itself but from which both ozone variations and cloud absorption can be determined. The wavelengths shorter than 330 nm detected by this meter (Figure 1 and Berger 1976) cause sunburn and after protracted exposure, non-melanoma skin cancers. They have also been shown to have myriad effects in the biosphere, primarily because they affect DNA, the basic control of all living organisms. The results of the network have therefore been used in the epidemiological study of non-melanoma skin cancer (Scotto et al. 1976). Meter results have also been used to study cloud absorption (Slomka 1976).

For the study presented here, monthly averages of sunburn dose for the 11-year period were derived from the semihourly meter printout. The deviation of each month from the mean of that month was plotted over the 11-year period; the sunspots for that month were plotted on the same abscissae. Smoothing of the data with a 12-month running average produced the top curve of Figure 2, which shows a clear correlation. The results are the sunburn ultraviolet variations over an 11-year period. From these variations, an estimate of ozone thickness variation due to sunspot activity is derived. The top curve in Figure 2 consists of two parts, the quasi-biennial fluctuations, an approximate 26-month oscillation and the 11-year cyclic variation due to sunspot activity. Separation of the top curve into its two component parts is shown on the lower curves of the figure.

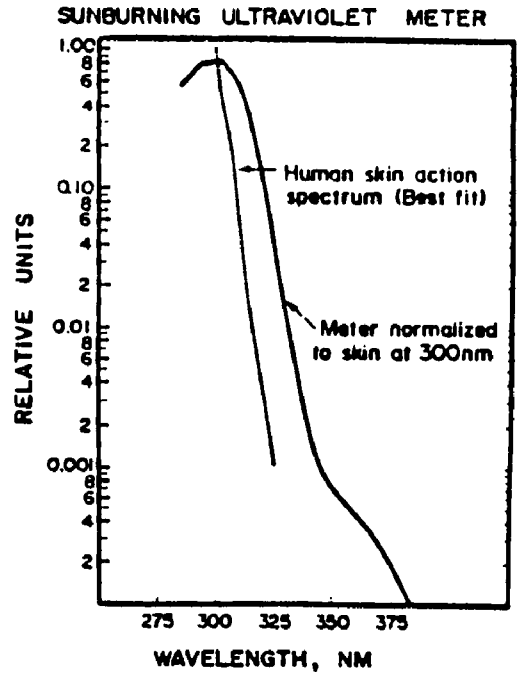

Figure 1. Erythema action spectrum and sunburn ultraviolet meter spectral response.

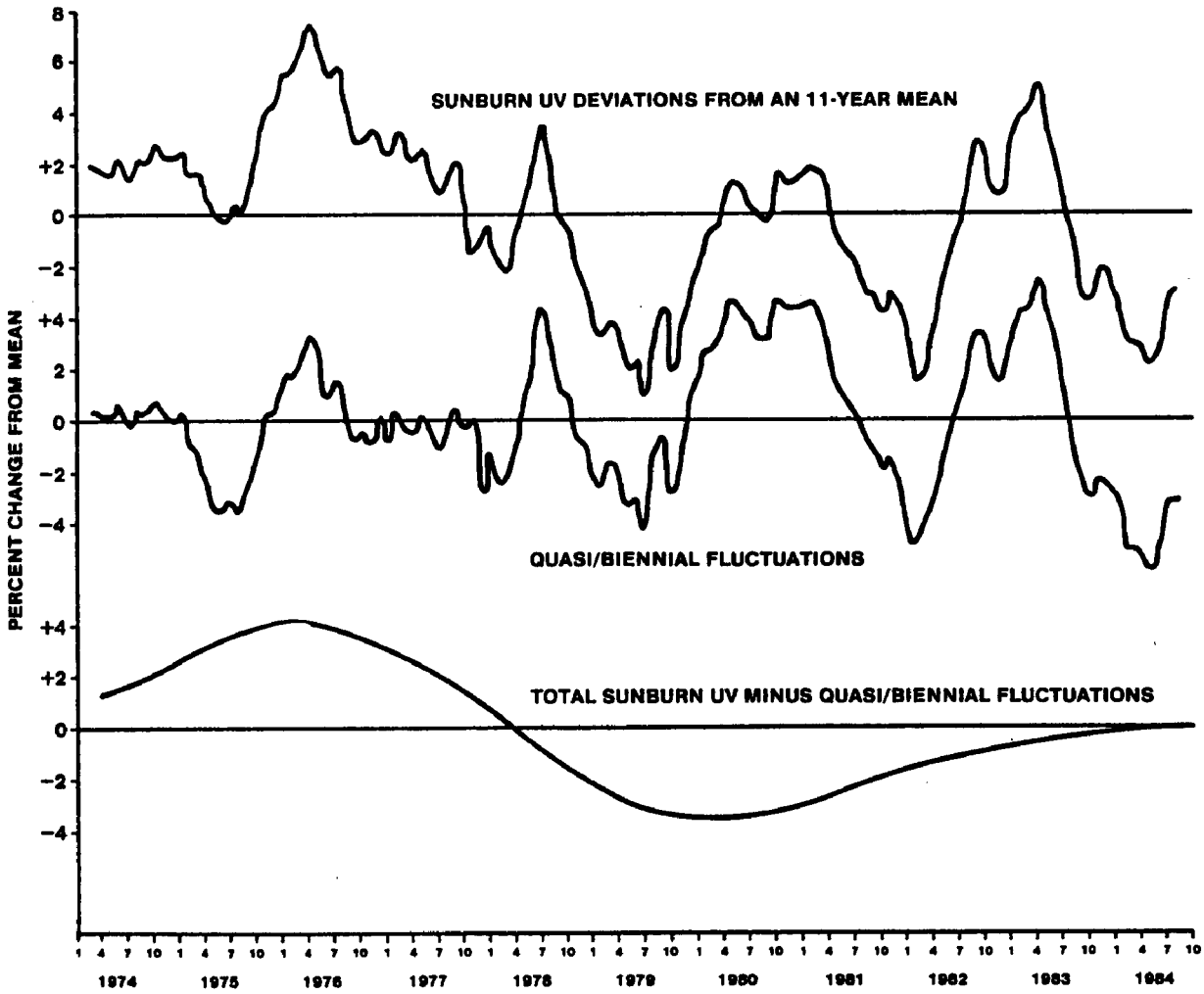

Figure 2.

The quasi-biennial fluctuation is a result of a variable rate at which ozone moves from the tropics into the temperate zone, in this case to 40° N latitude. No net change in stratospheric ozone occurs. Consequently, in a quasi-biennial period, the time integral should be zero. Deviations from zero are attributable to net changes of total ozone due to sunspot activity.

Comparison of the 11-year cyclic variation of sunburn ultraviolet shows a close correspondence to sunspot number, as shown in Figure 3.

There is also a relationship between the change of sunburn UV and the change in stratospheric ozone. If the meter had an exact erythema action spectrum, there would be about 1.7% change in meter reading per 1% change in ozone at 3 mm of ozone thickness. (See Figure 4.) Because the meter spectral response is long wave shifted, the meter change is more like 1.2% at 3 mm of ozone. Since there was an 8% maximum change in sunburn effect due to sunspots, the ozone change was about 6.5%. This change was due to 160 sunspots. Thus, 25 sunspots caused a 1% ozone change.

The constancy of extra-terrestrial radiation between 295 and 330 nm is assumed as is the complete decoupling of cloud variability from sunspot activity. The first assumption must be justified by extra-terrestrial spectro-radiometric observations. The extent to which the second assumption is true could be determined by a careful analysis of the meter record itself. Any cloud-sunspot coupling could be used to compensate the derived ozone variation.

It may appear naive to be making a calculation of the effect of a sunspot on ozone thickness based on so thin a reed as a non-thermostatted monitoring meter, but the unexpected and gratifying 11-year record that is shown here would have been considered naive to anticipate up until the time these calculations were completed.

The information-rich output, the almost perfect data captured, the long term stability, the low cost, and the ability of technicians without special training to provide the minimal maintenance required argue that there is a place for this device for long term monitoring of the ozone layer or of the ultraviolet levels in the biosphere required by environmental agencies.

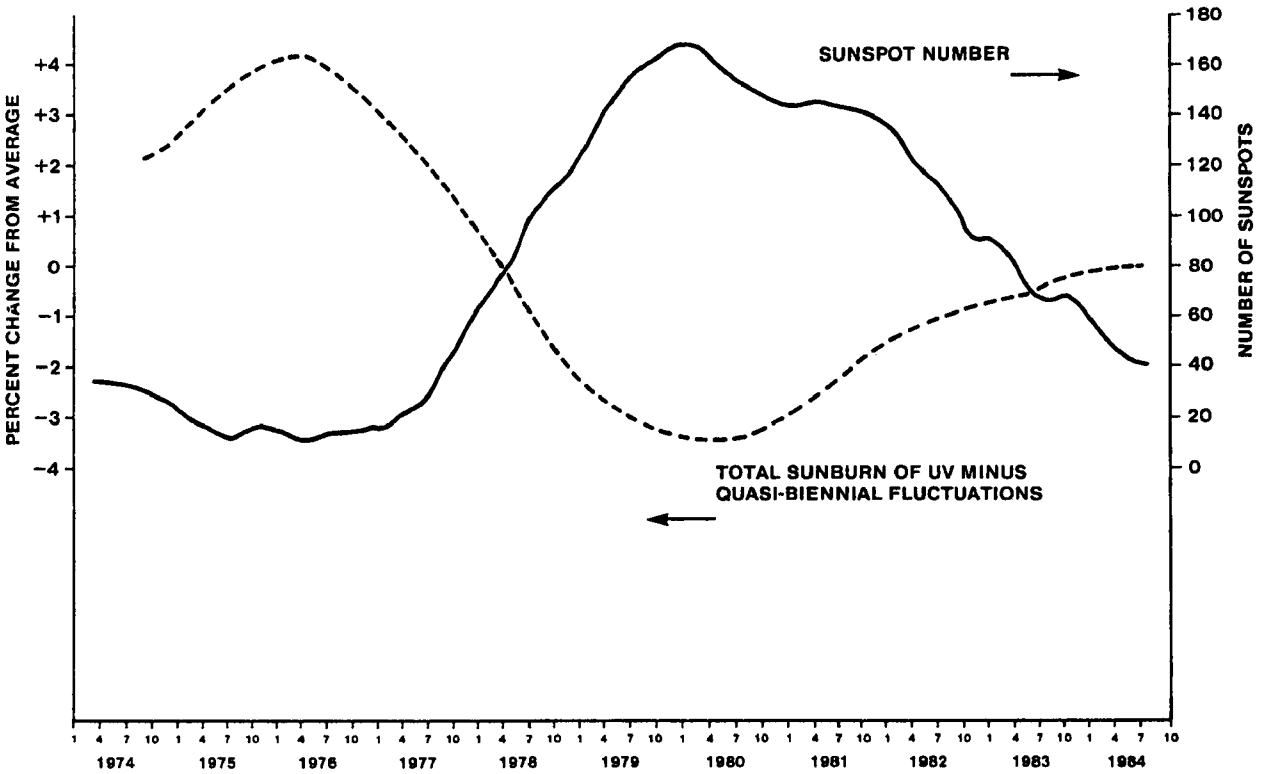

Figure 3.

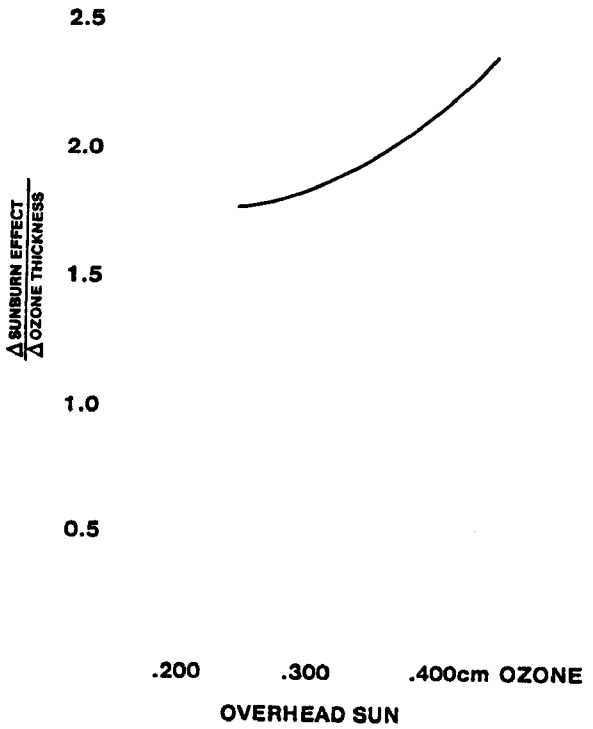

Figure 4. Rate of Change of Sunburning Capability of Sunlight with Ozone Variation

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Nonmelanoma Skin Cancer—UV-B Effects

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Since the early 1970s, the National Cancer Institute, in collaboration with other federal and nonfederal sources, has conducted special epidemiologic surveys of nomelanoma skin cancer and monitored ground level UV-B measurements at various geographic locations within the United States.

These studies responded to the need for basic data to be used in measuring the extent of certain human health effects that may result from stratospheric ozone depletion.

We focus on solar ultraviolet radiation in the range of 280 to 320 nanometers, called mid-UV or UV-B (see Figure 1). Under experimental conditions, UV-B has been shown to produce skin erythema (sunburn) in man and skin cancer in animals, and it is effective in altering DNA. Cumulative exposure to UV-B is also believed to be partially responsible for the "aging" process of the skin in humans. Except for preventing vitamin D deficiency rickets, which is now confined to populations with inadequate nutrition, UV-B is basically considered biologically harmful.

While no solar radiation below 295 nm ever reaches the earth's surface, a small quantity of UV-B does. Stratospheric ozone depletion may result in increases of UV-B energy reaching the earth and its populations. The physical amplification factor has been put at 2. This means that a 1% decrease in ozone may result in a 2% increase in solar ultraviolet, UV-B.

Basal cell carcinomas and squamous cell carcinomas of the skin, the nonmelanomas, are the most common malignant neoplasms occurring in the white populations of the world. Currently, annual incidence in the United States is estimated at about one-half million patients, and the rates are increasing at about 3% per year. Epidemiologic study has been limited by the fact that most patients are customarily seen and treated in the offices of physicians and not hospitalized. Cure rates are high (about 99%) and only a small percentage of skin cancers are metastatic or result in death.

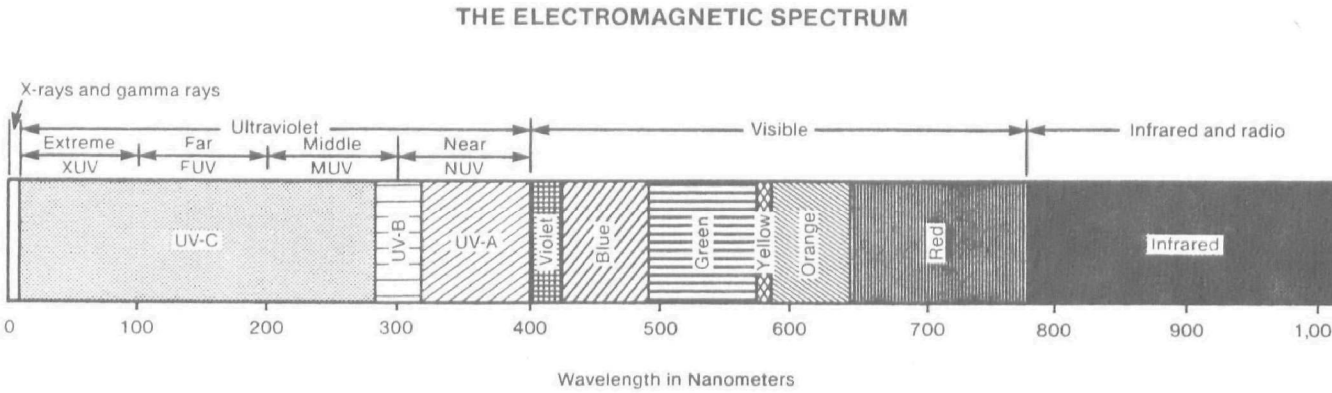


Figure 1

Because the primary source of data cancer registries is the inpatient hospital file, the statistics routinely collected on skin cancer are usually very incomplete and not comparable with other forms of cancer. Thus, population-based estimates of skin cancer incidence require special surveys to collect data from offices and outpatient files.

Figure 2 shows the geographic locations within the continental United States where skin cancer surveys were conducted and ground level measurements of UV-B were obtained. In 1971-72, there were four areas in the Third National Cancer Survey; in 1977-78, eight areas of NCI Surveillance Epidemiology and End Results Program (SEER) were surveyed.

Two locations, Minneapolis-St. Paul, Minnesota, and San Francisco-Oakland, California, were resurveyed in the late 1970s. Skin cancer incidence data from New Hampshire, Vermont, and San Diego, California, were most recently included. We do not have statistical details for other cancers from these locations, and we are just now receiving UV-B readings from Concord, New Hampshire, and Burlington, Vermont. Patient and general population interview studies were conducted in nine locations. These locations span the United States from Seattle in the north (47.5°N) to New Orleans (30°N). Most of the figures that follow display the epidemiologic details for the eight-area survey conducted in 1977-78. Seven of these are SEER locations where NCI has continuing surveys of all other malignancies. The results are similar for all surveys.

Skin cancer is a disease that rarely occurs in black and pigmented races. The age-adjusted rate of 242 per 100,000 for whites is more than 60 times that for blacks with a rate of under 4 per 100,000. Among Caucasians "Anglos" or non-Hispanics, are at greater risk than Hispanics by about 7-10 to 1.

Figure 3 shows the incidence of nonmelanoma skin cancer and all other cancers combined among whites for each of eight locations plotted according to latitude. For all other malignancy, there is no latitudinal gradient. In contrast, nonmelanoma skin cancer incidence rates were definitely lower at the higher latitudes.

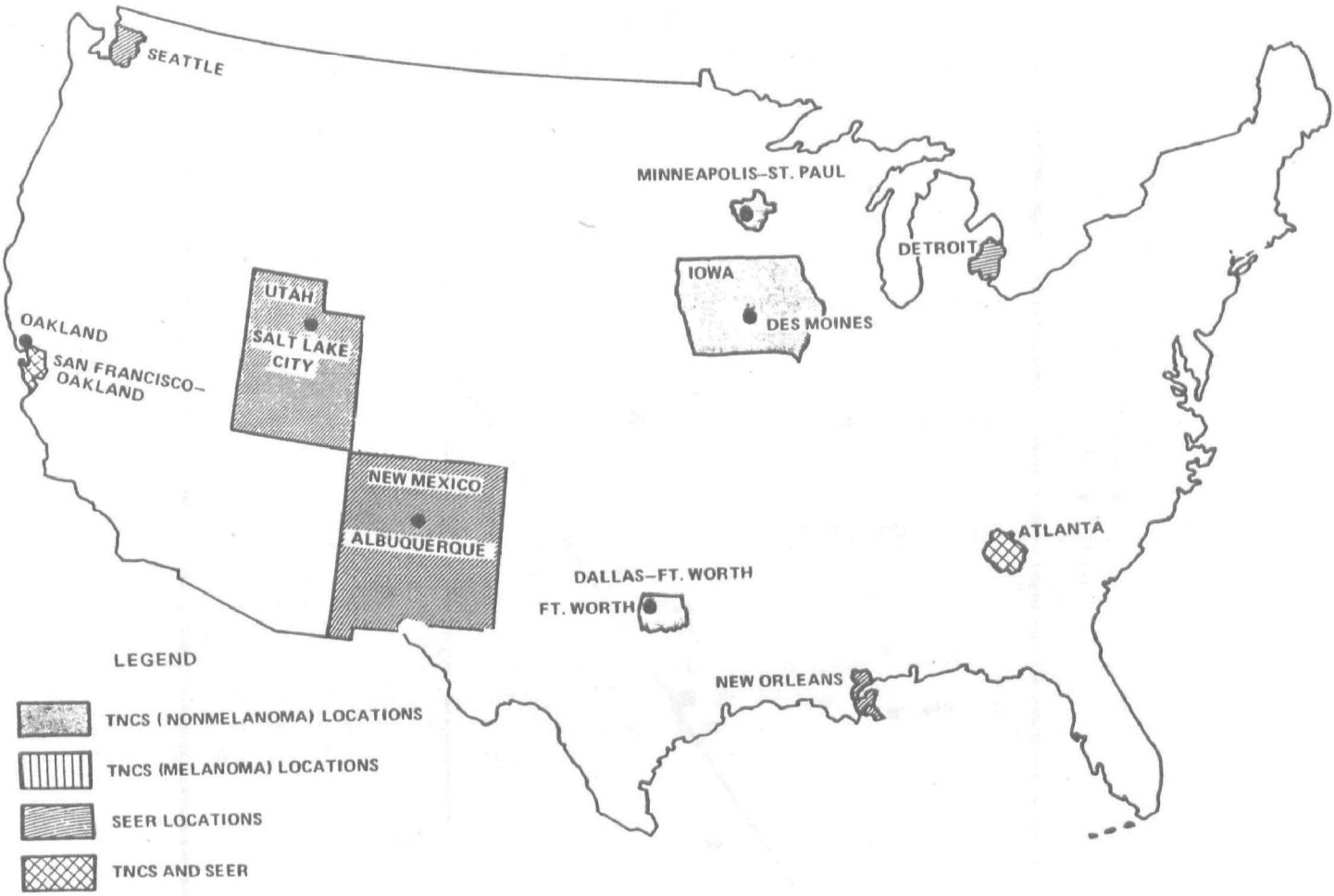


Figure 2. Skin Cancer Measurement Locations in the United States

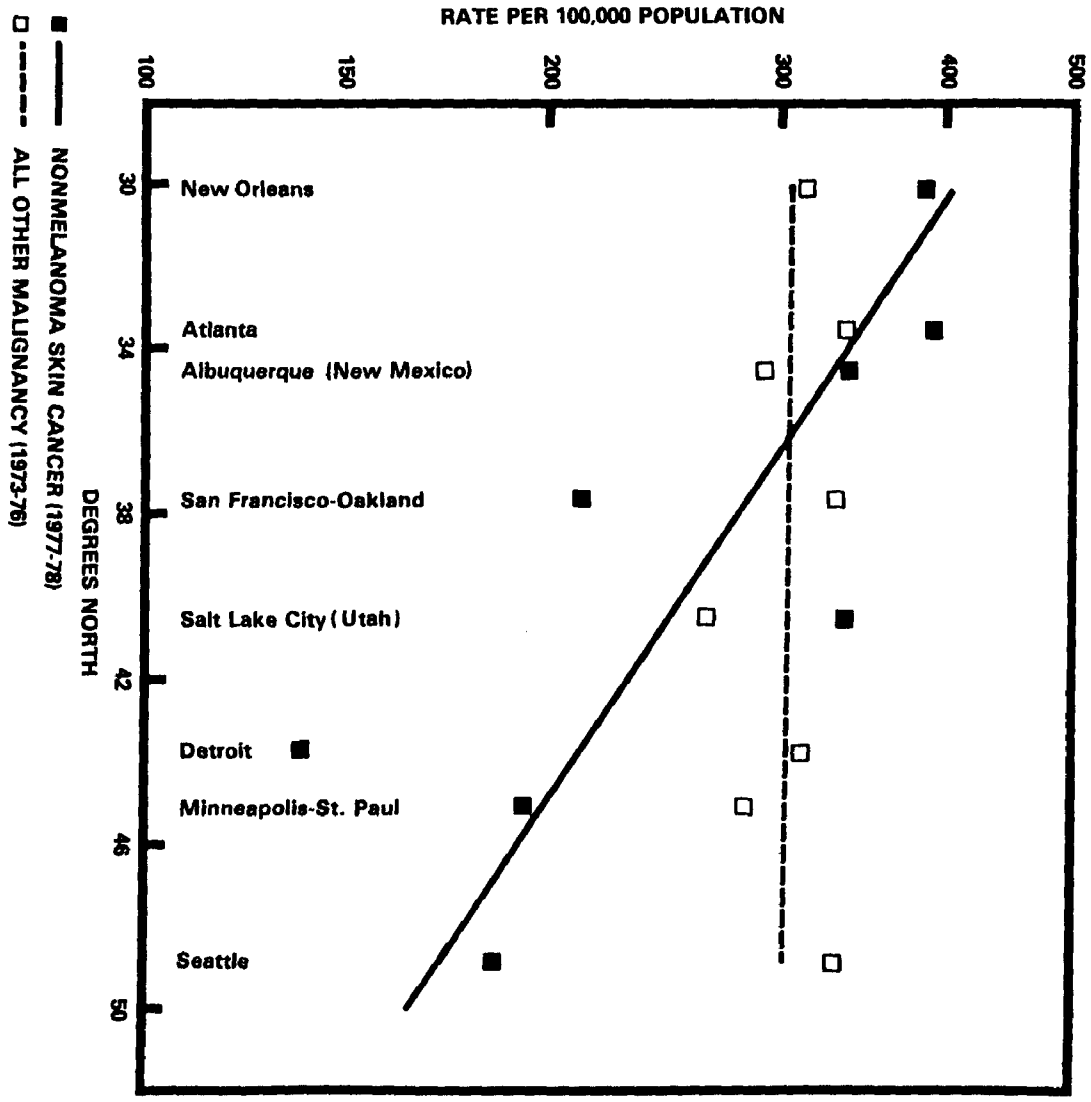


Figure 3. Age Adjusted Incidence of Cancer (U.S. 1970) Among Whites by Latitude

The age-adjusted incidence rates by geographic location for each sex are shown in Figure 4. The rates for males are always greater than those for females. Overall, the male/female ratio is close to 2 to 1 (1.83). Note there are two sets of bars for New Mexico. Because of the high proportion of highly pigmented Hispanics in that State, over one-third, the rates for all Caucasians are lower than that for Anglos only.

Figures 5 and 6 show age-specific rates according to region. The rates increased with age--the highest rates were seen in the oldest age groups. The southern region was clearly at higher risk than the northern region.

Figures 7 and 8 show age-specific rates by specific location, comparing males and females. At each location the male rates were lower or equal to the female rates at early ages. After age 45, in the northern and middle locations, male rates consistently exceeded those of females, and the differences were greatest in the oldest age groups (Figure 7). In the southern locations (Figure 8), the separation between male and female rates began a decade or two earlier, presumably because the UV threshold levels for skin cancer detection were reached sooner.

Tumors appear on the face, head, and neck in over 80% of nonmelanoma skin cancers. Among females, the nose is the most common site while, among males tumors of the nose, cheek, and scalp are equally high. Tumors of the ear occur more frequently among men, compared to women, by a factor of 10 or more, especially in the southern areas. In contrast, tumors of the legs are more common among females who have greater UV exposure and also more melanoma of the lower leg. In both sexes tumors are much more common in the lower lip than in the upper lip.

Age-specific incidence rates according to anatomical site are illustrated in Figure 9. Rates for face, head and neck, and upper extremities progressively increased, while those for trunk and lower extremities reached a plateau or declined at older ages.

With respect to histology, Figure 10 shows a composite of specific geographic locations depicting age-specific incidence patterns for basal cell carcinomas and squamous cell carcinomas of the skin. Overall the incidence rates for BCC was 4 to 5 times higher than SCC. Increases have been observed for each cell type as age increases. The rate of increase may be slightly higher for squamous cell carcinomas. But the squamous cell carcinomas begin at later ages. The ratio of BCC to SCC is greatest in the northern region and lowest in the southern region, ranging from over 12 to 1 in the north to over 2 to 1 in the southern locations.

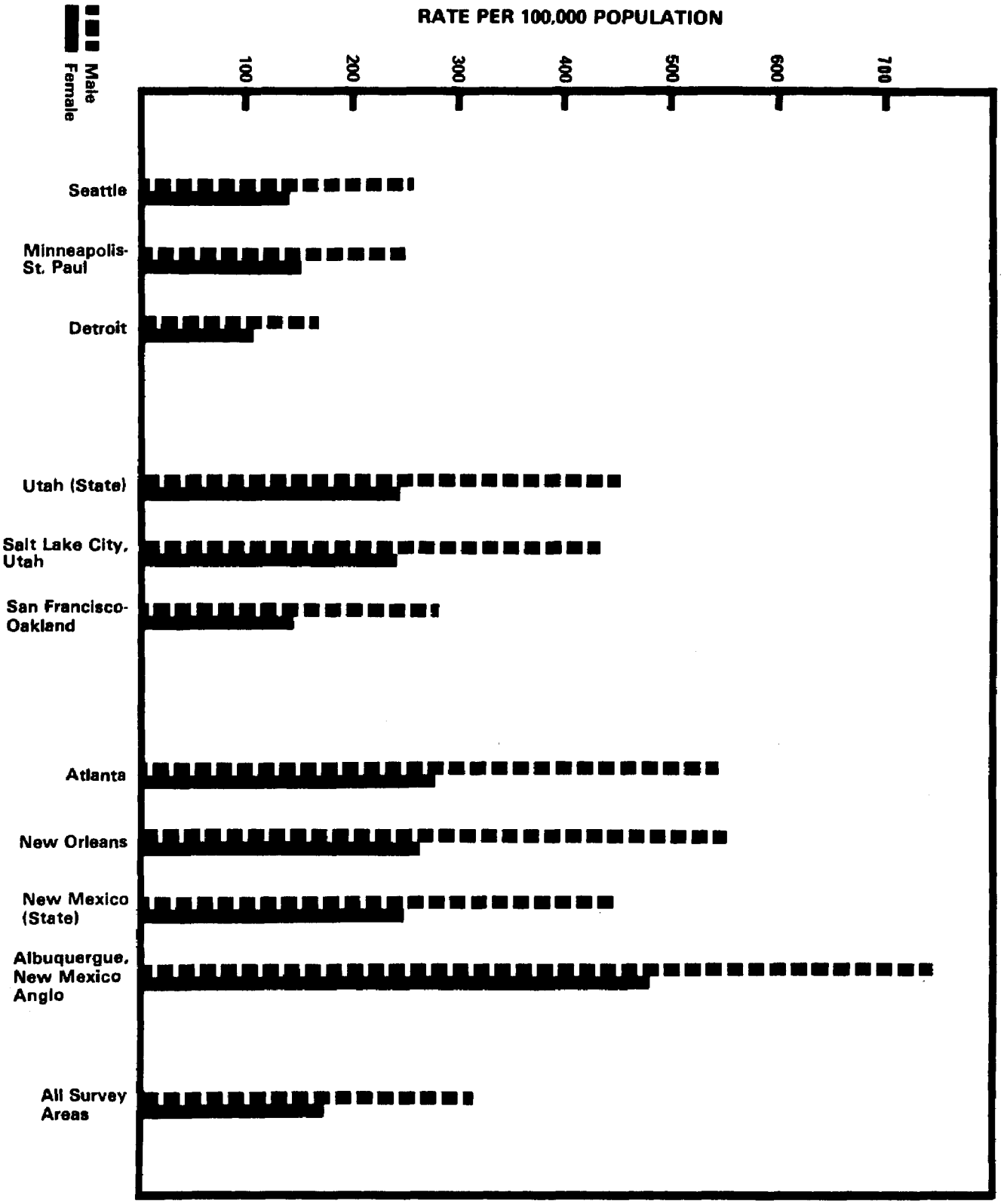


Figure 4. Age Adjusted Rates of Nonmelanoma Skin Cancer in Various Regions of the United States

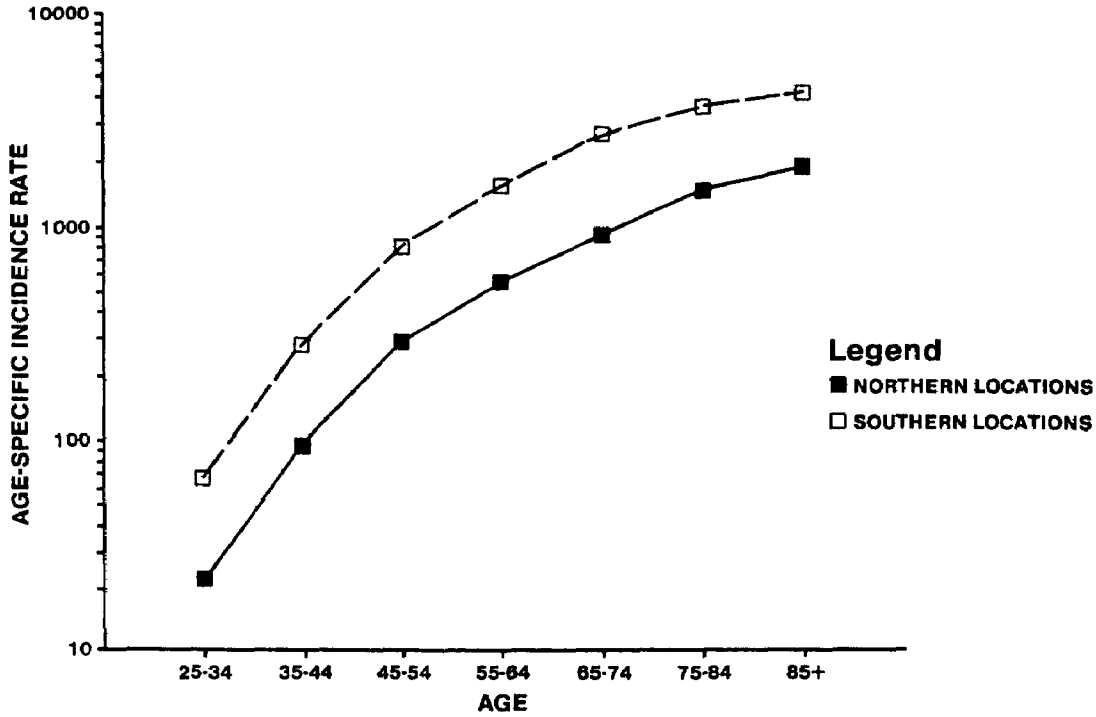


Figure 5. Age-Specific Nonmelanoma Skin Cancer Incidence by Region Among White Males

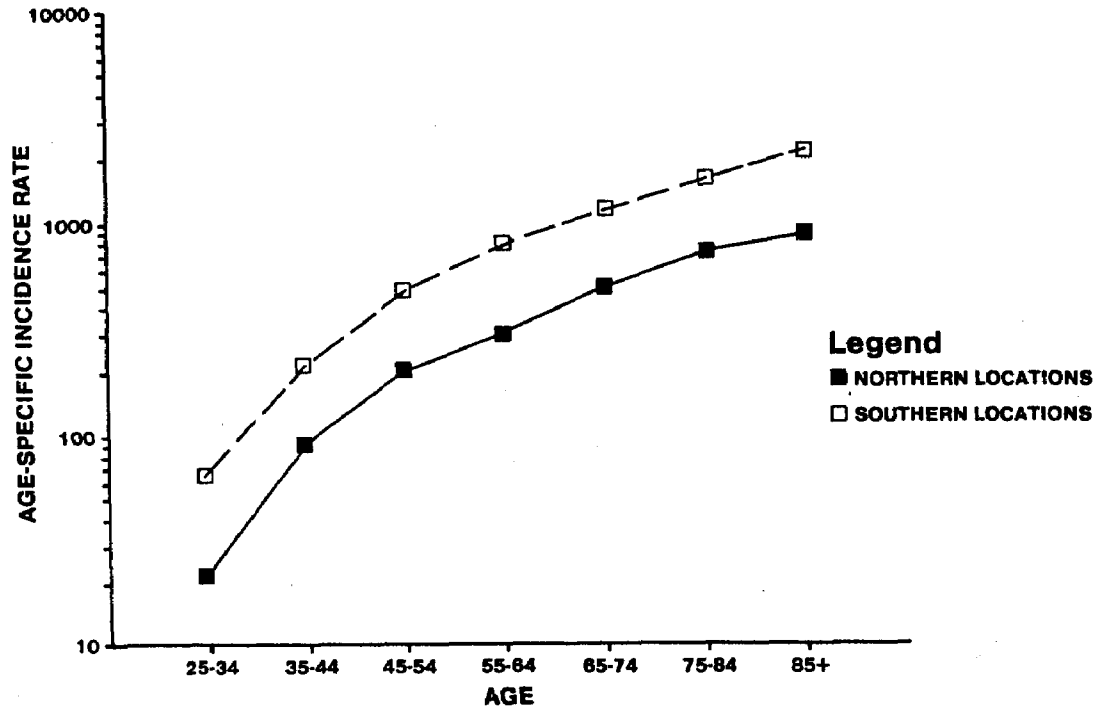
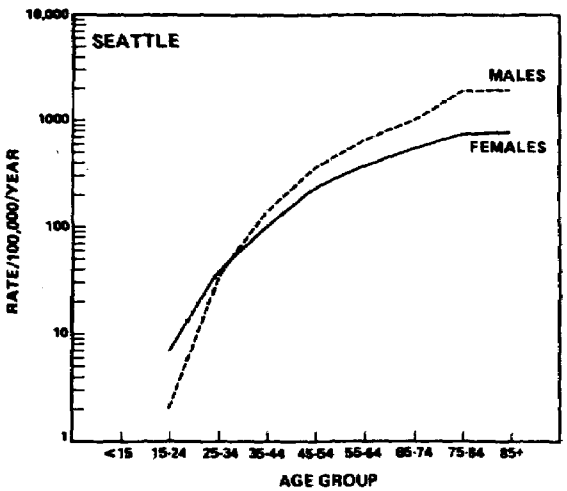
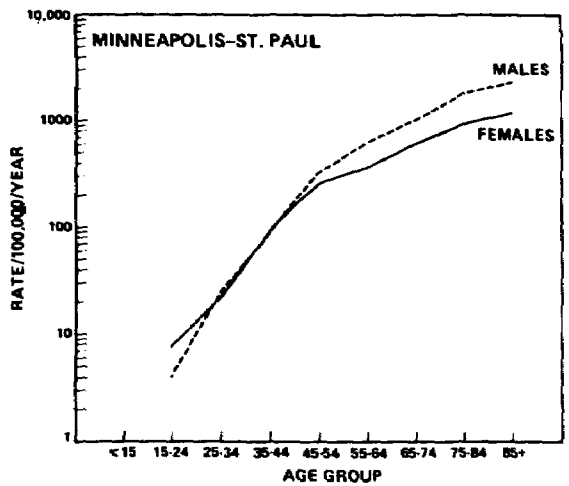
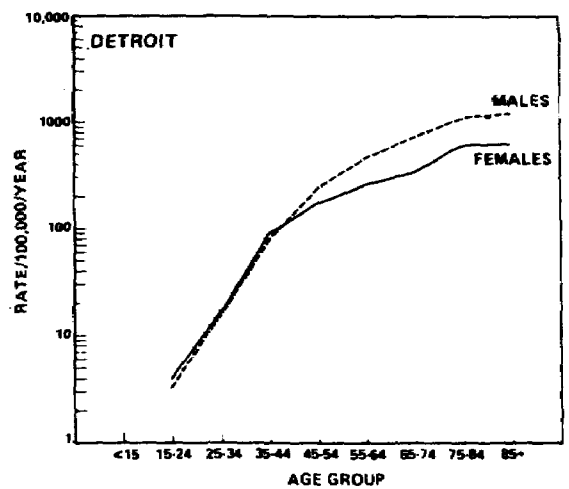


Figure 6. Age-Specific Nonmelanoma Skin Cancer Incidence by Region Among White Females

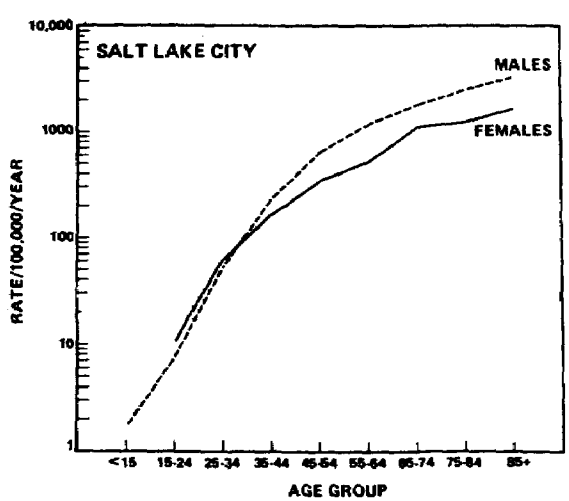
NORTHERN REGION (LATITUDES 40-50 DEGREES NORTH)







MID REGION (LATITUDES 35-40 DEGREES NORTH)



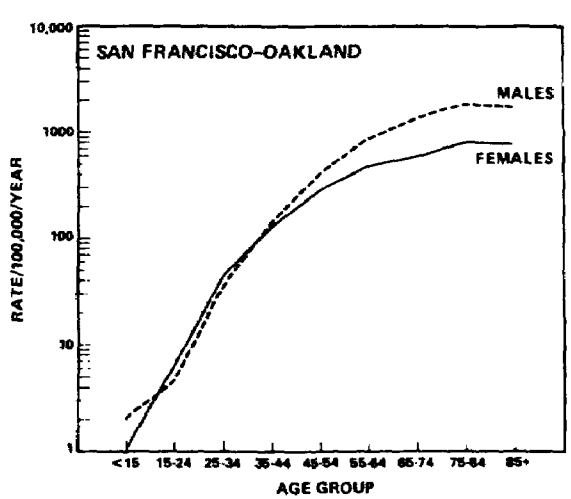


Figure 7. Age-Specific Nonmelanoma Skin Cancer Incidence Among Whites by Regions of the United States, 1977-78

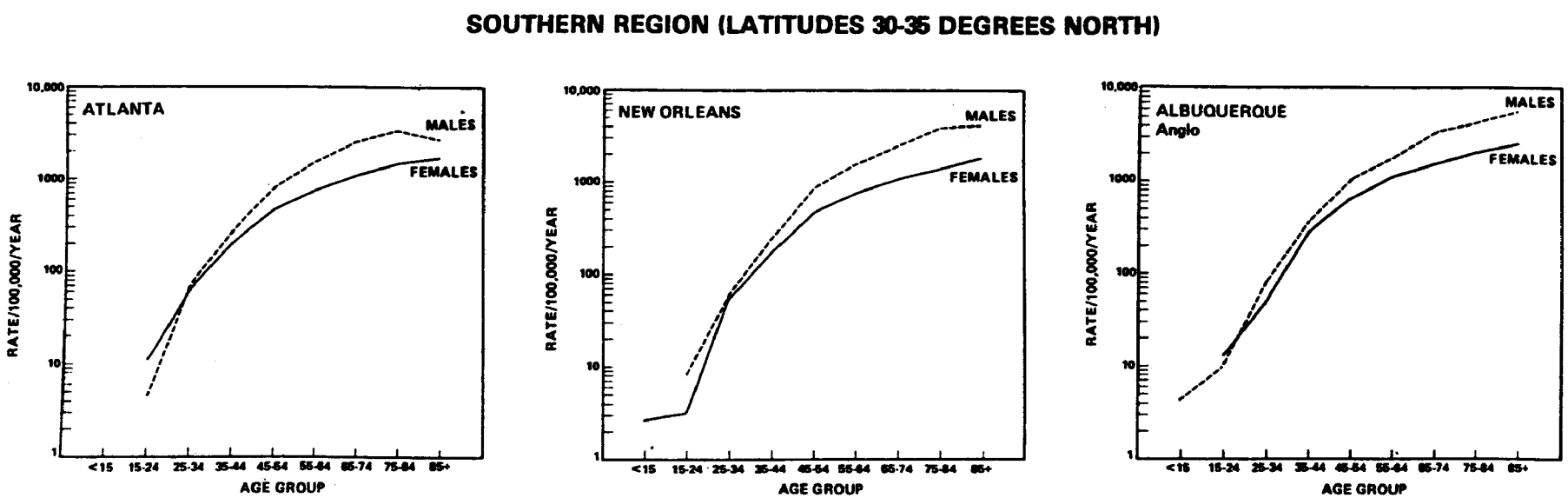


Figure 8. Age-Specific Nonmelanoma Skin Cancer Incidence Among Whites by Regions of the United States, 1977-78

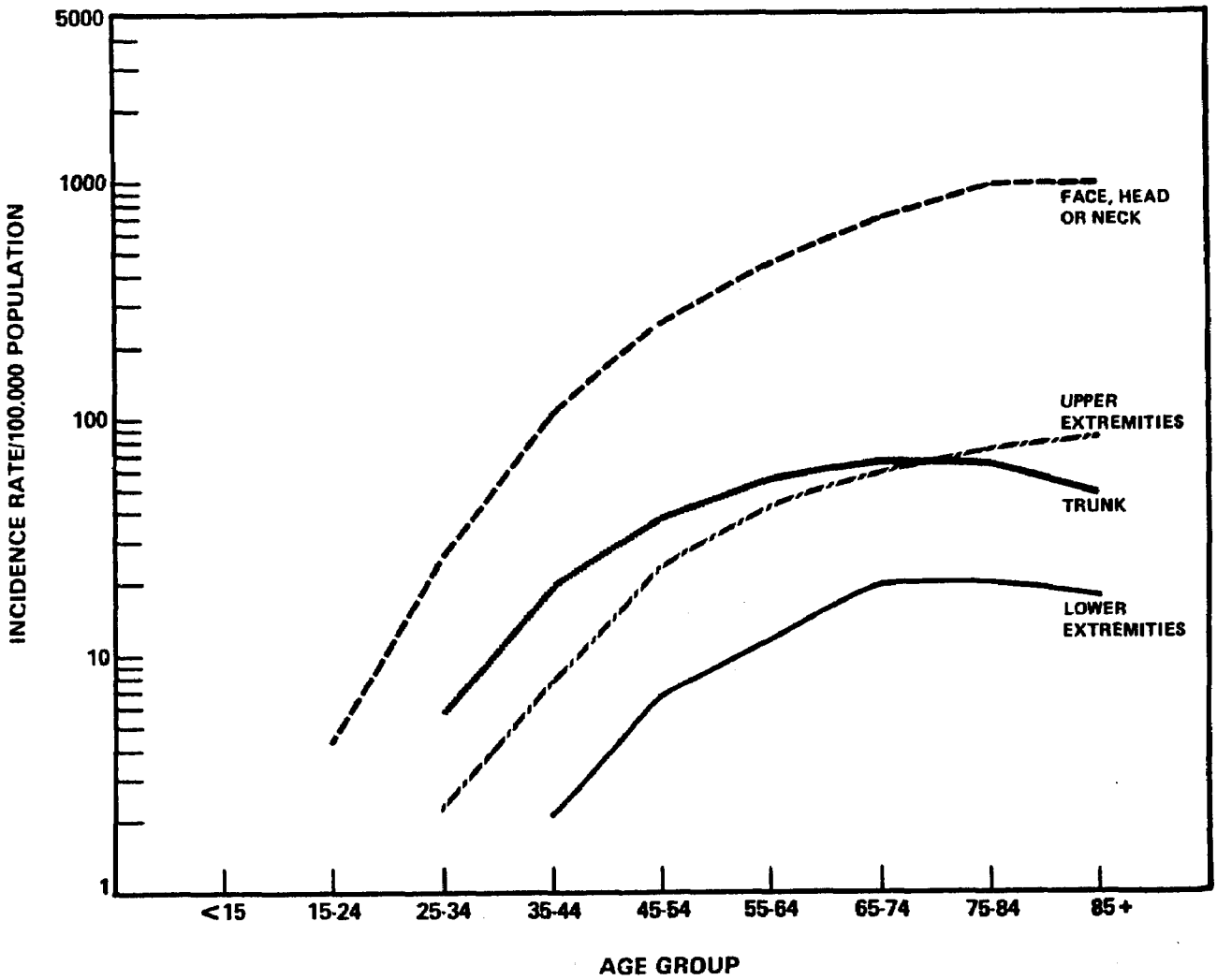


Figure 9. Trends in Annual Age-Specific, Nonmelanoma Skin Cancer Rates Among Whites, Both Sexes

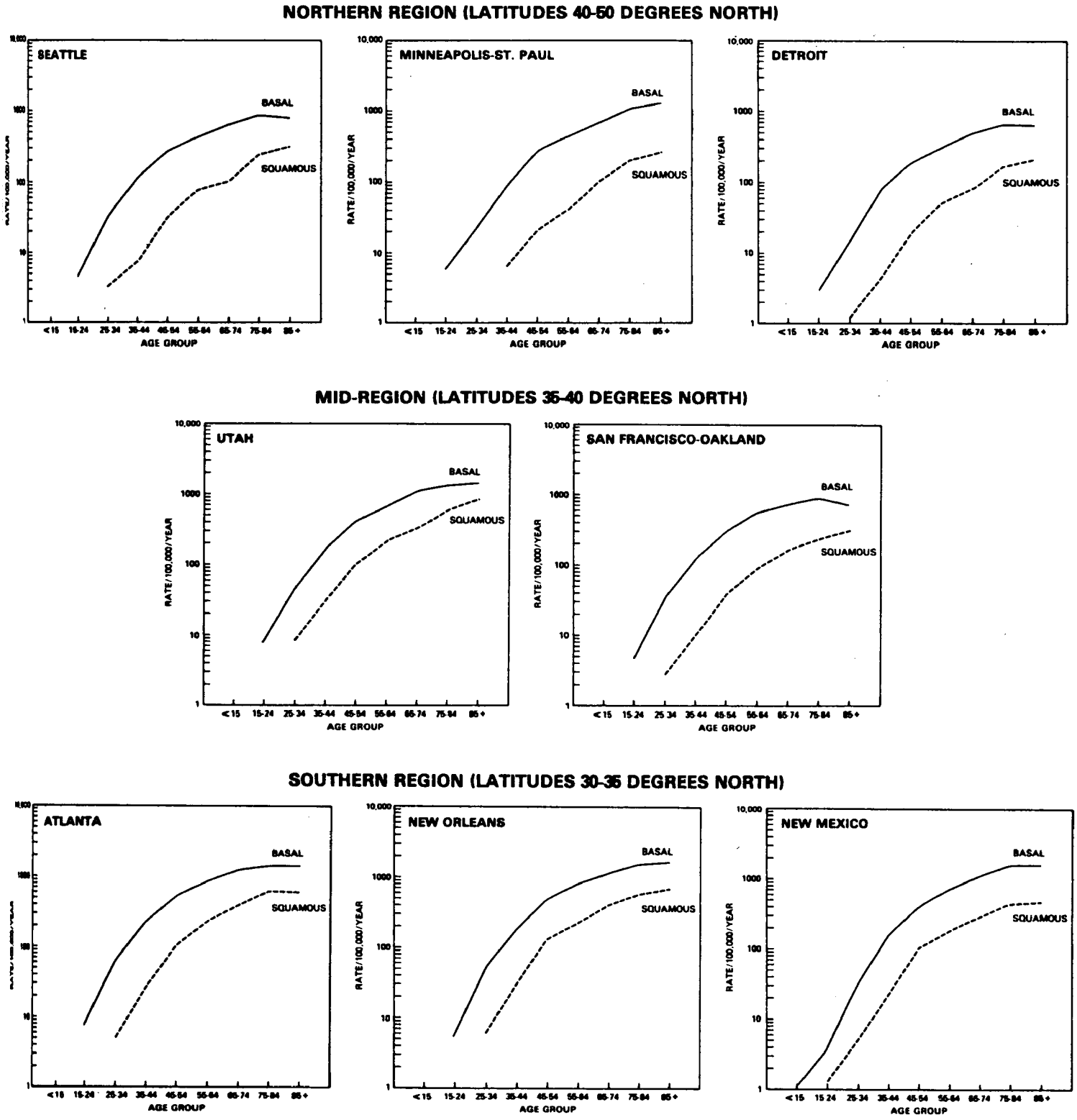


Figure 10. Age-Specific Nonmelanoma Skin Cancer Incidence Among Among Whites by Regions of the Unites States, Both Sexes

In regard to our estimates of ground level UV-B measurements, we have been monitoring and editing data in collaboration with researchers at the National Oceanic and Atmospheric Administration and their network of weather stations (see Berger in this volume for details of the Robertson-Berger meter).

Figure 11 shows, as expected, that the annual amounts of sunburn-producing UV-B correlated with latitude. To put these numbers in perspective, it is estimated that a count of 440 R-B units may produce a perceptible sunburn. It is possible to receive such a dose within 20 minutes on a midsummer day around noontime. It is important to note that these measurements are affected by altitude, cloud cover or water vapor, and other meteorological factors. At altitudes of over a mile high Albuquerque and Salt Lake City received greater amounts of solar UV-B than expected; while Tallahassee with relatively high humidity received less than the expected dose for that latitude. I should like to point out that current observations indicate a general downward trend in meter readings at several locations. The relative positions for Detroit and Minneapolis have changed, with current figures now lower at Detroit.

The monthly averages of R-B counts for Albuquerque, New Mexico, and Seattle, Washington, respectively, are shown in Figure 12. These were the highest and lowest exposure areas included in NCI's nonmelanoma skin cancer surveys. The ratio of UV-B exposure is about 2 to 1 for these locations.

Next my discussion focuses on our correlation studies of population-based skin cancer incidence and estimated UV-B dose.

The annual UV-B levels and age-adjusted incidence rates for nonmelanoma skin cancer in white males and females were determined in the two special surveys conducted in 1971-72 and 1977-78 (see Figures 13 and 14). The incidence data are plotted on a log scale so that a straight line with a positive slope represents a constant percentage increase in incidence. Mathematical models were used to describe dose-response relationships and do not reflect the mechanism by which UV causes skin cancer. Using an exponential model previously applied to the 1971-72 data, estimates of the biological amplification factor (that is, the relative change in skin cancer incidence due to a relative change in UV-B radiation) were derived. Assuming a common slope, the exponential model may be written as a logarithmic expression as shown:

$$\text{Ln } R_{ij} = a_i + b\,(UV_j) + R_{ij}$$

where u = 1, 2 denotes the two survey areas

j = 1, 2 ... 10 denotes the locations

R_{ij} = the age-adjusted incidence rate

UV_j = the annual UV-B count per 10,000

a_1, a_2 ^{b} = constants

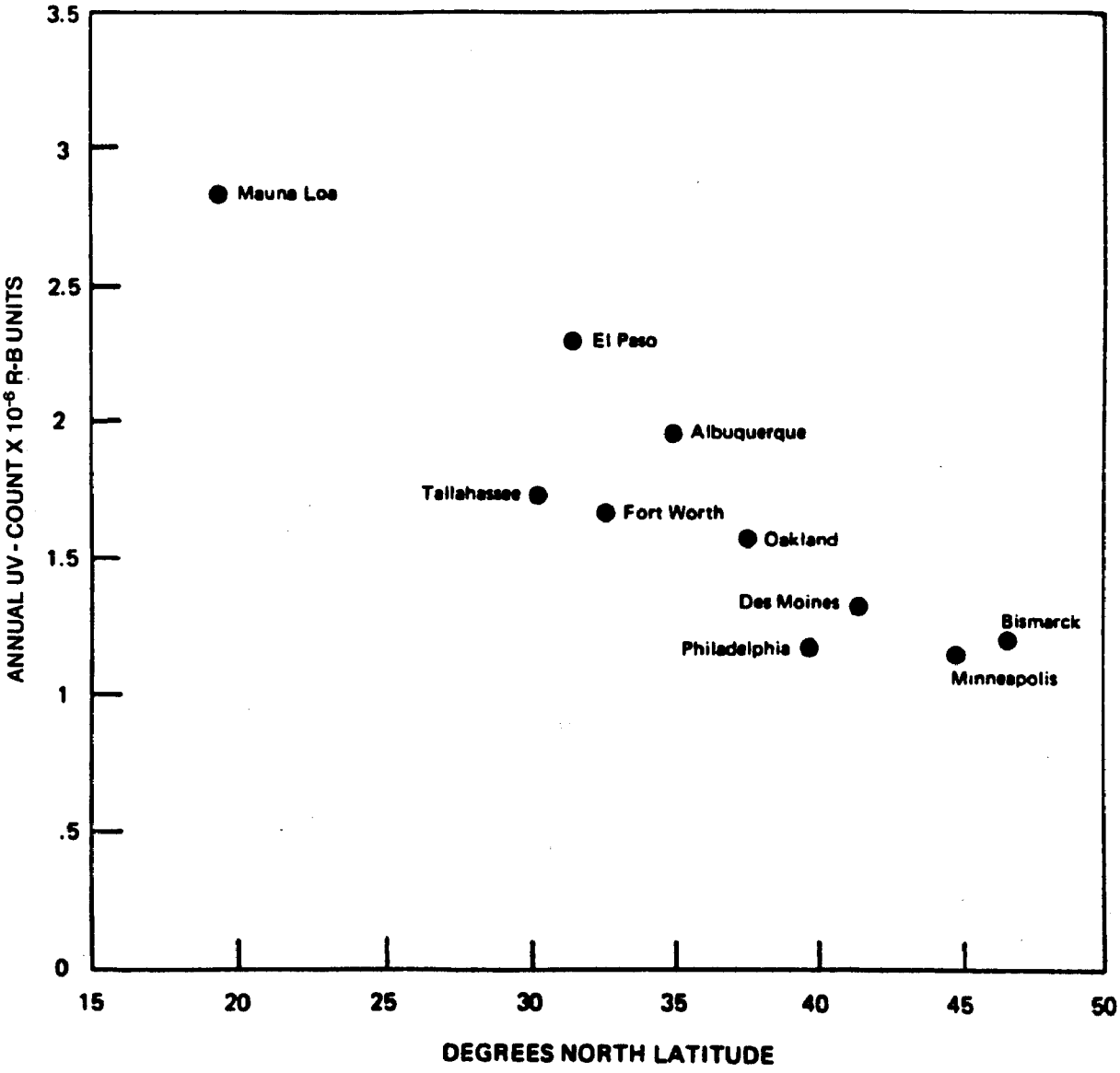


Figure 11. Annual UV Count by Latitude

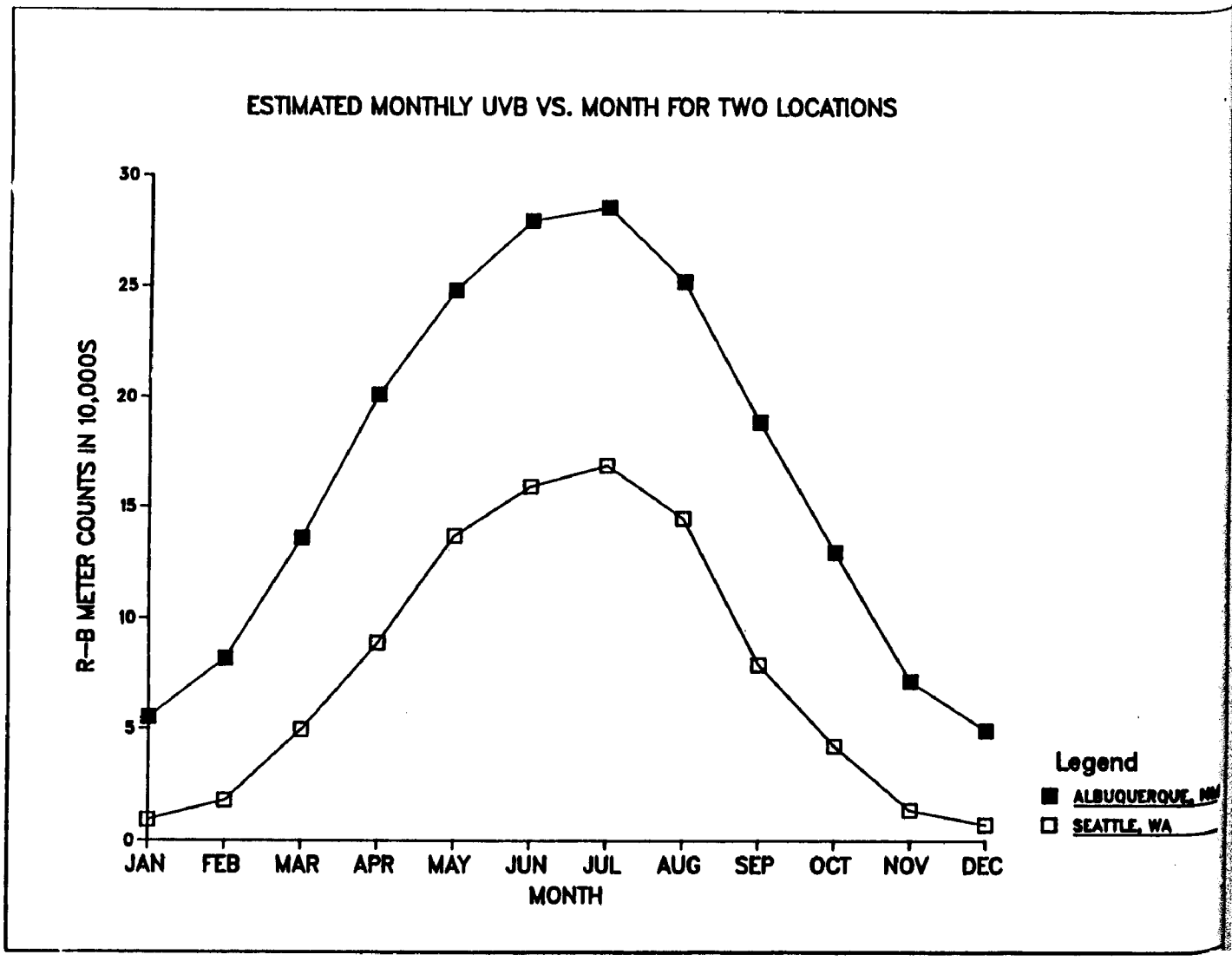


Figure 12. Estimated Monthly UV-B vs. Month for Two Locations

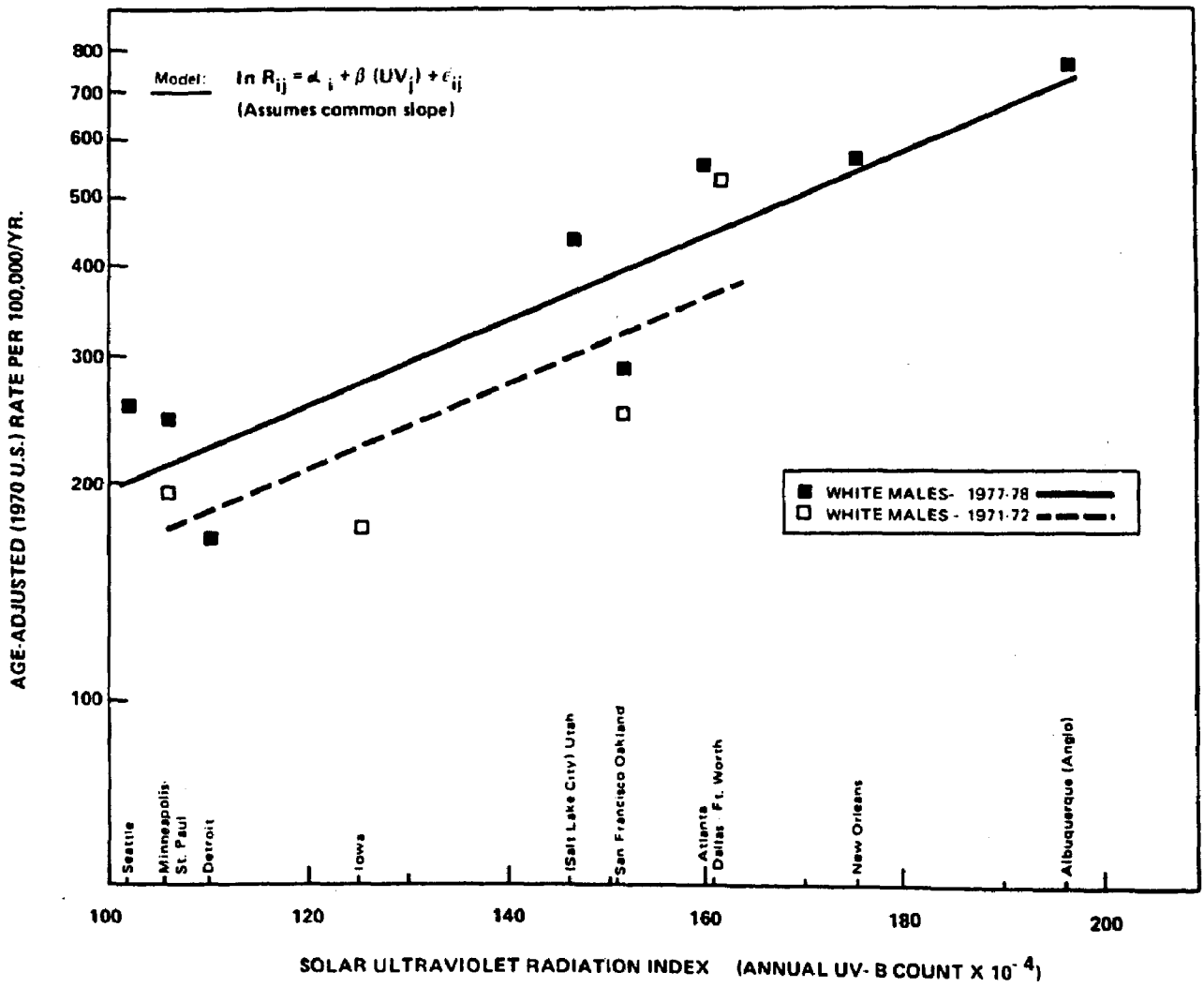


Figure 13. Nonmelanoma Skin Cancer Incidence Among White Males in the United States by Geographic Area and UV-B Exposure

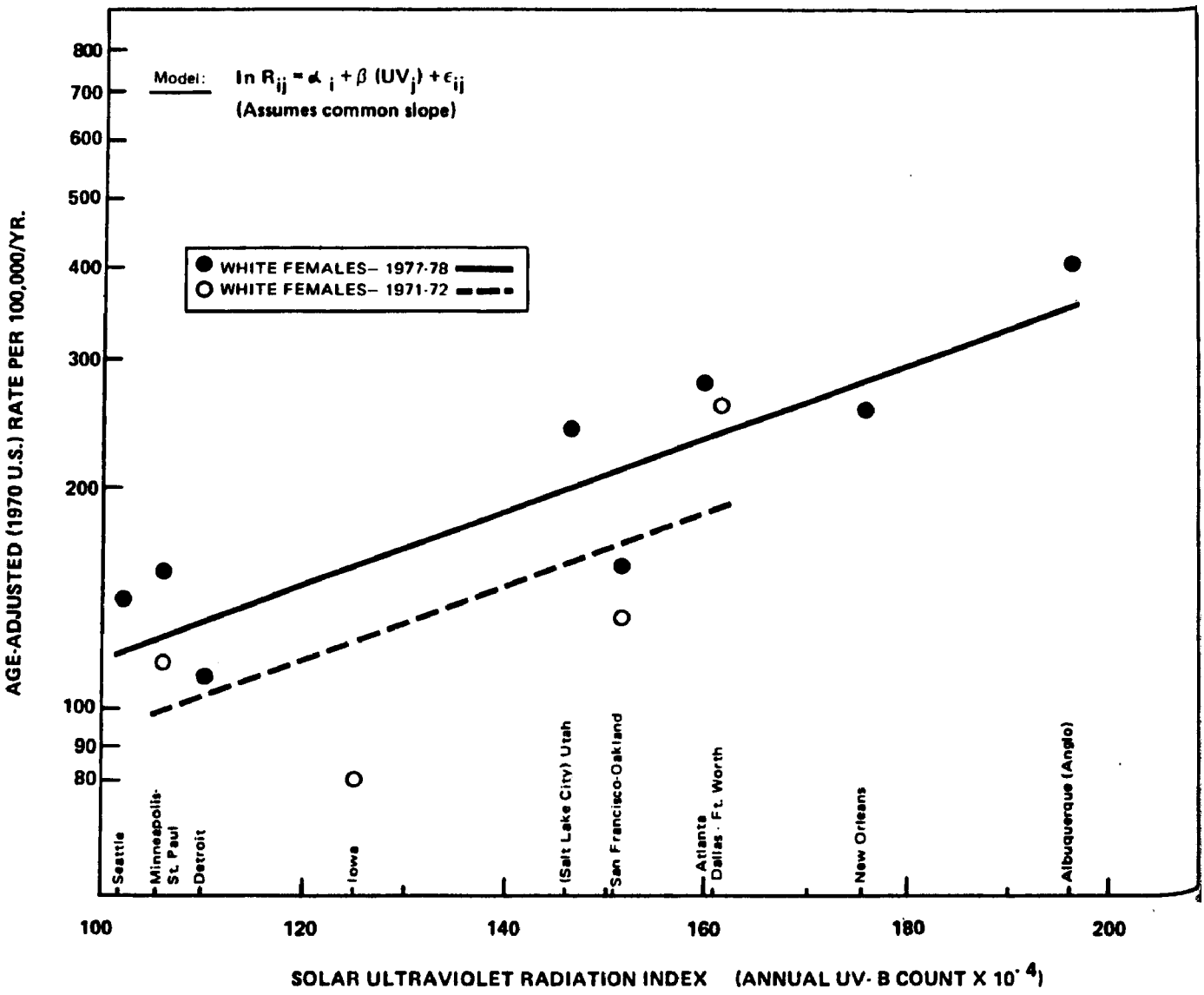


Figure 14. Nonmelanoma Skin Cancer Incidence Among White Females in the United States by Geographic Area and UV-B Exposure

In the regression analyses, the logarithms of the age-adjusted incidence rates were weighted by the inverse of their estimated variance. Assuming that the annual UV-B counts were to increase by 1% at each location, the relative effects on skin cancer incidence were found to vary from a low of 1.19% to a high of 2.88%. The estimates were lowest for females residing in areas of low UV-B exposure levels. Overall, the biological amplification factors were estimated to be roughly twofold, but steeper for squamous cell carcinomas.

An update of the incidence and UV-B correlations for males and females is shown in Figure 15. There are ten locations plotted, which include only the most recent surveys of 1977-80. We have tentatively estimated the UV-B index for NH/VT at 96 (x10,000 SU), and we use average annual counts for the years 1977 through 1981 for all other locations. In general, the average UV values are lower than those previously estimated for a one-year period.

Biological amplification factors (using exponential model) show no substantial changes; however, the range is now between 1.03 and 2.5. While these estimates are a little lower than those previously calculated, the degree of uncertainty has been reduced and the estimates for the 95% lower limits have in fact increased. Figures 16 and 17 depict these correlations with respect to cell type. The slopes are steeper for SCC compared to BCC. Correlations according to anatomical site are shown in Figures 18 and 19. The relationship of skin incidence and ground level UV-B exposure is consistent for each site group. However, there appear to be stronger associations and steeper slopes for the face, head, and neck, and upper extremities among white males and females. Keep in mind that over 87% of all nonmelanoma skin lesions occur on these relatively more exposed anatomical sites.

As many researchers have suggested, and as the results of our studies show, there are demographic factors that may reflect increased or decreased skin cancer risk in certain population groups similar to the differences we have noted for Anglos and Hispanics. So, in addition to the incidence surveys, telephone interview sample surveys of skin cancer patients and general population controls between the ages of 20 to 75 were conducted at nine locations. Information was sought on several host and environmental factors that may affect the risk of skin cancer. Examples follow showing the correlation of skin cancer incidence with UV-B radiation according to the presence or absence of certain constitutional factors.

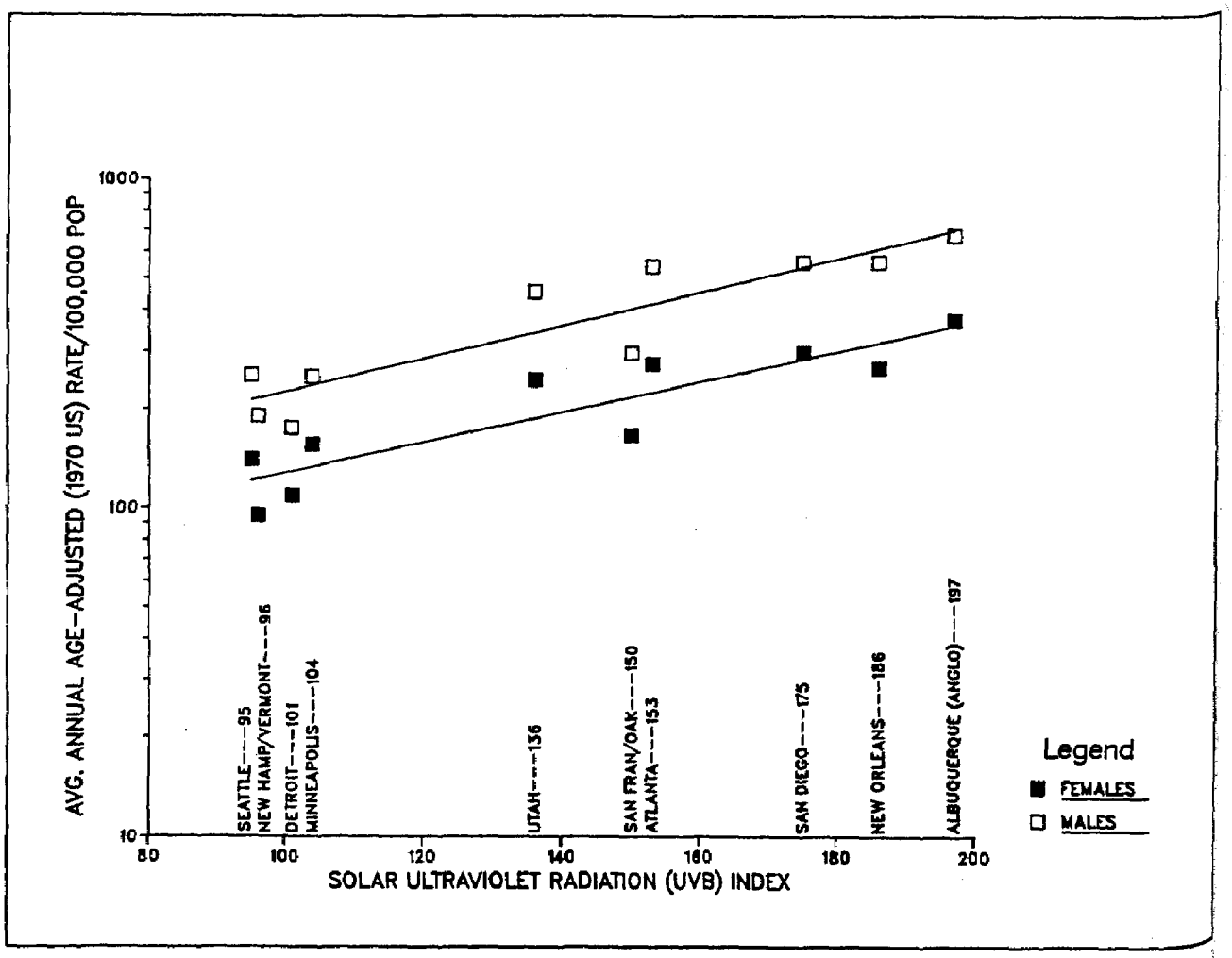


Figure 15. Nonmelanoma Skin Cancer Incidence According to UV-B Index

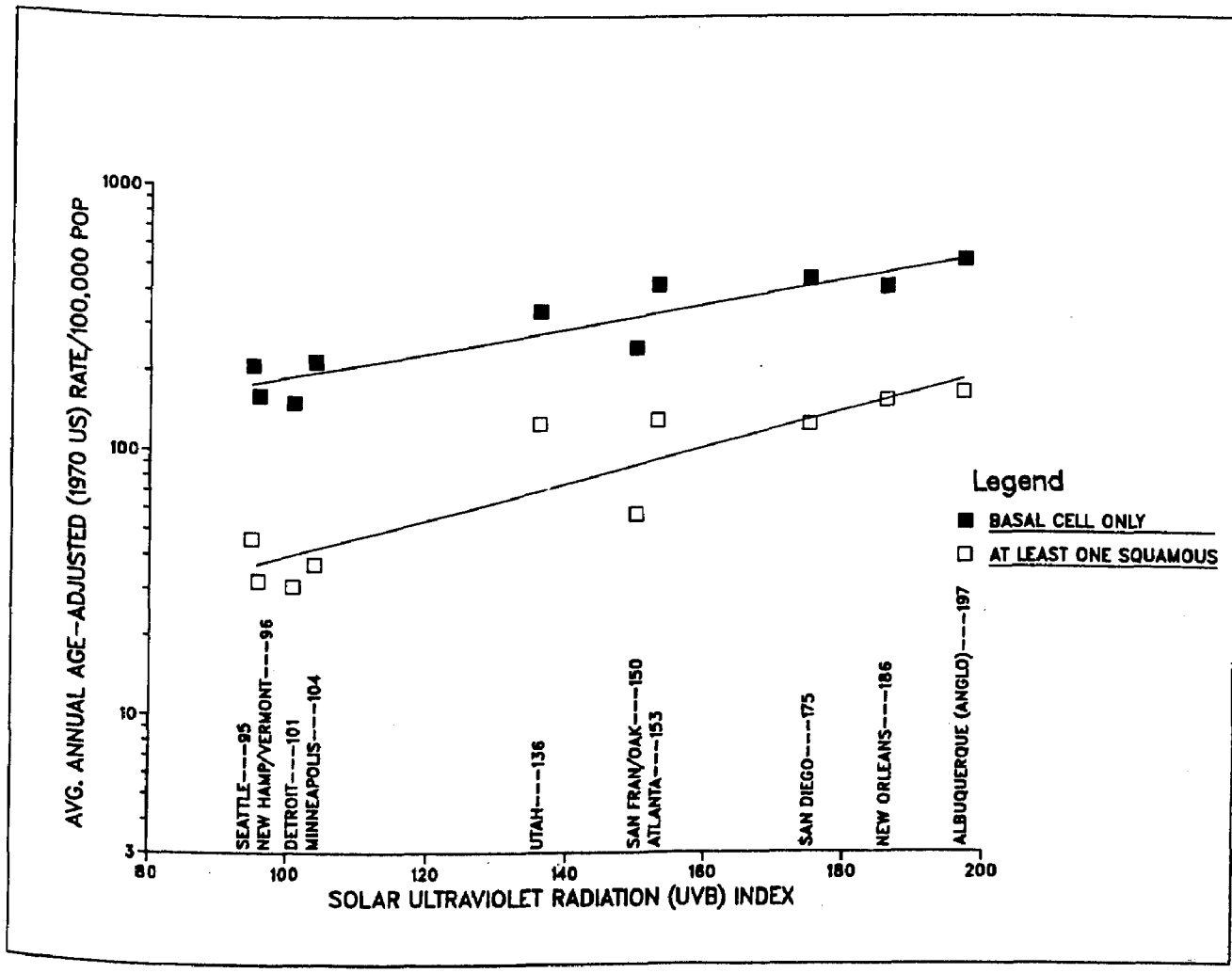


Figure 16. Nonmelanoma Skin Cancer Incidence by UV-B Index
White Males According to Cell Type

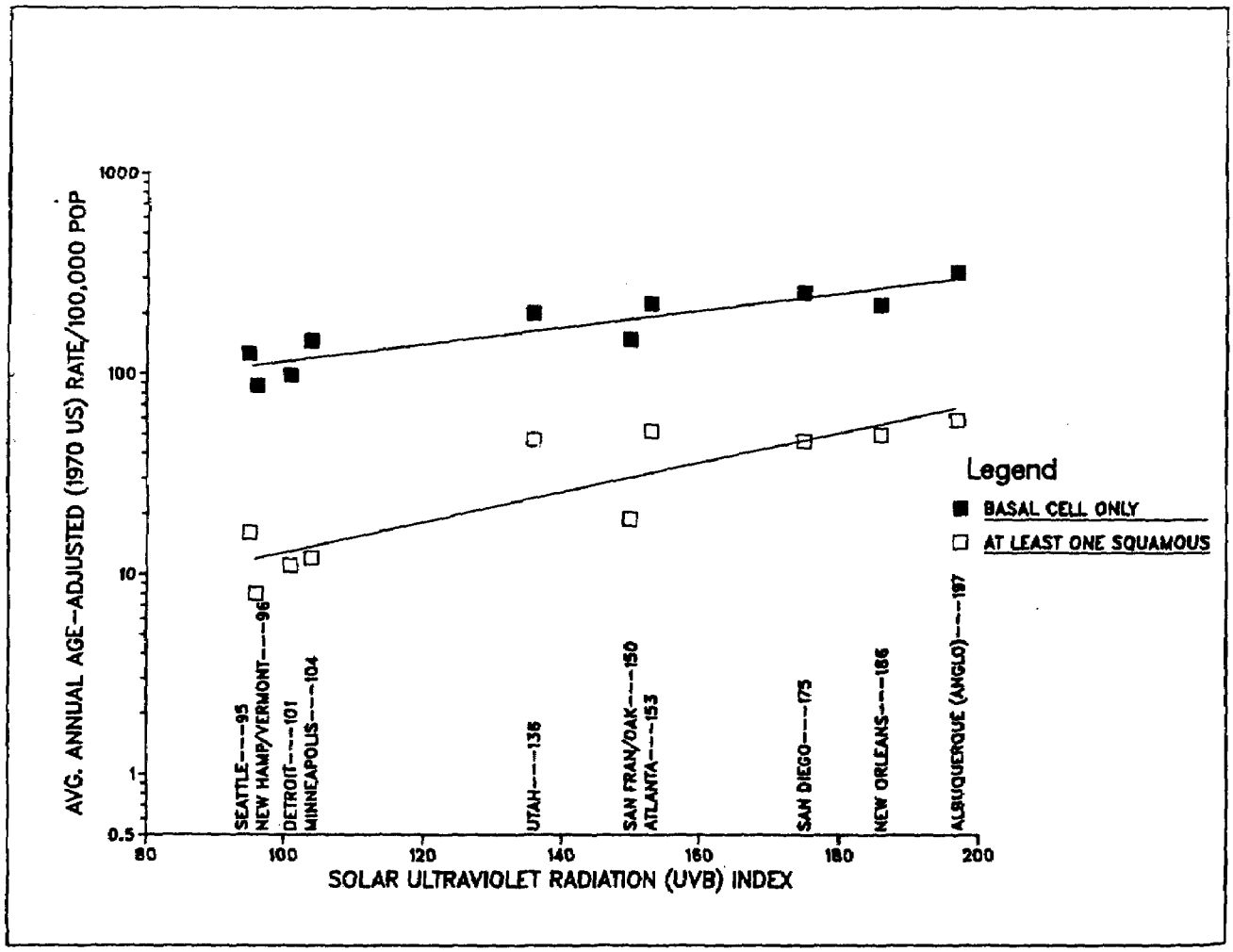


Figure 17. Nonmelanoma Skin Cancer Incidence by UV-B Index White Females According to Cell Type

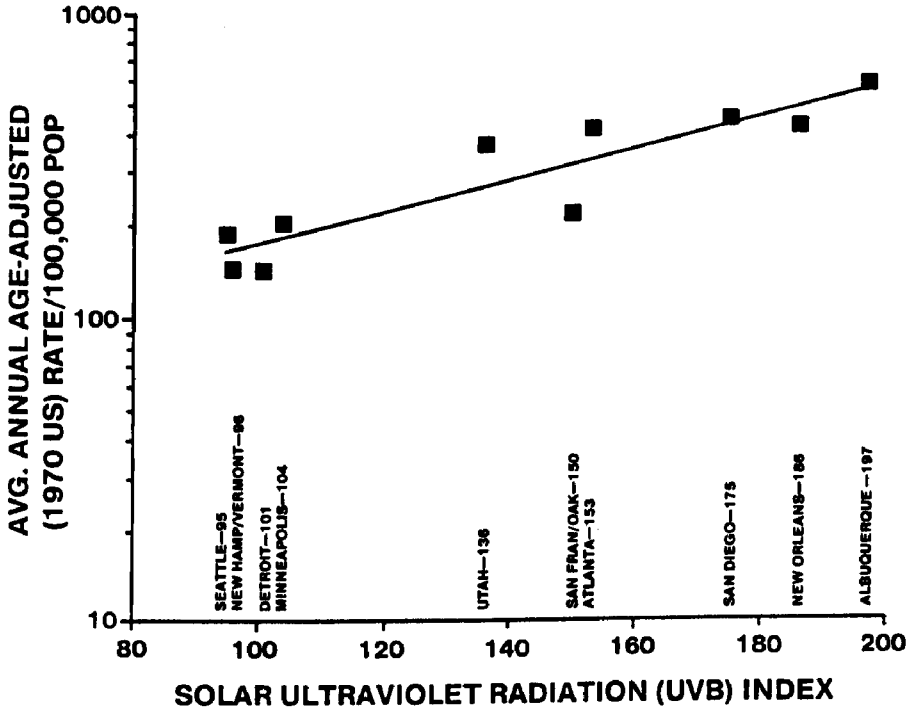


Figure 18a. Nonmelanoma Skin Cancer Incidence by UV-B Index According to Anatomical Site Group Among White Males Face, Head, or Neck

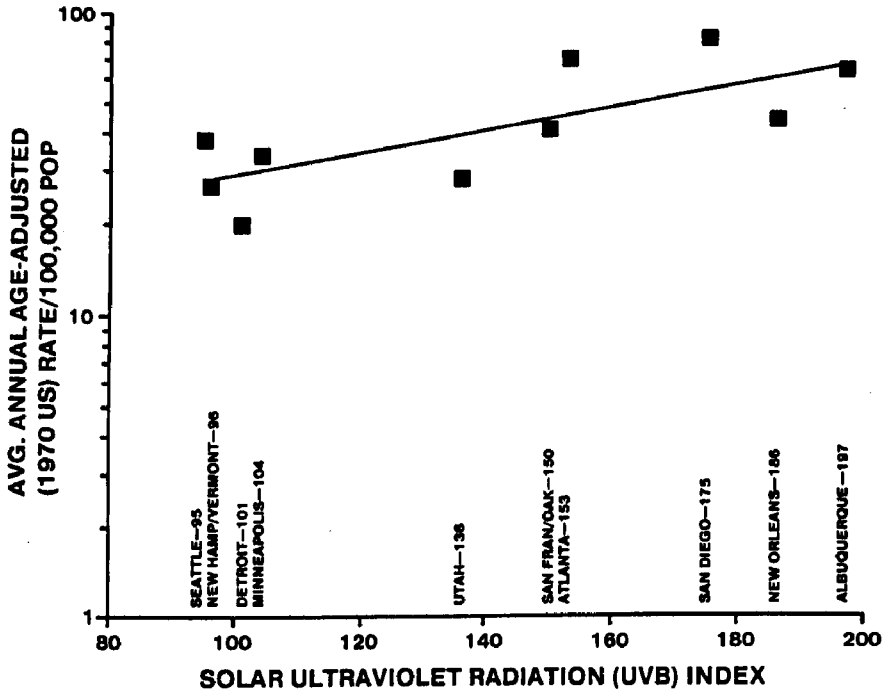


Figure 18b. Nonmelanoma Skin Cancer Incidence by UV-B Index According to Anatomical Site Group Among White Males Trunk

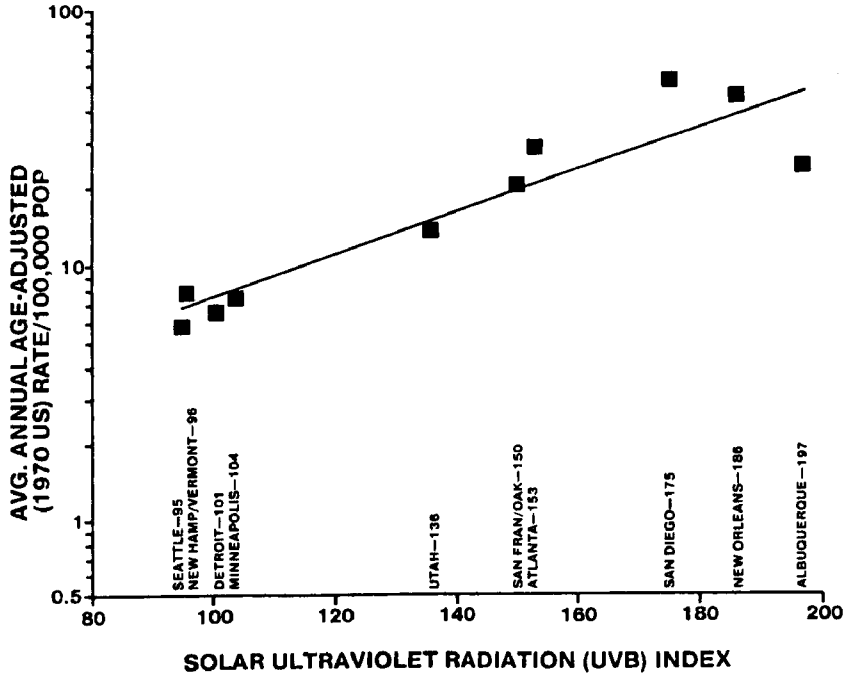


Figure 18c. Nonmelanoma Skin Cancer Incidence by UV-B Index According to Anatomical Site Group Among White Males Upper Extremities

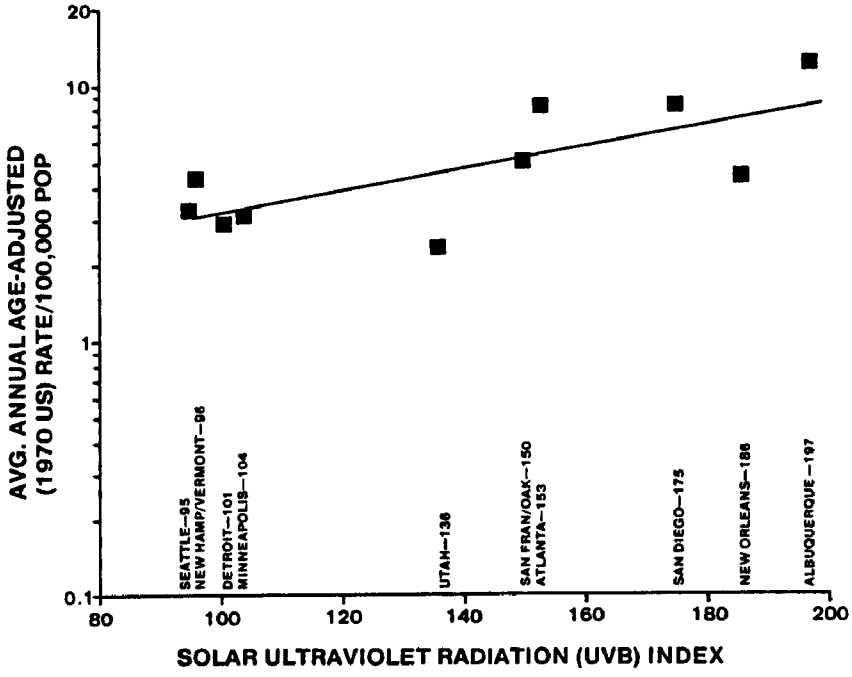


Figure 18d. Nonmelanoma Skin Cancer Incidence by UV-B Index According to Anatomical Site Group Among White Males Lower Extremities

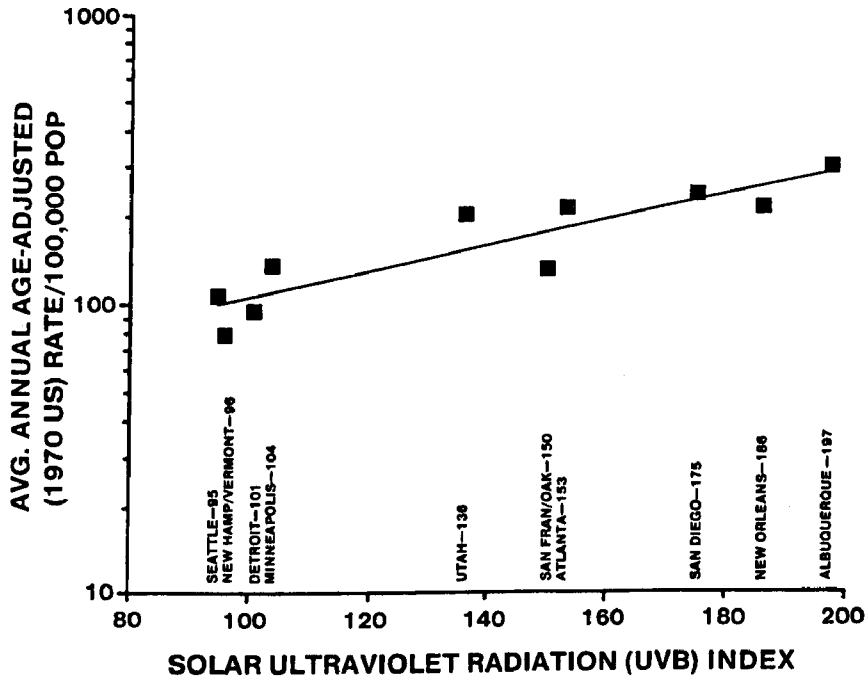


Figure 19a. Nonmelanoma Skin Cancer Incidence by UV-B Index According to Anatomical Site Group White Females Face, Head, or Neck

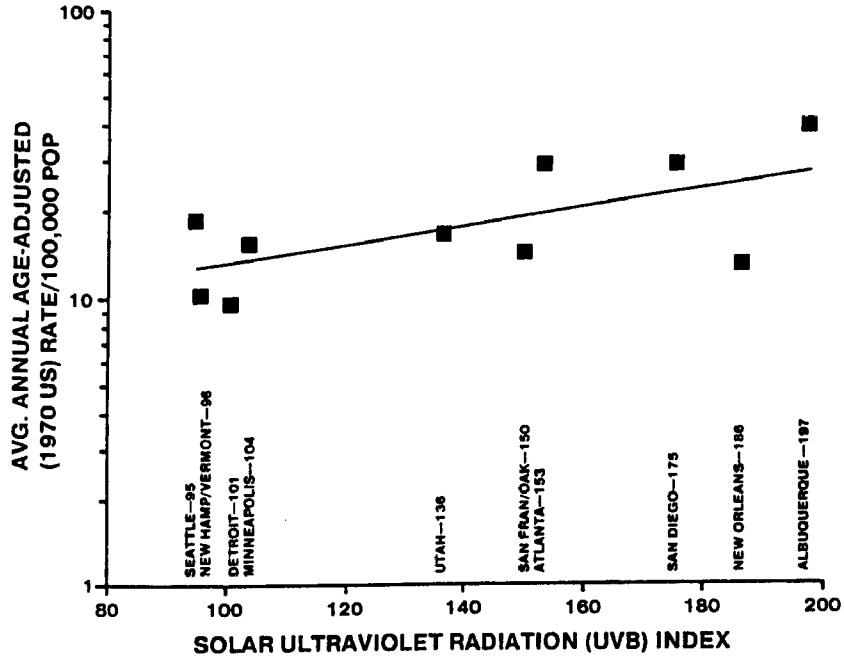


Figure 19b. Nonmelanoma Skin Cancer Incidence by UV-B Index According to Anatomical Site Group Among White Females Trunk

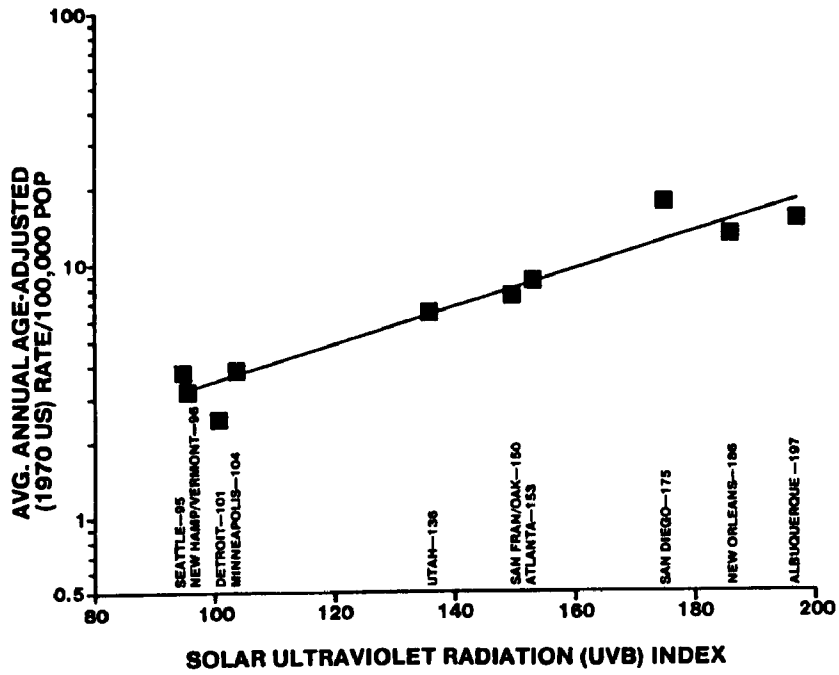


Figure 19c. Nonmelanoma Skin Cancer Incidence by UV-B Index According to Anatomical Site Group Among White Females Upper Extremities

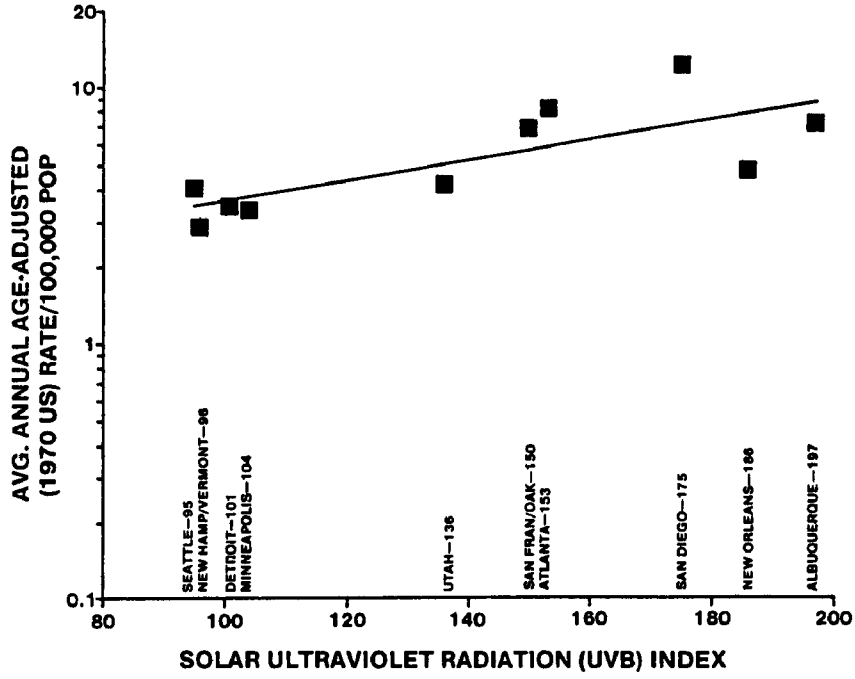


Figure 19d. Nonmelanoma Skin Cancer Incidence by UV-B Index According to Anatomical Site Group Among White Females Lower Extremities

In Figures 20 and 21 we see familiar patterns. Skin cancer incidence increases as UV-B radiation increases for white males with and without freckles. We also note that at each location the risk is greater for those with freckles. The estimated relative risk adjusting for age and location is 1.8 and 1.7 for men and women, respectively, compared to those without freckles.

The correlation for fair skinned complexion is illustrated in Figure 22. Again, the UV-B gradients are observed. Overall relative risk estimates were 2.6 for men and 1.6 for women, respectively, compared to those without fair skinned complexions.

Figure 23 shows the patterns for Celtics or those of Irish or Scottish ancestry. Estimates of relative risk were the same as those observed for individuals with freckles.

Other high-risk groups include individuals with blond or red hair color, blue or green eye color, and those who sunburn easily; individuals treated for acne, moles, warts or psoriasis; individuals exposed to radiation or radiation therapy, coal tar or pitch, and arsenic. Individuals at low relative risk include those of Mexican or Spanish ancestry and those who are never outdoors on their principal occupation and those who develop deep tans.

With respect to the consistent excesses in skin cancer risk observed for men compared to women, we noted that the average amount of time spent outdoors was greater for males by a factor of 1.5 to 2.

I am reminded of a statement made in a review article by our Chairman (Emmett, CRC 1973). It said:

> ...in the USSR where the occupations of women nearly parallel those of men, the incidence rates of skin cancer are the same for males as for females except in the elderly females who may retain more domestic occupations. On this basis, it may be unlikely that the male human has a biological predisposition to solar skin cancer.

In conclusion, I would add that this disease represents a major health and economic problem in the United States and other parts of the world. Twelve to thirty percent of the Caucasians in this country may expect to develop skin cancer in their lifetime if current rates and trends persist.

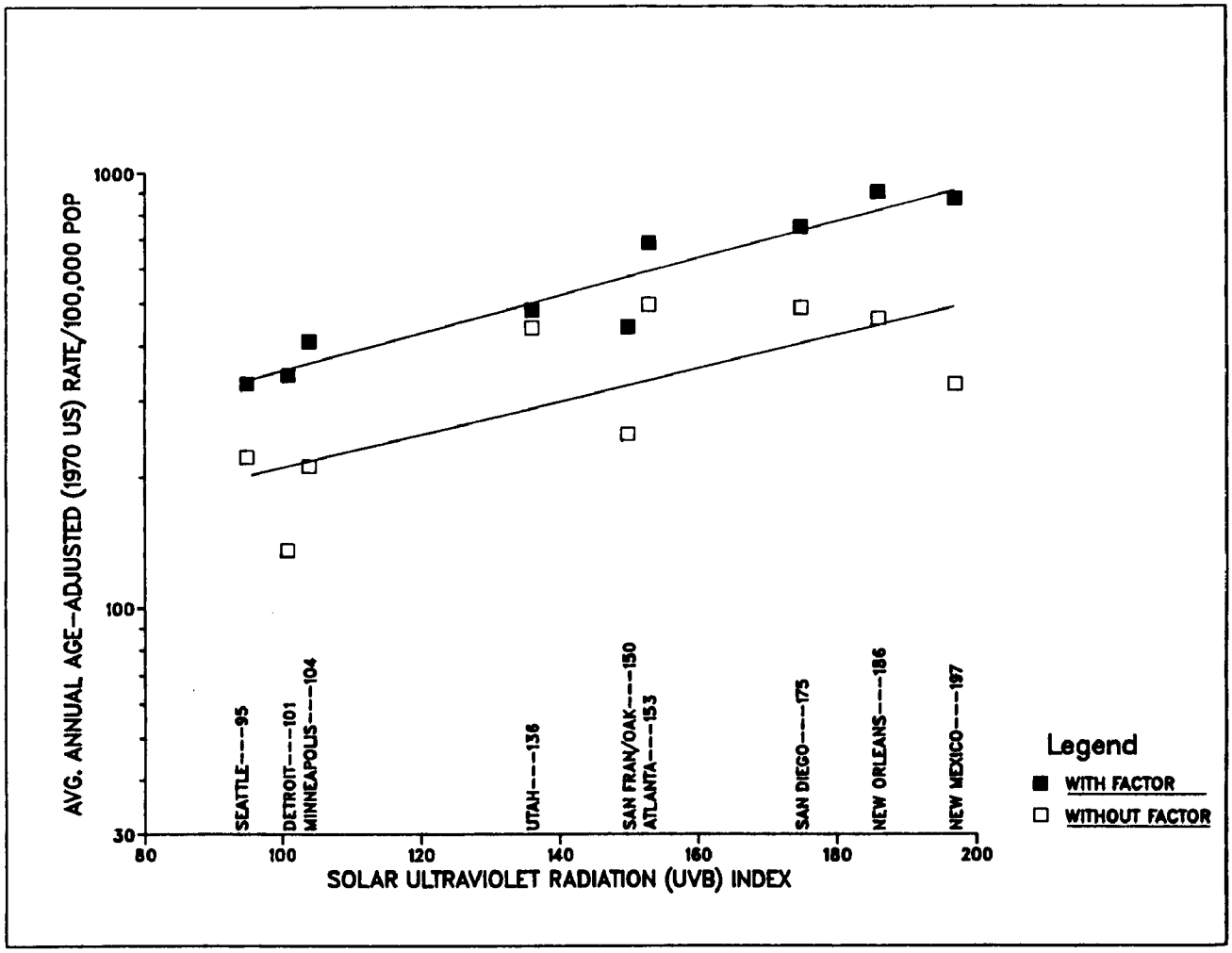


Figure 20. Nonmelanoma Skin Cancer Incidence by UV-B Index Among White Males According to the Presence or Absence of the Factor "Freckles"

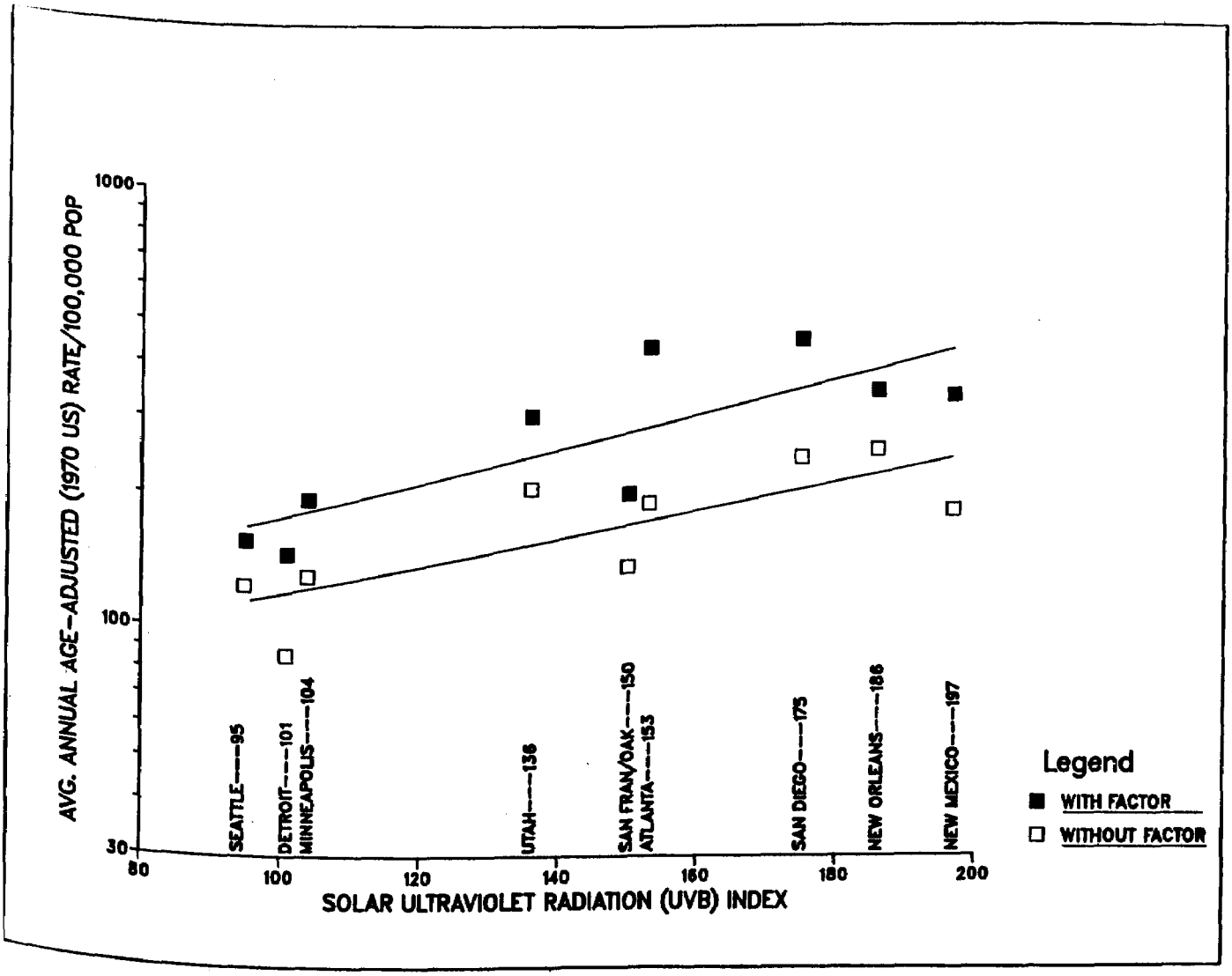


Figure 21. Nonmelanoma Skin Cancer Incidence by UV-B Index Among White Females According to the Presence or Absence of the Factor "Freckles"

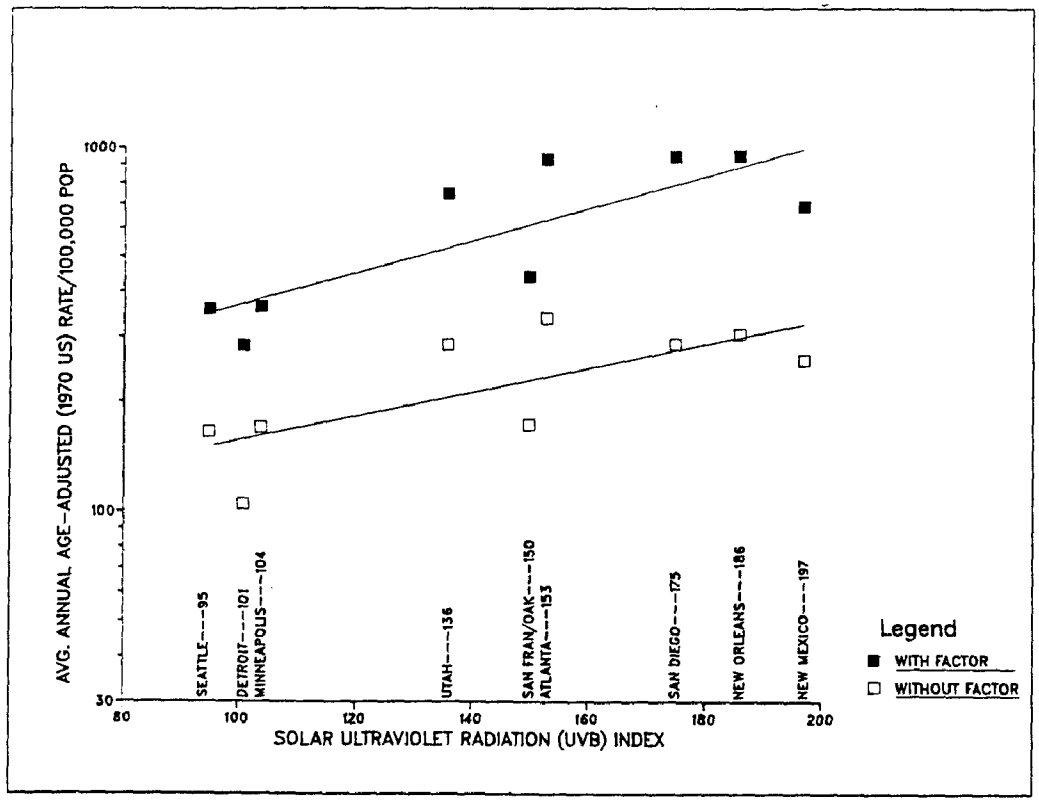


Figure 22a. Nonmelanoma Skin Cancer Incidence by UV-B Index Among White Males According to the Presence or Absence of the Factor Fair Complexion

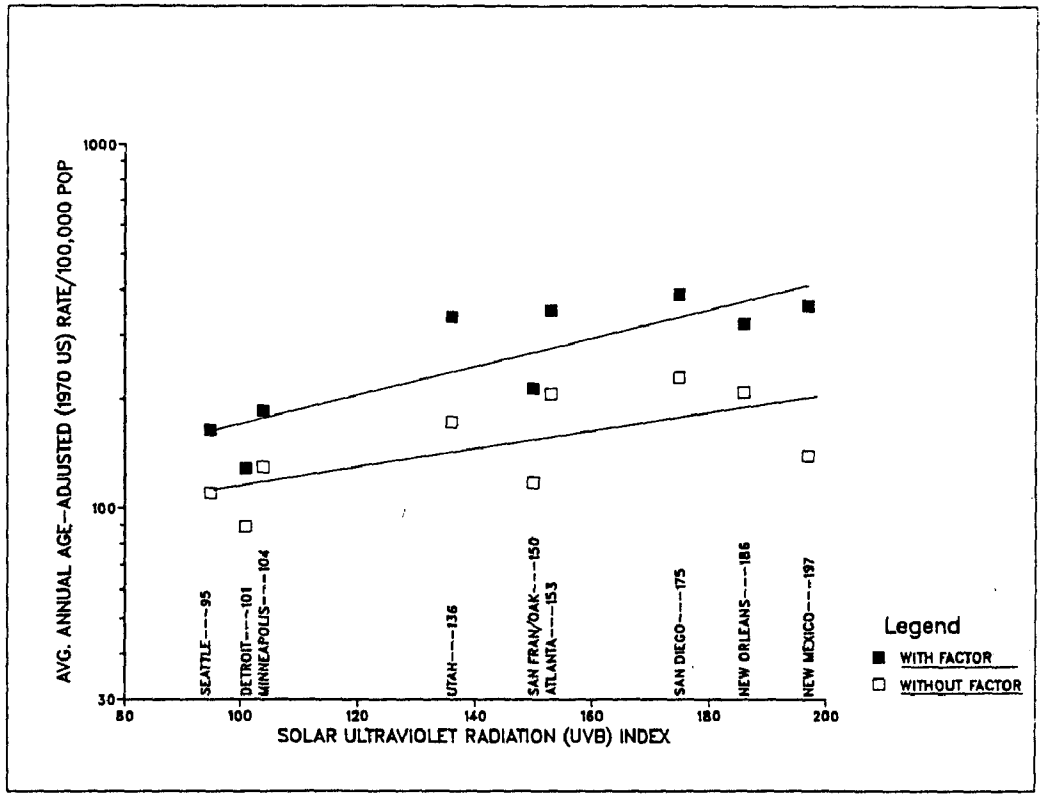


Figure 22b. Nonmelanoma Skin Cancer Incidence by UV-B Index Among White Females According to Presence or Absence of the Factor Fair Complexion

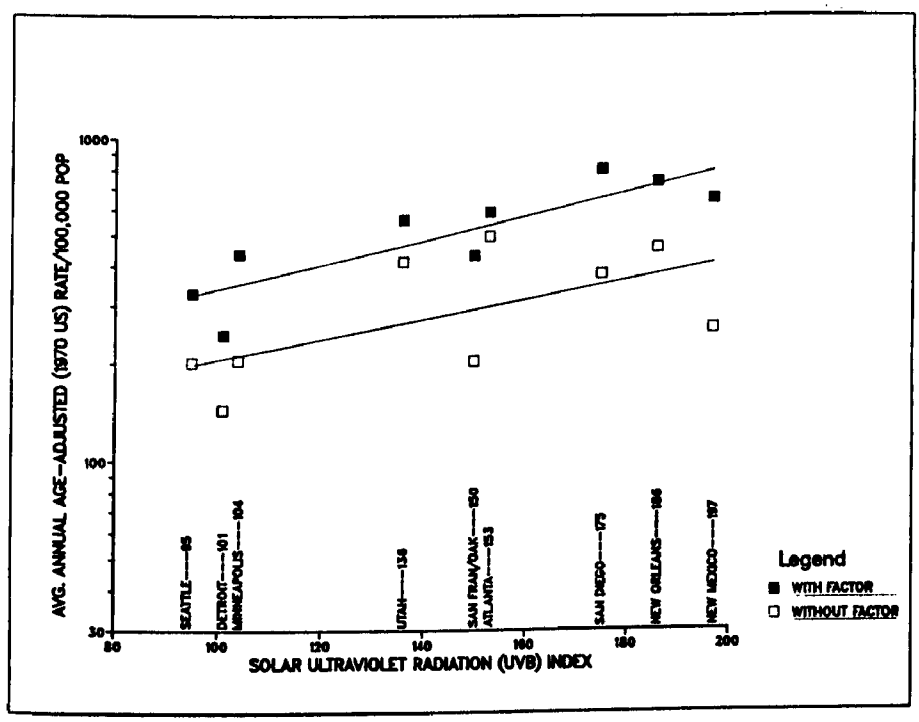


Figure 23a. Nonmelanoma Skin Cancer Incidence by UV-B Index Among White Males According to Presence or Absence of the Factor "Irish/Scot Descent"

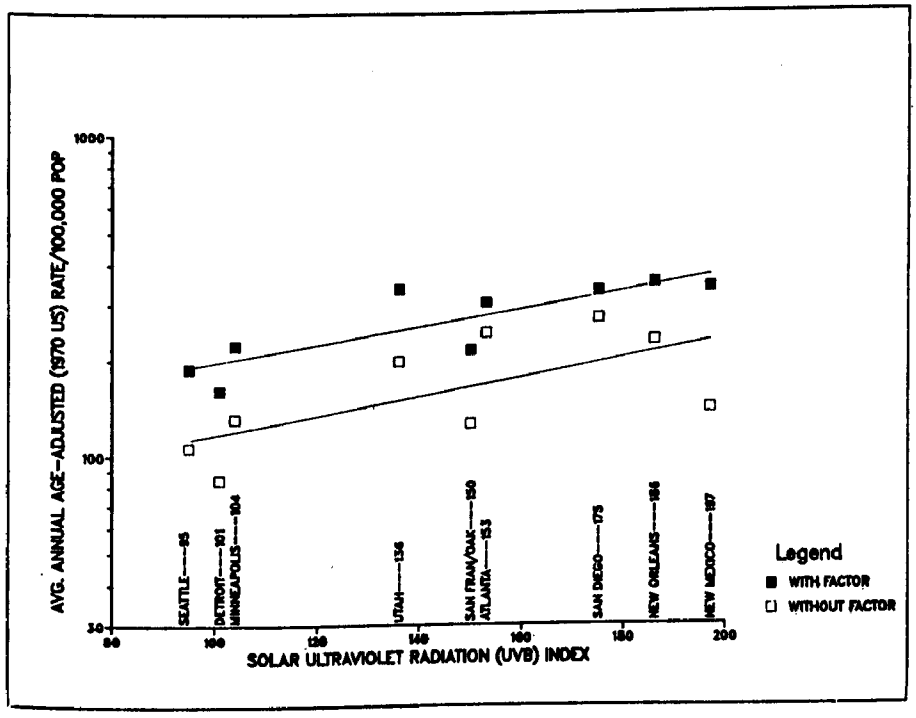


Figure 23b. Nonmelanoma Skin Cancer Incidence by UV-B Index Among White Females According to the Presence or Absence of the Factor "Irish/Scot Descent."

Immunomodulation by Ultraviolet Radiation: Prostaglandins Appear to be Involved in the Molecular Mechanisms Responsible for UVR-Induced Changes in Immune Function

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ABSTRACT

The experimental exposure of animals to sources of ultraviolet radiation (UVR) that emit their energy primarily in the UV-B region (280-320 nm) is known to result in a number of well-described changes in the recipient's immune competence. Two such changes include a depressed capacity to effectively respond immunologically to transplants of syngeneic UVR tumors and a markedly reduced responsiveness to known inducers of delayed-type (DTH) and contact hypersensitivity (CH) reactions. The results of experiments that were designed to elucidate the mechanisms responsible for UVR-induced immunomodulation have implicated: (a) an altered pattern of lymphocyt arecirculation, (b) suppressor T cells (Ts), (c) deviations in systemic antigen presenting cell (APC) potential, (d) changes in the production of interleukin-1-like molecules, and (e) the functional inactivation of epidermal Langerhans cells (LCs) in this process. The exposure of skin to UVR, therefore, causes a number of both local and systemic alterations to the ;normal host and its immune system. In spite of this seeming complexity and diversity of responses, our recent studies have established that each of the UVR-mediated changes is probably of equal importance to creating the UVR-induced imkmunocompromised state.

Normal animals were exposed to low-dose UVR on their dorsal surfaces under conditions where a 3.0 cm^2 area of skin was physically protected from the light energy. Contact sensitization of these animals with DNFB, to either the irradiated or the protected back skin, resulted in markedly reduced CH responses. This was observed in spite of a normal responsiveness following the skin sensitization to ventral surfaces of the UVR-exposed animals Systemic treatment of the low-dose UVR recipients with the drug indomethacin (1-3 micrograms/day) during the UVR exposures resulted in a complete reversal of the depression observed following DNFB sensitization to "protected" dorsal skin while the altered responsiveness found in the group exposed to the skin

reactive chemical through directly UVR-exposed site was maintained. These studies directly implicate the importance of LCs as effective APCs in the skin and also suggest that some of the systemic influences caused by UVR exposure involve the productin of prostaglandins. This concept was further supported by finding that indomethacin treatment was also capable of totally reversing the systemic depressions in CH responsiveness caused by high-dose UVR exposure (30 K joules/m^2) of mice. Attempts to analyze the cellular mechanisms responsible established that the spleens of all animals that demonstrated altered CH responses, regardless of whether sensitization was through a normal or an irradiated skin site, contained suppressor cells. Interestingly, we also found normal levels of T effector cells in the peripheral lymph nodes of the UVR-exposed mice that were contact sensitized through normal skin. No effector cells were found when skin sensitization took place through irradiated skin sites.

In spite of such an apparent paradox, insight into the probable mechanisms responsible for these observations was provided by establishing that UVR exposure of skin results in a striking and dose-dependent blockade of the efferent lymphatic vessels in all peripheral lymph nodes. Therefore, the afferent phases of immune responses can apparently take place normally in UVR-exposed animals when antigen is applied to normal skin. The final effector responses, however, appear to be inhibited in the UVR-exposed animals by an apparent block of effector cell mobility. This contrasts with findings in the normal animals. Following contact sensitization, normal animals were also found to simultaneously contain both antigen specific suppressor T cells and lymph node effector cells. However, these normal animals were fully capable of mobilizing their effector cells into the systemic circulation, thereby allowing a localization of these cells to peripheral sites of antigen challenge.

Our results suggest that UVR is probably not a significant inducer of suppressor T-cell activity to topically applied antigens. Rather, UVR exposure appears to modify the normal relationship that exists between effector and regulatory immune responses in vivo. It does so by one of two mechanisms: The first causes a direct reduction in the skin's APC function and results in an absence of effector cell generation to antigens applied to UVR-exposed skin sites, inhibiting the capacity of effector cells to gain access to skin sites of antigen challenge; the second sequesters the lymphocytes with effector cell potential into the draining peripheral lymph nodes. Each of these situations results in a similar effect on the UVR-exposed host--a reduced capacity to elicit a CH response. We hypothesize that altered DTH responses, altered alloresponses, and altered graft-versus-host responses (all of which have been observed in UVR-exposed animals) may result from similar mechanisms.

INTRODUCTION

Extensive literature describes the biologic changes that result from the exposure of experimental animals and man to the effects of UVR. Many of the reported studies focus on UVR-induced alterations of an exposed host's immune system--definable alterations that have led to the genesis of the term "photoimmunology" to describe this area of investigation. The field of photoimmunology is proving to represent a correlative scientific discipline that interrelates many areas of investigation including dermatology,

immunology, photobiology, and physiology. The goal of investigators in photoimmunology is to understand the immunobiologic consequences of UVR exposure to provide a clinically valid prediction of the potential benefits and/or detrimental effects that are associated with the continued exposure of skin to solar or artificially derived sources of UVR. Such information is important for a variety of reasons. These include both the probability that many biologic life forms on our planet may eventually be faced with increasing doses of UVR due to a reduction in the fidelity of the protective ozone layer and the recent interest by many individuals to maintain a year-round tan through the use of artificial sources of UVR.

The major objectives of this review will be to consolidate many of the observations that have been reported in the field of photoimmunology during the past few years. Many of the local and systemic changes that take place following the UVR exposure of skin will be described as they relate to changes in immunologic, histologic, pathologic, and physiologic processes. Our goal is to develop the hypothesis that many of the immunologic consequences that follow acute or chronic UVR exposure actually reflect the body's mobilization of a number of normal host defense mechanisms in response to the inflammatory effects of this physical agent. A large body of recently acquired experimental evidence will be presented to support this general hypothesis. While our results do not support the concept that UVR-mediated effects on immune function are unique, they do demonstrate the fact that the biologic changes that manifest following skin exposure to this physical agent are quite diverse. The immunomodulatory influences of UVR, therefore, appear to result from its capacity to affect a large number of interrelated biologic systems.

IMMUNOBIOLOGY OF EXPERIMENTAL UVR CARCINOGENESIS

UVR is a known carcinogen for the induction of skin tumors in both experimental animals and in man (Blum 1959; Fears, Scotto, and Schneiderman 1977; Tanenbaum et al. 1976). In addition to its carcinogenic properties, UVR can also function as a cocarcinogenic-promoting agent and an immunologic modulator (Epstein and Epstein 1962; Elmets and Bergstresser 1982). It must be appreciated that associated with the transformation event are the immunomodulatory effects of UVR that have a direct influence on the emergence and progression of skin neoplasia (Fisher and Kripke 1982; Roberts and Daynes 1980). Historically, the discovery that subcarcinogenic doses of UVR induce an immunologic state of UVR tumor susceptibility in syngeneic mice suggested that UVR was an immunomodulatory agent (Daynes et al. 1977; Kripke and Fisher 1976). It is the tumor-permitting immunomodulatory potential of UVR that distinguishes it from other carcinogenic agents.

UVR-induced tumors, like most experimentally induced tumors (e.g., chemically and virally induced), express tumor-associated antigens (TAA) that can elicit specific immune responses in their syngeneic host (Roberts, Lynch, and Daynes 1982; Pellis and Kahan 1976; Leffell and Coggin 1977; Rogers and Galetto 1985). For example, a state of tumor-specific immunity is induced in mice that are immunized with syngeneic UVR-induced tumors (either fragments or cell lines maintained in vitro) (Daynes et al. 1977; Roberts, Lynch, and Daynes 1982; Kripke 1974; Spellman and Daynes 1978; Roberts, Spellman, and Daynes 1980). This implies that the major rejection responses elicited in tumor-immunized hosts are directed toward unique tumor-specific transplantation antigens (TSTA). In addition, experiments employing hyperimmunized

animals have established that common tumor-associated transplantation antigens (TATA) are also shared by different UVR tumors (Roberts, Lynch, and Daynes 1982; Spellman and Daynes 1978; Roberts, Spellman, and Daynes 1980). Thus, it appears that any given UVR tumor expresses both unique and common TAA that are capable of becoming involved in the host immune response to varying degrees. The immune response elicited by these TAA dictates whether the tumor is rejected or allowed to grow.

Aside from a few reports, very little is known about the biochemical nature of the TAA expressed by UVR tumors. Employing tumor-reactive monoclonal antibodies, DeWitt has identified a membrane-bound TAA that appears to have a molecular weight of 200-300 KD (Daynes et al. 1985). Although the exact biochemical nature of these antigens is not understood, preliminary data suggest that one of these monoclonal antibodies recognizes a glycolipid moiety (DeWitt, personal communication). Fortner et al. (1982) have reported that UVR tumors express a virally encoded gp70 antigen on their cell surface. Similarly, DeLuca et al. (1979) have reported that cross-reactive antibodies in the serum of UVR tumor-immune mice have specificity for murine leukemia viral products. Beeson, Scott, and Daynes (1983) have demonstrated the presence of oncofetal antigens on UVR and chemically induced tumors that are also expressed on placental and fetal tissues. These particular viral-associated or oncofetal antigens could very easily function as cross-reactive TAA. Finally, Ristau et al. (1980) have reported that UVR tumors express a cross-reactive TAA that appears to be a 200 KD glycoprotein. Although these studies have begun to identify some of the common TAA that are expressed by UVR tumors, the exact biochemical nature and immunogenic properties of individual TAA are currently unknown.

Unlike the tumors that are experimentally induced by chemical carcinogens, a majority of the UVR-induced tumors are rejected when transplanted into normal syngeneic hosts (Daynes et al. 1977; Kripke 1974; Roberts, Bernhard, and Daynes 1984). These UVR-induced, regressor-type tumors will grow, however, when implanted into immunologically compromised hosts or syngeneic animals that have been exposed to subcarcinogenic doses of UVR (Daynes et al. 1977; Kripke and Fisher 1976; Kripke 1974). Thus, it would appear that the predominant immune response elicited by UVR tumors, when implanted into normal syngeneic animals, leads to their rejection. A number of mechanisms have been investigated to determine how UVR tumors are able to escape the immune surveillance capability of their host.

Tumor rejection or progression is a dynamic process involving complex interactions between the tumor and its host. Conditions that allow for tumor progression are related to the inability of the host to mount an effective immune response, as well as the capacity of the tumor to modulate its tumorigenic potential in response to immunologic pressures. A number of studies have been designed to investigate the ability of UVR tumors to modulate their tumorigenic potential. Wortzel, Urban, and Schreiber (1984) have analyzed immune responses elicited by the TSTA expressed on a UVR regressor tumor and a number of epitope loss variants derived from that tumor (Wortzel et al. 1983; Wortzel, Urban, and Schreiber 1984). Through the use of cloned tumor-specific cytotoxic T-cell (Tc-cell) lines, these investigators have provided compelling evidence that a number of TSTA epitopes are expressed by a single UVR tumor. Furthermore, the loss of specific TSTA epitopes by various tumor variants derived from the original tumor was found to be

associated with the acquisition of a progressor phenotype, i.e., the capacity to grow when implanted into normal syngeneic recipients. Thus, the loss of an immunodominant epitope(s) on a UVR-induced regressor tumor may represent a possible mechanism that allows for its progression. In this regard, tumorigenic heterogeneity of UVR tumors was observed by Schmitt et al., who isolated cloned lines from a UVR regressor tumor that was capable of progressive growth when transplanted into normal syngeneic recipients (Schmitt and Daynes 1981; Schmitt et al. 1983). In addition, these investigators determined that a UVR regressor clone could be converted from a regressor-type tumor to one capable of progressive growth in normal recipients subsequent to culture with normal lymphoid cells in vitro, or following passage through immunologically compromised hosts (Schmitt et al. 1983). These investigators also provided suggestive evidence that the regressor-to-progressor conversion process correlated with a somatic cell hybridization between cells of the tumor and those of host origin. This is not a unique concept, since in other tumor systems it has been shown that somatic cell hybridization correlates with increased metastatic potential of the tumor (Kerbel et al. 1983; Larizza, Schirrmacher, and Pfluger 1984).

Although these studies would suggest that a UVR tumor is capable of modulating its tumorigenic potential in response to the host immunologic pressures, it was necessary to confirm that UVR tumors were clonal in nature and not derived from the progressive growth of both progressor and regressor clones arising from multiple transformants with the tumor mass. To investigate this possibility, Burnham, Gathering, and Daynes (1986) employed the tool of x-chromosome inactivation mosaicism with the x-linked enzyme phosphoglycerate kinase 1 (PGK-1) to evaluate the clonality of UVR-induced tumors. Out of thirteen primary UVR tumors that were induced in (C3HxC3H.PGK-1^a)F_1 heterozygote female mice, which phenotype as PGK-1a/b, all were found to express only a single PGK-1 enzyme form. Based on this finding, it was concluded that the majority of UVR-induced tumors are monoclonal in origin and result from the progression of a single transformed cell. These experiments also suggest that the heterogeneity observed within a single tumor with regard to its tumorigenic potential must arise from events taking place subsequent to the original transformation process.

Although the ability to modulate TSTA epitopes and a certain level of tumorigenic heterogeneity within UVR regressor tumors indicates that these tumors may escape immunologic rejection through their ability to modulate their growth characteristics in an immunologically competent host, the exact mechanism of how these tumors are capable of emerging and progressing within their autochthonous host is unclear. It is intriguing that UVR regressor tumors are rejected when transplanted into normal syngeneic recipients, but are capable of progressive growth in the autochthonous host as well as syngeneic animals that have been exposed to subcarcinogenic doses of UVR, especially since UVR-exposed animals appear to possess virtually normal immunologic competency (Spellman, Woodward, and Daynes 1977; Norbury, Kripke, and Budmen 1977; Kripke et al. 1977). This suggests that UVR regressor tumors employ an escape mechanism(s) to evade the immunologic rejection response of their host. Transplantation studies have shown that the progressive growth of UVR regressor tumors is, in certain cases, dependent on a population of UVR-induced suppressor T cells (Ts-cells) (Spellman and Daynes 1978).

In contrast to the Ts-cells that arise in response to the TSTA expressed by progressor-type tumors (both UVR and chemically induced), the UVR-induced Ts-cell population possesses functional antigenic specificity for a common TAA that appears to be expressed by virtually all UVR tumors and some chemically induced tumors (Roberts, Lynch, and Daynes 1982; Spellman and Daynes 1978; Roberts and Daynes 1986). Because this population of Ts-cells arises prior to the appearance of overt tumors, it appears that these Ts-cells dictate the emergence and progression of these neoplasia. The functional characteristics of this Ts-cell population, as well as the process for their induction, have been the subject of recent investigations.

It has been shown that the regulation of immune responses to a number of complex multideterminant antigens is mediated through the recognition of distinct immunoregulatory epitopes (Yowell et al. 1979; Hashim et al. 1976). We have hypothesized that the immune response to UVR tumors may be viewed in a similar context, since epitopes associated with the various types of tumor antigens could function as either strong tumor rejection epitopes or weak immunoregulatory determinants (Roberts and Daynes 1980; Roberts, Lynch, and Daynes 1982; Roberts, Spellman, and Daynes 1980).

Thus, the UVR-induced Ts-cell population would function through an associative recognition mechanism. Through its ability to recognize common TAA expressed by virtually all UVR tumors, it would inhibit the development of immune rejection responses elicited by the stronger TSTA and other common TATA. This hypothesis implies that the UVR-induced Ts-cell population is homogeneous, i.e., consists of a single clone or limited number of clones of Ts-cells that are restricted in their ability to recognize a common TAA epitope. This has been confirmed by recent studies employing a number of interleukin-2 dependent Ts-cell lines derived from animals that have been exposed to subcarcinogenic doses of UVR (Roberts, Spellman, and Warner 1983; Roberts 1986). In these studies, it was shown that both the parental and cloned Ts-cell lines were capable of rendering normal syngeneic hosts susceptible to the growth of a battery of UVR regressor tumors. In vitro, these cloned Ts-cell lines appear to mediate their effect by inhibiting the differentiation of Tc-cells from the draining lymph node (DLN) cell populations obtained from tumor-immune mice. Thus, this Ts-cell population, through its ability to recognize these common TAA, would provide an immunologic environment that would allow for the emergence and progression of virtually any neoplastic cell that expresses these antigenic determinants, regardless of their expression of other stronger tumor rejection antigens.

Although the exact mechanism for the induction of the UVR-induced Ts-cell population is unknown, previous studies suggest that neoantigens, which are cross-reactive with TAA, are expressed in the skin of UVR-exposed mice (Palaszynski and Kripke 1983; Spellman and Daynes 1984; Sielstad et al. 1985). Palaszynski and Kripke (1983) demonstrated that normal syngeneic mice were rendered susceptible to the growth of UVR regressor tumors when grafted with large pieces (5 cm x 2.5 cm) of skin from UVR-treated donors. In contrast, Spellman and Daynes (1984) found that animals grafted with smaller pieces (1 cm diameter) of UVR-exposed skin were effectively immunized against a transplantable UVR tumor. In both studies, normal skin grafts failed to produce any detectable immunologic alterations. We have recently detected TAA cross-reactive antigens in cellular extracts from UVR-exposed epidermal cells employing TAA-specific monoclonal antibodies in an enzyme-linked immunosorbent

assay (Sielstad et al. 1985). Collectively, these studies strongly support the contention that UVR induces TAA cross-reactive antigens in the skin before the recognized emergence of neoplasia. It is conceivable that these skin-associated TAA are responsible for eliciting the Ts-cell response that is observed in animals that are exposed to subcarcinogenic doses of UVR.

We have recently conducted a series of experiments to further define the immunogenicity of the cross-reactive TAA that are expressed in the skin of UVR-exposed mice (Hong and Roberts 1986). A combined protocol of in vivo immunization and in vitro culture of DLN cells was employed for the analysis of TAA-specific Tc-cells. For these studies, mice were first immunized by injecting their footpads with viable tumor cells or skin cells. After eight to ten days, the DLN were removed from these animals, and the cells were suspended in tissue culture. After four days of tissue culture, the DLN cells were harvested and the Tc-cell activity was analyzed in a cell-mediated cytotoxicity assay. The results of these studies are summarized as follows:

- It was determined that Tc-cells capable of lysing cross-reactive tumors differentiate from the DLN of UVR tumor and UVR skin-immune, but not normal skin-immune mice.

- These Tc-cells are capable of lysing a range of tumor targets, including syngeneic and allogeneic UVR tumors and syngeneic methylcholanthrene-induced tumors. However, these cells did not lyse Con A activated syngeneic lymphoblasts, thioglycolate-induced peritoneal exudate cells, or YAC 1 lymphoma cells, which are sensitive targets for the lysis by natural killer cells.

- Cold cell inhibition experiments demonstrated that these Tc-cells recognize common cross-reactive TAA.

- The expression of these cross-reactive TAA in the skin of UVR-exposed mice appears to be the direct effect of UVR exposure, since the only cells that were effective in immunizing syngeneic mice against TAA were those that were obtained from skin that was directly exposed to the effects of UVR.

These studies were further expanded to characterize the immunogenic potential of the cross-reactive UVR tumor and UVR skin-expressed TAA. In these experiments, cellular extracts (obtained by CHAPS detergent) from UVR tumors and epidermal cells of either UVR-exposed or normal mice were incorporated into liposomes for the immunization of normal syngeneic animals. The lytic activity of four-day cultured DLN cells from these immunized animals was compared with the DLN cells derived from mice immunized with either whole cells or nonincorporated soluble cell extracts. The results of these experiments indicated that the cross-reactive TAA expressed by UVR epidermal or UVR tumor cells can elicit a Tc-cell response when incorporated into liposomes and used to immunize normal syngeneic animals.

Although the levels of lytic activity were different, similar results were obtained when whole cells were used for immunization. In this study, normal epidermal cells did not display any appreciable level of TAA expression. Interestingly, soluble cell extracts alone were incapable of eliciting a Tc-cell response in the DLN of immunized mice. Therefore, in vivo

experiments were conducted to identify the difference in the immunogenic potential of whole cells, liposome-incorporated TAA, and soluble cell extracts for the induction of TAA-specific Tc-cells. The results of these experiments suggested that presentation of the antigens on a membrane surface is required to elicit an effective immune response, while presentation of soluble antigen alone may induce tolerance. In the first part of this study, groups of mice were immunized repeatedly with viable epidermal cells obtained from UVR-exposed or normal syngeneic donors. These immunized animals were then challenged with a UVR progressor tumor that was capable of progressive growth in untreated normal hosts, as well as in those animals that were immunized with normal epidermal cells. However, mice immunized with UVR skin cells rejected the UVR tumor implants. In a second series of experiments, groups of mice received intravenous injections of soluble epidermal cell extract obtained from UVR-exposed or normal syngeneic donors. These animals were then challenged with a UVR regressor tumor. This tumor grew progressively in UVR-treated hosts and was retained for an extended period of time in recipients of soluble UVR skin cell extract. The tumor was rejected at a similar rate by normal mice and recipients of soluble normal skin cell extracts. From these studies, we have concluded that, similar to what was observed in vitro, cross-reactive UVR skin expressed TAA that was capable of eliciting an in vivo immune response. When presented to their host in a soluble form, these antigens elicit a UVR tumor rejection response. When presented to their host in a soluble form, these antigens elicit a UVR tumor tolerance reaction. This supports the possibility that by shedding a soluble cross-reactive TAA, UVR-exposed skin cells may elicit the TAA-specific Ts-cells that arise in UVR-treated mice before the appearance of any overt neoplasia.

In conclusion, the UVR-induced Ts-cell population appears to arise in response to neoantigens in the skin of UVR-exposed animals. These Ts-cells, by an unknown mechanism, suppress the ability of their host to mount an effective immune rejection response to emerging neoplasia. As a result, highly immunogenic tumors can emerge and progress to the death of the host that would have otherwise been eliminated had the individual possessed its normal immunologic potential. We have hypothesized that this particular immunoregulatory network may have arisen as a mechanism to protect the host during repair of UVR-damaged skin, i.e., that through an immunosuppressive mechanism which is mediated by antigen-specific Ts-cells, damaged skin can undergo repair processes without the elicitation of an autoimmune type response. Through the ability of emerging neoplasia to express a common TAA that is also expressed by UVR-damaged skin cells, they are able to use this particular Ts-cell population as an immune surveillance escape mechanism. Although not necessarily the sole mechanism for providing the growth of UVR-induced tumors, this particular Ts-cell population does appear to be responsible for the emergence and progression of a majority of UVR-induced neoplasia.

THE EFFECTS OF UVR EXPOSURE ON THE SKIN AND ITS ASSOCIATED LYMPHOID TISSUE

It is now appreciated that many types of specialized immune responses are initiated whose effector responses are primarily restricted to specific anatomic compartments within the body. For example, those immune responses that are associated with the gut-associated lymphoid tissue (GALT) and the bronchial-associated lymphoid tissue (BALT) represent two well-described examples of immunologic responses whose effector arms are anatomically

compartmentalized. This anatomic restriction to both the afferent and efferent mechanisms in GALT- and BALT-associated immune responses provides for a means to achieve a marked enhancement to the protective capabilities of these systems (Guy-Grand, Griscelli, and Vassalli 1974; Bienstock, Johnson, and Perey 1979).

It has been proposed that an immunologic circuit exists that is dedicated exclusively to the immune surveillance and protection of the skin (Streilein 1978). This specialized circuit has been given the acronym SALT for skin-associated lymphoid tissue. The evidence to support the existence of such a specialized system is somewhat indirect and includes: the observed epidermotropism for some subsets of normal and transformed lymphocytes, a demonstration of the antigen presentation capabilities of certain epidermal cell subsets, and a demonstration that antigen recognition and responsiveness of lymphocytes can occur exclusively in the skin. In addition, this concept is supported by the knowledge that the integrating and regulatory elements capable of controlling certain types of immune responses can be found, both in the skin itself, as well as in the draining peripheral lymph nodes (Streilein 1985). It is quite probable that UVR-induced skin damage can mediate a pronounced alteration to the fidelity of the SALT system.

The elegant experiments performed by Macher and Chase (1969) established that cells and/or soluble factors derived from hapten-sensitized skin were required in the development of a contact hypersensitivity (CH) response. This work was followed by the finding, greater than ten years later, that the majority of antigen presenting cell (APC) capacity of normal skin resides with the epidermal Langerhans cell (LC) (Stingl et al. 1978). Furthermore, the exposure of experimental animals (Toews, Bergstresser, and Streilein 1980) or man (Cooper et al. 1985) to low doses of UVR in the UV-B range (280-320 nm) was found to markedly reduce the number and functional properties of LC in the exposed skin, with a parallel depression in the capacity of these individuals to elicit a CH response to skin-reactive chemicals applied directly to the UVR-exposed skin sites (Toews, Bergstresser, and Streilein 1980). Interestingly, the exposure of animals to high doses of UVR (>10 KJ/m^2) causes a systemic depression in their ability to elicit a CH response, a condition which does not correlate with a reduction in LC presence or function at the non-UVR-exposed sites of hapten application (Noonan, DeFabo, and Kripke 1981; Lynch, Gurish, and Daynes 1983). These two phenomena, local and systemic suppression of CH responses, have been the object of intense investigative effort over the past few years. In a later section of this review we will describe the results of recent experiments that implicate the formation of arachidonic acid metabolites in the mechanism(s) responsible for these changes in immune function.

Greene et al. (1979) demonstrated that the UVR exposure of animals results in a decrease in their splenic APC potential. They concluded that this UVR-induced APC reduction was ultimately responsible for many of the immunomodulatory influences of UVR, including the acquisition of tumor susceptibility, the observed depressions in humoral and cellular immune responses, and the reduced capacity of UVR-exposed animals to elicit CH responses. Subsequent investigations by Gurish, Lynch, and Daynes (1982) established that the UVR-induced reduction in splenic APC function was paralleled by a marked increase in the APC activity within peripheral lymph nodes that drain the skin sites of UVR exposure. The results of these

experiments suggested that the reported changes in APC function following UVR exposure actually reflect a redistribution of APC from central to peripheral lymphoid compartments. This was further supported by the finding that splenectomized UVR-exposed animals did not demonstrate an increase in their peripheral lymph node APC activity. Investigations by Lynch, Gurish, and Daynes (1983) in mice and Cooper et al. (1985) in humans established that Ia-positive cells with APC activity regain access to the epidermis by three days following an acute UVR exposure. These cells may not be LC since the results with humans indicate that the epidermis infiltrating cells are DR positive and T6 negative. Collectively, the results of these experiments indicate that Ia-positive cells with APC function are highly mobile in vivo. Under normal conditions, the cell types having this functional and surface phenotype are distributed among a number of distinct anatomic compartments. These include the peripheral blood, spleen, lymph nodes, skin, as well as other tissue sites. Exogenous stimulation of an animal with an inflammatory agent (i.e., UVR) results in: a functional inhibition of any directly UVR-exposed immunocompetent cells and a marked anatomic redistribution of the cells with APC potential, with enhanced numbers going to the tissue sites of inflammatory insult. This is manifested by increased numbers of APC entering the skin, and eventually localizing to draining peripheral lymph nodes following their entrance via the afferent lymphatic vessels. This hypothesis is fully consistent with the recent findings of Hendricks and Eestermans (1983) who have analyzed the recirculation patterns of rat macrophages in vivo and concluded that their entry into peripheral lymph nodes is predominantly through afferent lymphatic drainage.

Studies performed by Spangrude et al. (1983) have established that the exposure of normal mice to UVR results in a marked change in the lymphoid tissue localization properties of recirculating lymphocytes. This is reflected experimentally by a significant increase in the percentage of intravenously injected radiolabeled lymphocytes recovered from the draining peripheral lymph nodes of UVR-exposed animals. This UVR-induced alteration in lymphocyte localization patterns is quite protracted and persists for greater than six weeks following the cessation of UVR exposure. Recent experimental evidence has now provided us with an appreciation of the mechanisms that are responsible for this effect. Immunohistologic staining of peripheral lymph node sections with the monoclonal antibody MECA-325, which is specific for high endothelial venules (HEV), revealed that the HEV content of lymph nodes from UVR-exposed donors is far greater than the HEV content of a similar lymph node from a normal animal (Samlowski and Daynes, unpublished). Since the quantity of HEV in a lymph node dictates the rate of lymphocyte entry into the tissue, an enhancement of HEV expression would result in an increase in lymphocyte localization potential. This is due to the fact that HEV contain the lymphocyte recognition structures that are essential for the entrance of lymphocytes into various types of lymphoid and nonlymphoid tissues (Gallatin, Weissman, and Butcher 1983). Specific lymphocyte-HEV interactions have also been proposed to control the distribution of lymphocytes between peripheral, mucosal, and central lymphoid organs (Butcher 1983).

It is now appreciated that the integrity of peripheral lymph node HEV (both the presence and the magnitude) is controlled by humoral or cellular factors that drain into the nodes via the afferent lymphatics. This conclusion was based on the results of studies that established that severing of the afferent lymphatic vessels resulted in a disappearance of HEV

structures in the draining lymph nodes (Hendriks and Eestermans 1983). This reduction in HEV expression was followed by a markedly decreased rate of lymphocyte entry into these manipulated nodes from the blood. Such experiments suggest that the skin actually functions as a transducer, in some way converting exogenous inflammatory stimuli into humoral and/or cellular signals that have an influence on the presence and extent of vascular endothelium that can function as lymphocyte-receptive HEV.

There are a couple of obvious candidates that can be considered important in the transmission of HEV regulating signals from the skin to the lymph nodes. These include the highly mobile macrophages and APC or their products, as well as soluble mediators produced directly by cells within the skin in response to an exogenous stimulation. Recent evidence from our laboratory emphasizes each of these factors in the regulation of the lymphocyte content of a peripheral lymph node. We now appreciate that subsequent to the treatment of animals with agents capable of interfering with macrophage function (silica or carrageenan) or following surgical splenectomy, exogenous stimulation with UVR does not result in an enhanced HEV expression. Such an activity can be returned to a splenectomized animal by the adoptive transfer of adherent splenocytes prior to stimulation with UVR exposure (Chung and Daynes, unpublished). Therefore, the extent to which peripheral lymph node HEV can be stimulated via an exogenous inflammatory insult appears to depend on the integrity of the animal's macrophage/APC function.

The lymphocyte content of a given lymphoid organ is a reflection of not only the rate at which blood-borne cells are capable of entering the tissue, but is also dependent upon the length of time that the lymphocytes are sequestered within the lymphoid organ prior to gaining recirculation potential. We have recently determined that the exposure of animals to UVR causes blockade in the efferent lymphatic vessels, a situation which results in the sequestration of lymphocytes within the lymph nodes draining the site of UVR exposure (Chung et al., submitted). A similar condition can be induced by the intravenous injection of murine and interferon into normal mice. Of interest was the parallel finding that treatment of mice with indomethacin (1-3 μg/day) totally abrogated the development of the efferent blockade by UVR and α/β interferon. The efferent blockade caused by the direct injection of prostaglandin E2 was not influenced by indomethacin injection, suggesting that prostaglandins were involved in the responsible mechanism.

In summary, the exposure of animals to UVR is capable of modifying many components of the SALT system. Both macrophage/APC and the recirculation and tissue localization properties of lymphocytes are equally affected. The role played by such changes in the modulation of immune responses by this physical agent are currently unresolved, although the parallelism that exists certainly suggests a cause-effect relationship to some of the observed changes.

PROINFLAMMATORY PROPERTIES OF ULTRAVIOLET RADIATION

It is well recognized that animals exposed to UVR (especially UV-B) undergo a pronounced and dosage-dependent inflammatory response. While the cellular and molecular alterations in the skin that ultimately result in a sunburn reaction are both numerous and complex, it is now appreciated that the generation of biological mediators and the elaboration of cytokines by

epidermal cells or infiltrating cell types may play an important role in the immunomodulatory changes that are observed following UVR exposure.

Keratinocytes of the skin are capable of producing protein mediators termed epidermal-derived thymocyte activating factor (ETAF), a group of multifunctional hormones that are functionally, physiochemically, and structurally identical to macrophage-derived interleukin-1 (IL-1) (Luger et al. 1983). ETAF/IL-1 is now appreciated to function as a major mediator in both immune and inflammatory responses (Luger and Oppenheim 1983). Based upon the knowledge that number of inflammatory processes are initiated by UVR and that the epidermal cells of the skin are a major target of this physical agent, we have analyzed whether modifications in the levels of ETAF production, plus a number of known host responses to this mediator, were affected by UVR exposure. Our results established that UVR exposure does not adversely affect ETAF production at subcytotoxic doses (Gathering et al. 1984).

Many biologic effects have been ascribed to the stimulation and release of ETAF/IL-1 in vivo. ETAF and IL-1 can stimulate numerous target organs throughout the body including the brain, bone marrow, liver, and lymphoid organs. Target cell interactions with ETAF/IL-1 result in elevations in core body temperature, the number of circulating neutrophils, the enhanced production of acute phase proteins by the liver, as well as lymphocyte activation and chemotaxis (Powanda and Beisel 1982; Moissec, Chai-Li, and Ziff 1984). Exposing mice to UVR results in an elevation in the number of peripheral blood neutrophils as well as an increase in plasma concentrations of several of acute phase proteins (Gathering et al. 1984; Dinarello 1984). Further, ETAF/IL-1 can be detected in the serum of these animals 24 hours after exposure to UVR. Since UVR exposure is inflammatory, and mononuclear cells have been known to migrate into irradiated skin sites, we cannot discriminate whether the observed elevation in acute phase proteins in vivo is due to an increase in the production of ETAF by keratinocytes or is due, in part, to IL-1 produced by macrophages that have infiltrated the sites of inflammation.

A recent observation in our laboratory has provided insight into another possible source of ETAF/IL-1. Normal murine and human stratum corneum contains a substantial amount of ETAF/IL-1 (Gathering, Buckley, and Daynes 1985). The physiologic role of this stratum corneum-associated ETAF/IL-1 remains to be determined; however, one could speculate that it provides a preformed pool of this mediator that is capable of initiating inflammatory responses following wounding or subsequent to protocols that increase the rate of percutaneous absorption. UVR is known to be capable of increasing the percutaneous absorption of small molecular weight substances through the skin. Therefore, keratinocyte production of ETAF, percutaneous absorption of preformed mediator, and/or mononuclear cell production of IL-1 may all influence the generation of a UVR-induced systemic inflammatory response.

We have demonstrated that daily exposure of mice to UVR results in an increase in the production of acute phase proteins, and have speculated that this is due to elevations in ETAF/IL-1. It is interesting to note that this heightened response is followed by an eventual return to normal plasma levels of these acute phase proteins despite a continued UVR exposure of the animal. The cause of this "desensitization" to the effects mediated by UVR

exposure is currently unknown. We have, however, established that keratinocytes obtained from a skin site exposed to daily UVR are still fully capable of producing ETAF in vitro. While some control of ETAF/IL-1 effects in vivo may reside at the level of the keratinocyte, the possibility that regulation takes place at the site of action (e.g., liver, brain, etc.) or in the delivery system (plasma) of this important mediator also needs to be considered.

RELATIONSHIP BETWEEN UVR EXPOSURE-INFLAMMATION AND MODIFICATIONS TO NORMAL IMMUNOLOGIC POTENTIAL

In the preceding sections of this review article a number of known immunologic, physiologic, and pharmacologic changes were described that take place following the exposure of experimental animals to UVR. These alterations include the acquisition of tumor susceptibility, a condition that parallels overt tumor induction, suppressor T-cell induction, alterations in macrophage and lymphocyte components of the SALT system, plus the stimulation of both prostaglandin and ETAF/IL-1 mediated effects. When experimentally analyzed individually, each of these known changes which take place could individually be hypothesized to have a modifying influence on the immunologic potential of an UVR-exposed host.

It should be appreciated that UVR exposure of skin is capable of initiating all of the reported changes simultaneously in the exposed host, making it highly probable that significant overlaps, cause-effect relationships, and a number of biologic generalizations can be formulated. Furthermore, many of the changes inducible by UVR are not unique to this physical agent as evidenced by the knowledge that a large number of distinct inflammatory stimuli are capable of initiating a similar cascade of immunologic and physiologic changes. In an attempt to support the concept that many of the apparently distinct immunomodulatory effects of UVR exposure significantly overlap with one another mechanistically, we will focus this section on two immunologic changes that take place subsequent to UVR exposure.

The experimental exposure of mice to UVR results in a marked reduction in their capacity to elicit contact hypersensitivity (CH) responses to epicutaneously applied skin-reactive chemicals. Mice exposed to low doses of UVR (4 x 400 J/m^2/day) demonstrate a reduced capacity to elicit CH responses that are site specific (localized to the skin areas of direct UVR exposure), whereas high-dose exposure of mice to UVR (15-30 KJ/m^2) causes systemic alterations that leave the animals hyporesponsive to CH induction regardless of the skin site of hapten application (Toews, Bergstresser, and Streilein 1980; Noonan, DeFabo, and Kripke 1981). Both of these types of UVR-mediated alterations have been previously reported to be associated with suppressor T-cell induction (Toews, Bergstresser, and Streilein 1980; Noonan, DeFabo, and Kripke 1981). The capacity of low-dose UVR to depress the capacity of animals to respond to skin-reactive chemicals has been concluded to be due to a direct functional inactivation of LC by this physical agent (Toews, Bergstresser, and Streilein 1980). The state of systemic suppression requires high doses of UVR for induction, and while it too is associated with the generation of hapten-specific suppressor T-lymphocytes, it is not dependent upon a functional or phenotypic modification of the resident LC at the site of hapten application (Lynch, Gurish, and Daynes 1983; Morison, Bucana, and Kripke 1984). Due primarily to their ease of manipulation, these phenomena now serve as

prototypes to help establish the mechanisms responsible for the immunologic changes that follow UVR exposure.

It was originally concluded that a cause-effect relationship exists between the functional inactivation of epidermal LC and the depression in CH responsiveness that is observed following the exposure of animals to low doses of UVR. While attractive, the validity of this hypothesis was dependent upon the generalized capacity of low-dose UVR-exposed animals to respond normally to skin sensitization at non-UVR-exposed sites. We therefore undertook an experiment to test this prediction formally. Groups of normal mice were exposed on their dorsal surfaces to UVR in low doses (400 J/m^2/day). Each of the animals had a small 3.0 cm^2 patch of UVR opaque tape applied to a specified dorsal skin site just prior to the irradiation treatment. A phenotypic analysis of epidermal LC in the exposed, protected, and unexposed skin sites confirmed that the tape treatment had protected the covered area from UVR exposure. Subsequent to the UVR exposures, the treated animals were divided into three groups and contact sensitized with DNFB. The first group was sensitized through an unirradiated skin site on the abdomen. The second group of animals was contact sensitized through an irradiated dorsal skin site and the third group had hapten applied to the tape-protected dorsal skin site. An additional group of normal animals were contact sensitized through a normal dorsal skin site to serve as a positive control. All animals were challenged with DNFB five days later. The results of this experiment (Table 1) established that UVR-exposed animals that had been sensitized through their abdominal wall skin responded normally, a finding that is fully consistent with the original observation (Toews, Bergstresser, and Streilein 1980). Likewise, the animals that were contact sensitized through the irradiated dorsal skin (LC deficient) exhibited a marked reduction in their capacity to elicit a CH response. Interestingly, the animals that were contact sensitized through the UVR-protected dorsal skin site were also found to be hyporesponsive, in spite of the fact that LC density in this area of the skin was totally normal. This finding suggested that LC inactivation by UVR is not solely responsible for the reductions in CH responses elicited by these animals.

We next questioned whether the known capacity of UVR to stimulate prostaglandin synthesis was involved in the immunologic changes that were taking place in low-dose UVR-exposed animals. This was based on the known capacity of UVR to stimulate an increase in the systemic release of IL-1-like molecules and to also cause both inflammation and pain (Gathering et al. 1984; Eaglstein, Sakai, and Mizuno 1979). Two large groups of skin patch protected mice were prepared and exposed to low doses of UVR. One group of the animals to be UVR-exposed received a subcutaneously implanted pellet of indomethacin, designed to deliver a daily dosage of 1.25 μg over a 20-day period. After subjecting all experimental animals to the four daily UVR exposures, they were segregated into four separate groups. One group of indomethacin-treated and one group of untreated UVR-exposed mice were contact sensitized with DNFB through irradiated dorsal skin sites. Likewise, a second group of indomethacin-treated and a parallel group of untreated UVR-exposed animals were contact sensitized through the protected area on their dorsal skin surface. To establish the effect of the indomethacin treatment, all experimental and control groups were challenged with hapten five days later. The results of this experiment (Table 2) clearly demonstrate that the <u>in vivo</u> inhibition of prostaglandin synthesis had no effect on the reduced capacity of

Table 1. The Effect of Low-Dose UVR Exposure on Contact Hypersensitivity Responses Elicited to DNFB

Group	Sensitization Site[a]	Treatment[c]	Percent Depression of CH Responses[d]
1	Abdomen	UVR	0
2	Back	UVR	0
3	Abdomen	UVR	7.1
4	Back	UVR	64.1
5	UVR-Shielded Back[b]	UVR	61.5
6	None	None	-

[a] Normal C3H mice were contact sensitized with a solution of DNFB on day 0 and day 1 by topical application to their shaved ventral or dorsal skin surfaces.

[b] The UVR-shielded site was created by applying UVR-opaque tape to a specified area on the dorsal skin of normal C3H mice.

[c] Animals were exposed to UVR by irradiating them with a bank of six FS-40 bulbs which emit approximately 2.5 J/m^2/sec of UV-B energy. All experimental animals received 400 J/m^2 of total energy per day for four consecutive days.

[d] Percent depression was calculated as a relationship to the positive normal control.

animals that were hapten sensitized directly through an irradiated skin site to elicit a CH response. In contrast, the reduced capacity of UVR-exposed animals to elicit a CH response following sensitization through a patch-protected dorsal skin site was not observed in the indomethacin-treated group. These animals responded normally to the DNFB sensitization, suggesting that the diminished CH response to haptens applied to normal (UVR-protected) skin sites depends on the mobilization of arachedonic acid and the biosynthesis of its metabolites. Further studies confirmed that the plasma of UVR-exposed animals contained elevated levels of PGE2 and that the indomethacin treatment of such animals inhibited this elevation in PGE2 production.

We next turned our attention to the phenomenon of systemic suppression of contact hypersensitivity and hypothesized that the induction of this condition might also require the in vivo stimulation of prostaglandins. Two groups of animals were exposed to high-dose UVR (30 KJ/m^2). Each individual from one of these groups was given an indomethacin pellet to inhibit their capacity to synthesize prostaglandins. After a three-day rest following the UVR exposure, all experimental animals plus a group of normal controls were contact

Table 2. Indomethacin Treatment Abrogates the Immunodepression Observed Following Contact Sensitization of Low-Dose UVR-Exposed Animals Through Protected Skin Sites

Group	Sensitization Site[a]	Treatment[c]	Indomethacin[d]	Percent Depression of CH Responses[e]
1	Back	None	-	0
2	Back	UVR	-	61.8
3	Back	UVR	+	62.3
4	UVR-Shielded Back[b]	UVR	-	60.5
5	UVR-Shielded Back	UVR	+	3.6
6	None	None	-	-

[a] Normal C3H mice were contact sensitized with a solution of DNFB on day 0 and day 1 by topical application to their shaved ventral or dorsal skin surfaces.

[b] The UVR-shielded site was created by applying UVR-opaque tape to a specified area on the dorsal skin of normal C3H mice.

[c] Animals were exposed to UVR by irradiating them with a bank of six FS-40 bulbs which emit approximately 2.5 $J/m^2/sec$ of UV-B energy. All experimental animals received 400 J/m^2 of total energy per day for four consecutive days.

[d] Indomethacin treatment was accomplished via the subcutaneous implantation of a pellet designed to release the drug at a constant rate of 1.25 µg/day for 20 days.

[e] Percent depression was calculated as a relationship to the positive normal control.

sensitized with DNFB on their unirradiated ventral skin surfaces. Five days later, all animals were challenged and the extent of ear swelling was evaluated after 24 hours. The results of this representative experiment (Table 3) clearly demonstrate that the ability of high-dose UVR exposure to cause a systemic depression in CH responsiveness is inhibited by the treatment of the test animals with indomethacin. These results strongly suggest that the mechanisms responsible for UVR-induced systemic suppression of CH responses involves the stimulation of prostaglandin synthesis.

Previous investigations have implicated hapten-specific T-suppressor (Ts) cells in the mechanisms responsible for high-dose UVR-induced suppression of CH responses (Noonan, DeFabo, and Kripke 1981). These conclusions were drawn from the finding that the spleens of hapten-sensitized UVR-exposed animals contained hapten-specific Ts which, upon adoptive transfer to naive recipients, were capable of causing a depression in the recipients' capacity

Table 3. Indomethacin Treatment Prevents the Development of Systemic Suppression of Contact Hypersensitivity Responses by High-Dose UVR Exposure

Group	Sensitization Site[a]	Back Skin Treatment[b]	Indomethacin[c]	Percent Depression of CH Responses[d]
1	Abdomen	None	-	0
2	Abdomen	High-Dose UVR	-	56.6
3	Abdomen	High-Dose UVR	+	6.4
4	None	None	-	-

[a] Normal C3H mice were contact sensitized with a solution of DNFB on day 0 and day 1 by topical application to their shaved ventral or dorsal skin surfaces.

[b] Animals were exposed to UVR by irradiating them with a 100-watt mercury arc lamp which emits approximately 500 J/m^2 of UV-B energy. All experimental animals received 15000 J/m^2 of UV-B energy.

[c] The indomethacin pellets used in this study delivered 2.5 μg of drug per day.

[d] Percent depression was calculated as a relationship to the positive normal control.

to elicit a CH response to the immunizing hapten (Noonan, DeFabo, and Kripke 1981). We therefore questioned whether suppressor cell activity could be demonstrated in indomethacin-treated UVR-exposed animals subsequent to skin sensitization. The results of our study determined (Table 4A) that the induction of splenic suppressor cells represented a normal consequence of skin sensitization with the hapten DNFB. Not only was suppressor cell activity present in the spleens of high-dose UVR-exposed donors, regardless of indomethacin treatment, but suppressor cell activity could also be found in the spleens of the DNFB-sensitized normal animals as well. Normal splenocytes adoptively transferred into naive recipients were without inhibitory effect. In parallel to an analysis of suppressor cell activity, the draining lymph nodes were removed from both normal DNFB-sensitized donors and high-dose UVR-exposed DNFB-sensitized donors for adoptive transfer to naive recipients. This protocol tests for the development of effector cell potential. Recipient animals were immediately challenged with hapten. The results (Table 4B) demonstrate that DNFB-sensitized high-dose UVR-exposed animals and DNFB-sensitized normal animals generate an equivalent effector cell activity in their peripheral lymph nodes. Our results, therefore, suggested that the depressed capacity of high-dose UVR-exposed animals to elicit a CH response was not due to: apparent modifications in suppressor cell generation or a depression in the capacity of these animals to generate effector cells in response to hapten application. Subsequent experiments, where UVR-exposed animals were contact sensitized through irradiated or UVR-protected skin

Table 4. Contact Sensitization of High-Dose UVR-Exposed Animals Results in the Simultaneous Induction of Both Suppressor and Effector Cells for CH Responses

A.

Group	Donor Treatment[a]	Recipient Treatment[b]	Percent Suppression[c]
1	-	Challenge	-
2	-	Sensitization Plus Challenge	0
3	None	Sensitization Plus Challenge	54.6
4	High-Dose UVR	Sensitization Plus Challenge	64.0
5	High-Dose UVR Plus Indomethacin	Sensitization Plus Challenge	62.1

B.

Group	Donor Sensitization[d]	Donor Treatment[e]	Percent Normal Response[f]
1	-	-	0
2	+	+	100
3	+	High-Dose UVR	93.1

[a] Donor animals were untreated or exposed to high-dose UVR (30 KJ/m^2) in the presence or absence of indomethacin.

[b] Recipient animals received 10^8 splenocytes from DNFB-sensitized donors. The recipient animals were subsequently skin sensitized with DNFB.

[c] Percent suppression was calculated as a relationship to normal mice or mice that received an adoptive transfer of normal cells.

[d] Donor animals were footpad and belly sensitized with DNFB. Peripheral lymph node lymphocytes (30×10^6) were adoptively transferred to naive recipients that were immediately challenged with DNFB.

[e] Certain donors were exposed to high-dose UVR (30 KJ/m^2) three days previously.

[f] The capacity to elicit a CH response was calculated as a percentage of the positive (normal) control.

sites, determined that splenic suppressor cell activity was induced under all conditions employed, while adoptively transferrable effector cell activity was only found when skin sensitization took place through a skin site having normal LC function (data not shown).

Insight into the mechanisms responsible for the depressed capacity of UVR-exposed animals to elicit CH responses was derived from experiments where normal, UVR-exposed, and indomethacin-treated UVR-exposed animals served as recipients of CH-effector cells obtained from normal hapten-primed donors. Both normal animals and the indomethacin-treated UVR-exposed groups were capable of eliciting a demonstrable CH response. However, the UVR-exposed recipients of normal CH-effector cells exhibited a tremendous reduction in their capacity to manifest a CH response (Table 5). Therefore the capacity of UVR to inhibit the elicitation of a CH response to agents initially applied to normal skin sites does not appear to represent the result of a diminished capacity of the animal to generate an immune response to the inducing hapten. Rather, the depressions in the intensity of CH responses observed in UVR-exposed animals appear to reflect some prostaglandin-induced alteration in the capacity of the effector arm of the CH response to function, possibly through a modification in lymphocyte receptiveness of the microvascular endothelium associated with the blood vessels at the specific site of a given immunologic or inflammatory response.

GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

The major objective of this review was to present the interrelationships that exist between the seemingly diverse effects that UVR has on normal immunological and physiological processes. Each of these inducible changes may be similar mechanistically to those elicited by other types of inflammatory stimuli. Ultimately, the changes caused by UVR-exposure produce a condition in which immunologic responsiveness of animals is dampened, either through the dominance of specific suppression following antigenic stimulation, or due to intrinsic mechanisms that exist to functionally inhibit certain types of effector cell responses. We have hypothesized that these immunoregulatory responses are reflections of normal host defense mechanisms. Such mechanisms may exist to protect the individual against the possible development of autoimmune conditions during the essential repair processes taking place subsequent to the UVR-mediated damage to skin. While serving to protect a host from possible autoimmune manifestations represents a beneficial aspect of such processes, the creation of a condition where the development of immune responses is continually being suppressed would clearly be detrimental. This represents the condition that would take place under situations of chronic UVR exposure. These could come about from either changing mores, where individuals increase their daily exposure to solar or artificial UVR for personal or cosmetic reasons, or from a reduction in the function of the ozone layer which currently provides a degree of protection from UVR.

Photoimmunology is providing the basic and clinical scientists with fresh insight into the types of mechanisms which can operate <u>in vivo</u> to control immunologic responses. It has long been appreciated that the molecular characteristics and the mode of presentation of a given antigen to an immunocompetent host can play a significant role in the immunologic outcome of such an interaction. Likewise, it is known that an immune response can take

Table 5. UVR Inhibits the Capacity of Animals to Execute a Contact Hypersensitivity Response in the Presence of Normal Immunologic Potential

Experiment Number	Group	Treatment of Recipients[a]	Adoptive Transfer of DNFB-Primed Lymphocytes[b]	Ear Swelling[c]	Percent Depression of CH Response
I	1	None	-	4 ± 1	0
	2	None	+	29 ± .5	0
	3	Indomethacin	+	27 ± .5	8
	4	15 KJ/m^2 UVR	+	8 ± 2	84
	5	15 KJ/m^2 UVR + Indomethacin	+	25 ± .5	16
II	1	None	-	3 ± 1	0
	2	None	+	28 ± 2	0
	3	Indomethacin	+	43 ± 4	0
	4	15 KJ/m^2 UVR	+	9 ± 1	76
	5	15 KJ/m^2 UVR + Indomethacin	+	22 ± 1	24

a. UVR was administered 3 days prior to the adoptive transfer of DNFB-primed lymph node cells. Indomethacin pellets were employed which release 2.5 μg of drug/day.

b. Normal animals were sensitized with DNFB on their ventral surface, ears and footpads. Lymph nodes were excised after 4 days and 30 x 10^6 cells adoptivelly transferred to the normal and treated recipients.

c. Ear swelling was measured 24 hours subsequent to DNFB challenge. % depression of the CH response was calculated by comparing it to the positive control.

many forms, with both humoral and cell-mediated components functioning simultaneously to either effectuate or regulate the type or intensity of a given adaptive immunologic response. Photoimmunology, the effect of light energy on immunologic responsiveness, has served to expand our understanding of immunologic control mechanisms. The importance of anatomic compartmentalization, the relationship between inflammation and adaptive immunity, and the means by which suppressor cell dominated responses can be preferentially stimulated are all apparent from the immunobiologic analysis of the UVR-exposed host. Further, the use of UVR to alter a host's immunologic potential is now uncovering the role played by interleukin-1, prostaglandins, steroids, and other hormones in many forms of immunologic control processes. Such information should prove useful, both in the elucidation of the diverse range of processes that interact to ultimately control the immune response, as well as in the clinical manipulation of given immunologic situations.

While it is impossible to accurately predict the future of this interesting field of study, the tremendous advancements made during the past decade might be used as an indication of the future. Based upon the number of diverse areas of investigation that are involved in the field of photoimmunology, one might hypothesize that the future will bring significant advances from both the basic and clinical sciences. These advances should provide valuable information concerning the means by which environmental influences can modulate the immunologic potential of a normal host, as well as important insight into mechanisms that can be used to manipulate certain types of immunologic responses for clinical benefit.

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Stratospheric Ozone Depletion: Immunologic Effects on Monocyte Accessory Function in Humans

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ABSTRACT

Predicted increases in the amount of ultraviolet-B (UV-B) radiation reaching the earth due to stratospheric ozone depletion are likely to have significant impact on human health. One recently recognized target of UV-B radiation is the immune system. In particular, UV-B has been shown to render mononuclear phagocytes deficient in their ability to activate T lymphocytes to alloantigen and to soluble antigen. However, nearly all studies dealing with the deleterious effects of UV-B on immunological function have been conducted in experimental animals. The objective of this study was to characterize the influence of UV-B radiation on human monocyte accessory function, which is essential for the initiation of cell-mediated immune responses. Human peripheral blood monocytes obtained by plastic adherence were used as accessory cells and were exposed in vitro to UV-B radiation from FS20 lamps. Their ability to induce a blastogenci response to either antigen (tetanus toxoid) or mitogen (OKT3, PHA) when co-cultured with autologous peripheral blood T lymphocytes that had been rigorously rendered accessory dependent was used as an index of their accessory cell function. Exposure of monocytes to UV-B doses as low as 50 J/m^2 inhibited the blastogenesis response to antigens and mitogens by 90% ($p = 0.001$). Viability of UVB-irradiated cells did not differ significantly from unirradiated monocytes over the initial 72 hours of culture, indicating that inhibition was not a result of a lethal effect on this cell population. The reduction in accessory activity was associated with a 90% decline in IL-1 activity in supernatants of UVB-irradiated, LPS-stimulated monocytes. However, addition of exogenous IL-1 to cultures was insufficient to completely restore the blastogenesis response. UVB-irradiation also rendered monocytes deficient in antigen processing. When they were exposed to antigen for 30 minutes before UV-B rather than after UV-B, no loss of accessory activity was observed. Thus, UV-B radiation adversely affects the accessory function of human peripheral blood monocytes in antigen- and mitogen-induced activation of T lymphocytes. Deficient IL-1 production and impaired antigen processing are at least partially responsible for this effect. These findings suggest that modification of the stratospheric ozone

layer with resultant increases in UV-B penetration may have deleterious effects on monocyte accessory activities in humans.

INTRODUCTION

Chronic exposure of the skin to solar ultraviolet radiation has a variety of deleterious effects on human health (Harber and Bickers 1981). It causes premature aging of the skin and is a well-recognized etiologic agent for cutaneous squamous cell and basal cell cancers. In addition, there is an increasing awareness that sun exposure may play an important role in the development of malignant melanoma (Kopf, Kripke, and Stern 1984).

Recent studies performed in experimental animal models have demonstrated that solar ultraviolet radiation also profoundly influences cell-mediated immunity (Elmets and Bergstresser 1982; Daynes and Krueger 1983; Parrish, Kripke, and Morison 1983). When animals are exposed to various doses of ultraviolet radiation, they develop impaired immune responses to UVB-induced skin cancers (Kripke 1974), to contact sensitizing agents (Toews et al. 1980; Jessup et al. 1978), and to Herpes simplex virus (Howie, Norval, and Maingay 1986). A UVB-induced alteration in the function of accessory cells appears to form the basis for such disturbances in cell-mediated immunity (Greene et al. 1979; Toews et al. 1986). These cells, of which macrophages and monocytes, epidermal Langerhans cells, and splenic dendritic cells have been the most thoroughly studied, are essential for the activation and expansion of helper T lymphocytes, an obligatory step in the initiation of immune responses.

Action spectrum studies have been performed to determine the wavelength bands of solar ultraviolet radiation that are most efficient at producing these immunologic alterations (Elmets, LeVine, and Bickers 1985; Schacter et al. 1983; DeFabo and Noonan 1983). They have demonstrated that wavelengths within the UV-B exert the greatest inhibition of cell-mediated immune function. This is of imminent concern because the widespread use of chloroflurocarbons in aerosols and refrigerants has been predicted to deplete the atmosphere of ozone, which filters out solar UV-B radiation (Molina and Rowland 1974). It is likely that stratospheric ozone depletion with resultant increases in the amount of UV-B reaching the earth's surface will have a significant impact on several aspects of human health, including an increase in the incidence of diseases that require the immune system for protection.

The vast majority of evidence implicating UV-B as a causative agent in deficient cell-mediated immune function, however, is derived from studies conducted in experimental animals. Relatively little is known about the immunological effects of UV-B in humans. We recently have conducted a number of experiments to determine whether UV-B inhibits the function of immunocompetent cells of humans in a manner similar to its inhibitory effect on the immunocompetent cells of animals. The cell type we chose to examine was the human peripheral blood monocyte and the immunological function we elected to observe was its accessory activity. Studies in animals have suggested that the accessory function of macrophages and monocytes is profoundly altered following UV-B exposure (Greene et al. 1979). Accessory activity is an obligatory first step in the initiation of all cell-mediated immune responses. This function is vitally important for a broad spectrum of sensitivity reactions, including immunity against tumors, and immunity to certain microbial agents including viruses and fungi.

METHODS

Blastogenesis Assays

We assessed the function of accessory cells in antigen- and mitogen-induced blastogenesis assays. Accessory-dependent purified T lymphocytes served as the responding cell population; peripheral blood monocytes were employed as accessory cells; and tetanus toxoid (for antigen-induced assays) and PHA or OKT3 (for mitogen-induced assays) served as the initiating stimuli.

T lymphocytes were purified by a three- or four-step procedure from peripheral blood mononuclear cells (PBMC) obtained from the heparinized blood of healthy human volunteers by Ficoll-Hypaque (Pharmacia, Piscataway, New Jersey) gradient centrifugation. PBMC were first allowed to adhere to 100 mm tissue culture grade plastic petri dishes (Falcon, Oxnard, California) for 1-2 hours at 37°C in a 5% CO_2 incubator. Then the plastic non-adherent cells were pipetted off and incubated on acid-washed nylon wool columns (Fenwall Laboratories, Deerfield, Illinois) for 45 minutes at 37°C. Finally, the resulting T lymphocyte-enriched population was treated with a 1:50 dilution of a monoclonal antibody to a framework determinant of the HLA-DR antigen (OKIA, Ortho Pharmaceuticals, Raritan, New Jersey) and low toxicity rabbit complement (Cedarlane Laboratories, Hornsby, Ontario). In some experiments, cells were further depleted of accessory cells by treatment with a 5 mm concentration of L-leucine methyl ester (L-LME, Sigma Chemical Co., St. Louis, Missouri) for 40 minutes at 22°C. When L-LME was employed, it was used prior to treatment with anti-HLA-DR and complement. The resulting population of T cells was accessory-dependent and was completely unresponsive to antigen (tetanus toxoid) and mitogen (OIT3, suboptimal doses of PHA).

Accessory cells were obtained by dislodging those cells that remained adherent to the plastic petri dishes in the first step of the T lymphocyte purification procedure. This population was 85%-90% peroxidase positive and for the purposes of this discussion will be called peripheral blood monocytes.

Tetanus toxoid (a gift from Lederle Laboratories, Pearl River, New Jersey) was used as the antigen in soluble antigen-induced T cell blastogenesis assays. Suboptimal concentrations of phytohemagglutinin (PHA, Gibco, Grand Island, New York) or OKT3 (Ortho Pharmaceuticals, Raritan, New Jersey) were used as mitogens in mitogen-induced blastogenesis assays.

In the blastogenesis assays, 2 x 104 monocytes were cultured with 1 x 10^5 T lymphocytes and with either tetanus toxoid, PHA, or OKT3 in 96 well microtiter plates (Corning, Corning, New York). In all the experiments described, only autologous combinations of monocytes and T cells were employed. All variables were performed on triplicate wells. Cultures in which tetanus toxoid as used as the stimulus were incubated for 5 days; those in which OKT3 or PHA was used as the stimulus were cultured for 3 days. Each well was pulsed with 1 microCurie of tritiated thymidine for the final 18 hours of the culture period. Incorporation of tritiated thymidine into proliferating cells was used as an index of T lymphocyte activation. Data were expressed as cmp = (cmp of cultures containing antigen or mitogen) - (cpm of unstimulated cultures).

UV Irradiation of Cells

One x 10^6 cells in 1 ml of colorless HBSS without Ca^{+2} and Mg^{+2} (KC Biological, Lenexa, Kansas) were irradiated in 35-mm tissue culture plates with a bank of four FS20 fluorescent UV-B bulbs (Westinghouse Electrical Corp., Bloomfield, New Jersey). These lamps have an output primarily in the UV-B range. Output in the UV-B at a tube to target distance of 21 cm was approximately 1.7 x 10^{-5} W/cm^2. Output was monitored with an IL700 Research Radiometer and SEE 240 UV-B photodetector (International Light, Newburyport, Maine).

Interleukin-1 Production and Bioassay

Peripheral blood monocytes were prepared as described above and were cultured at a concentration of 5 x 10^5/ml in RPMI-1640 plus 2% FCS. One ml was cultured per 16 mm Linbro plate well in the presence or absence of E. coli lipopolysaccharide (LPS) 10 ug/ml (Difco, Detroit, Michigan). After 24 hours, the well contents were aspirated and the supernatants filtered through 0.22 micron filters.

Interleukin-1 (IL-1) activity was assessed by placing the supernatants in the thymocyte proliferation bioassay. Briefly, 100 microliters of supernatant was added to round bottom microtiter wells containing 1.5 x 10^6 thymocytes prepared from the thymuses of 8-12 week old female C3H/HeJ mice (Jackson Labs, Bar Harbor, Maine). The plates were incubated for 72 hours, and the amount of tritiated thymidine incorporated into proliferating cells during the final 6 hours of culture was used as an index of IL-1 activity. IL-1 activity was calculated by modified probit-type analysis and was expressed as U/ml according to the method of Luger et al. (1982).

RESULTS

Requirement for an Accessory-Cell-Dependent System to Demonstrate UV-B Effects on Monocyte Accessory Function

In our initial studies we found that it was essential to employ an accessory-cell-dependent system to demonstrate UVB-induced defects in monocyte accessory function (Table 1). Cells that had been adhered to plastic and then passed over nylon wool were not accessory-dependent; some proliferation of T cells occurred to tetanus toxoid even without the addition of accessory cells (group I). The addition of unirradiated autologous monocytes to cultures, however, significantly augmented the response (group II). Only moderate inhibition of blastogenesis could be demonstrated when UVB-irradiated monocytes were added to nonaccessory-cell-dependent nylon wool nonadherent T cells (group III). On the other hand, when cells were additionally treated with anti-HLA-DR and complement, there was a reduction in T cell proliferation T background levels (group IV). The response could be reconstituted by the readdition of autologous monocytes (group V).

Table 1. Requirement for an Accessory Dependent System to Demonstrate UV-B Effects on Monocyte Accessory Function

Group	Cell Cultures[1]		Tritiated Thymidine Incorporation ($\times 10^{-3}$ CMP ± S.D.)
	Responding T Cells	Accessory Cells	
I	NWE[2]	None	5183 ± 2592
II	NWE	Unirradiated Monocytes	11,523 ± 1591
III	NWE	UVB Irradiated Monocytes[3]	9311 ± 1735
IV	HLA-DR Treated[2]	None	712 ± 624
V	HLA-DR Treated	Unirradiated Monocytes	12,185 ± 2103
VI	HLA-DR Treated	UVB-Irradiated Monocytes[3]	965 ± 743

1. 10^5 T cells were cultured with 2×10^4 monocytes with optimal amounts of tetanus toxoid for 5 days.
2. NWE - nylon wool enriched T lymphocytes; HLA-DR treated - T cells purified by plastic adherence, nylon wool columns and treatment with HLA-DR and complement.
3. Monocytes were exposed to 50 J/m^2 UVB.

UVB-irradiated monocytes were unable to reconstitute the response to tetanus toxoid when T cells had been rendered accessory-dependent (group VI). This contrasts with what was observed when UVB-irradiated monocytes were added to cultures containing responding nonaccessory-cell-dependent T lymphocytes (group VI vs. group III).

Inhibition of Human Monocyte Accessory Function by UV-B Radiation

Using this accessory-dependent system, we examined whether in vitro exposure of human peripheral blood monocytes to UV-B radiation inhibited their ability to serve as accessory cells for T cell responses to the soluble antigen tentanus toxoid. As shown in Table 2, there was a dose-dependent inhibition in T cell responses when monocytes were irradiated with increasing doses of UV-B. A dose of 50 J/m^2 was sufficient to inhibit the response by greater than 90%. This is less than one half the UV-B dose that would cause a mild sunburn in a fair-skinned individual following outdoor recreational activity and can be easily achieved in the dermal vasculature as monocyte cells circulate through the skin.

Inhibition of accessory cell function was due to a nonlethal effect of UVB. At 50 J/m^2 there was no significant difference in viability, as assessed by trypan blue exclusion, between unirradiated and UVB-irradiated monocytes over the entire duration of the culture period.

These experiments indicated that, similar to animal models in which UV-B radiation had been shown to preferentially inhibit the accessory function of macrophages and epidermal Langerhans cells for soluble antigen, this form of radiant energy also altered the accessory function of human peripheral blood monocytes for tetanus toxoid.

Effects of UV-B Radiation on Accessory Signals Required for Human T Lymphocyte Activation by Tetanus Toxoid

Monocytes and other accessory cells contribute at least three essential signals for T lymphocyte activation (Unanue et al. 1984). First, they express large amounts of HLA-DR antigens on their cell surface. These molecules serve as interaction structures between the accessory cell and the responding T lymphocyte. Second, they produce the soluble cytokine interleukin-1, which is a necessary co-factor required for T cell activation. Finally, accessory cells take up antigen, degrade it, and re-express it on the cell surface in an antigen processing step. Because UV-B radiation inhibited the accessory function of monocytes by a non-lethal insult to the cell, we next performed studies directed at examining which of these signals essential for T lymphocyte activation was inhibited by UV-B.

We first assessed the capacity of human peripheral blood monocytes to produce interleukin-1 following UV-B exposure. Peripheral blood moncytes were exposed in vitro to doses of UV-B ranging from 0 to 300 Jm^2 and were then placed in culture with LPS, which served as a stimulus for IL-1 production. Twenty-four hours later the supernatants were removed from these cultures and were examined for IL-1 activity in the thymocyte proliferation bioassay. As

Table 2. Dose-Dependent Inhibition of Tetanus Toxoid-Induced T Lymphocyte Blastognesis by UV-B[1]

UVB Dose (J/m^2)	Tritiated Thymidine Incorporation ($\times 10^{-3}$ CPM ± SD)
0	24650 ± 11466
10	16370 ± 4182
25	14760 ± 3392
50	2556 ± 2042
150	731 ± 138
300	469 ± 114

[1] Monocytes were used as accessory cells, were exposed to various doses of UV-B, and placed in culture for 5 days with purified T cells and tetanus toxoid in the tetanus toxoid-induced T lymphocyte blastogenesis assay.

shown in Figure 1, unirradiated monocytes were able to produce large amounts of IL-1 when stimulated by PLS. There was a marked decline in IL-1 activity, however, in supernatants derived from UVB-irradiated monocytes. A dose of 50 J/m^2 UV-B reduced the activity by greater than 95%, which corresponded to the UV-B dose that also caused marked inhibition of proliferation in the blastogenesis assay.

To determine whether deficient IL-1 production was the only accessory defect that was present in UVB-irradiated monocytes, experiments were performed in which recombinant murine IL-1 was added back to the blastogenesis cultures in an attempt to reconsititute the response (Figure 2). Murine recombinant IL-1 was not able to substitute for monocytes in the blastogenesis assay (group II). It also failed to augment the response in cultures containing unirradiated monocytes, T lymphocytes, and tetanus toxoid (group IV). Only partial reconstitution of the tetanus toxoid response was observed when this reagent was added to cultures containing UVB-irradiated monocytes, purified T cells, and tetanus toxoid (group VI). We thus concluded that although UV-B radiation inhibited IL-1 production by monocytes, deficient IL-1 production was not the sole accessory defect that occurred following UV-B exposure.

Next, we tried to examine the effect of UVB on antigen processing. In these experiments, monocytes were preincubated with tetanus toxoid for 30 minutes before UV-B exposure. Following irradiation, responding T lymphocytes were added to the cultures. The reasoning behind this experiment was that exposure of monocytes to antigen prior to UV-B might allow the accessory cells to take up and to process antigen before UV-B inhibited the antigen processing step; and therefore reconstitution of the response might occur. As shown in Figure 3, only slight inhibition of the response occurred when accessory cells were treated in this manner. The fact that monocytes became resistant to inhibition by UV-B when exposed to tetanus toxoid for a period of time sufficient to allow for antigen uptake and processing, suggests that UV-B may have an effect on antigen processing.

Effects of UV-B Exposure on the Ability of Human Monocytes to Activate T Lymphocytes to Mitogen

Accessory cells are also required for mitogenic activation of T lymphocytes. However, the accessory signals required for T cell activation by mitogen differ somewhat from those required for antigens. While both antigens and mitogens are thought to require IL-1 production for optimal T cell activation, mitogens, unlike antigens, do not require a processing step. We therefore performed experiments to assess whether UV-B irradiation of accessory cells inhibited the mitogenic activation of T lymphocytes. In these studies, OKT3 or PHA served as the mitogenic stimulus. Mitogens were placed in culture with UVB-irradiated monocytes and purified T cells and the blastogenesis response was determined three days later. Similar to what was observed for tetanus toxoid, there was a dose-dependent inhibition of T cell proliferation to suboptimal doses of PHA when increasing UV-B doses were administered (Figure 4). Dose-dependent inhibition also occurred when OKT-3 was used as the mitogenic stimulus (Figure 4). With both of these mitogens, a UV-B dose between 50 J/m^2 and 100 J/m^2 was sufficient to inhibit the T cell

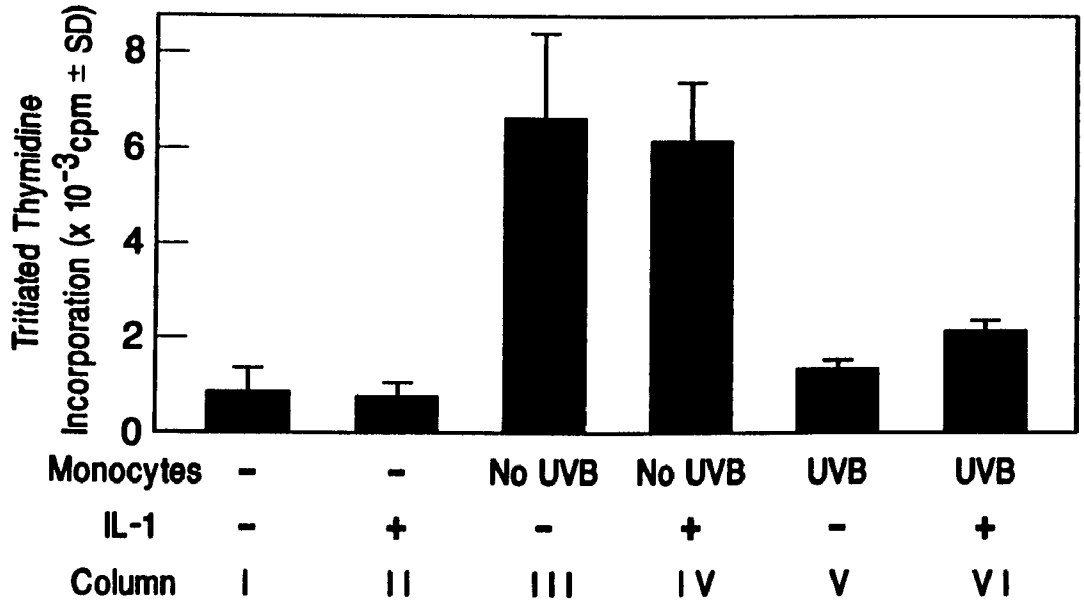

Figure 1. IL-1 production by UVB-irradiated monocytes. Peripheral blood monocytes were UVB-irradiated and then placed in culture with LPS. Twenty-four hours later the supernatants were removed and assayed for IL-1 activity in the mouse thymocyte bioassay.

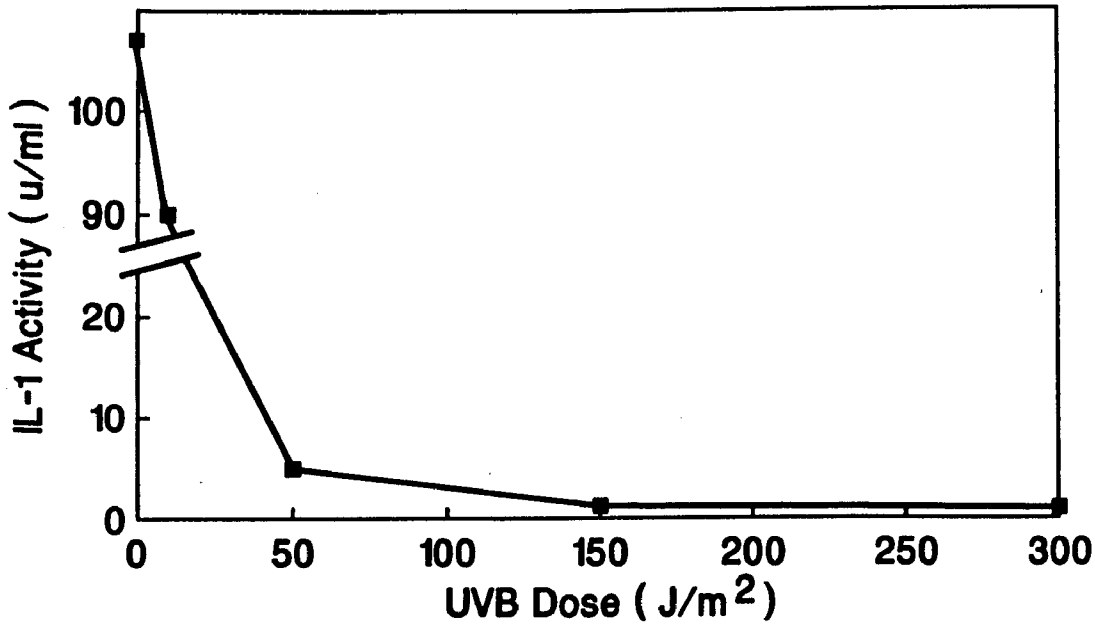

Figure 2. Incomplete reconstitution of UVB-induced inhibition of tetanus toxoid-induced T lymphocyte blastogenesis by recombinant murine IL-1. Accessory cells (unirradiated or UVB-irradiated) and/or 10/Um[1] recombinant murine IL-1 were added to cultures containing purified T cells and tetanus toxoid. Cultures were incubated for 5 days in the tetanus toxoid-induced T lymphocytes blastogenesis assay.

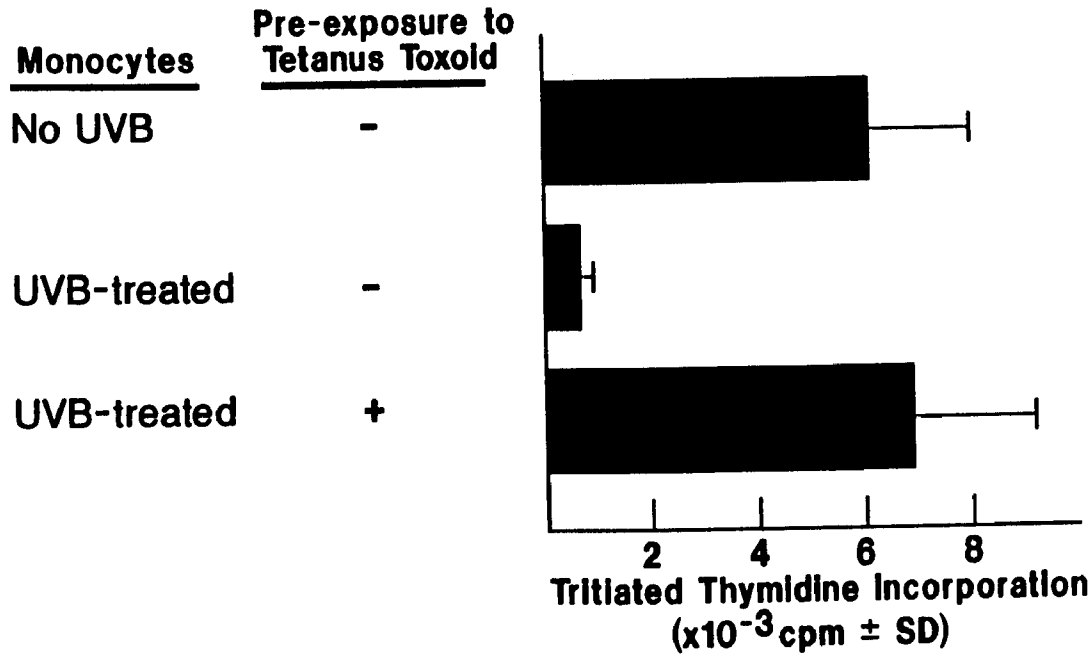

Figure 3. Development of resistance by monocytes to UV-B effects on accessory function by preculture with antigen. Accessory cells were placed in culture with tetanus toxoid (Panel C). Thirty minutes later the cells were UVB-irradiated with 50 J/m^2. T lymphocytes were then added and cultured for 5 days in the tetanus toxoid-induced T lymphocyte blastogenesis assay. Control panels were included in which accessory cells were unirradiated and received no pre-exposure to tetanus toxoid (panel A) or were UVB-irradiated and received no pre-exposure to tetanus toxoid (panel B).

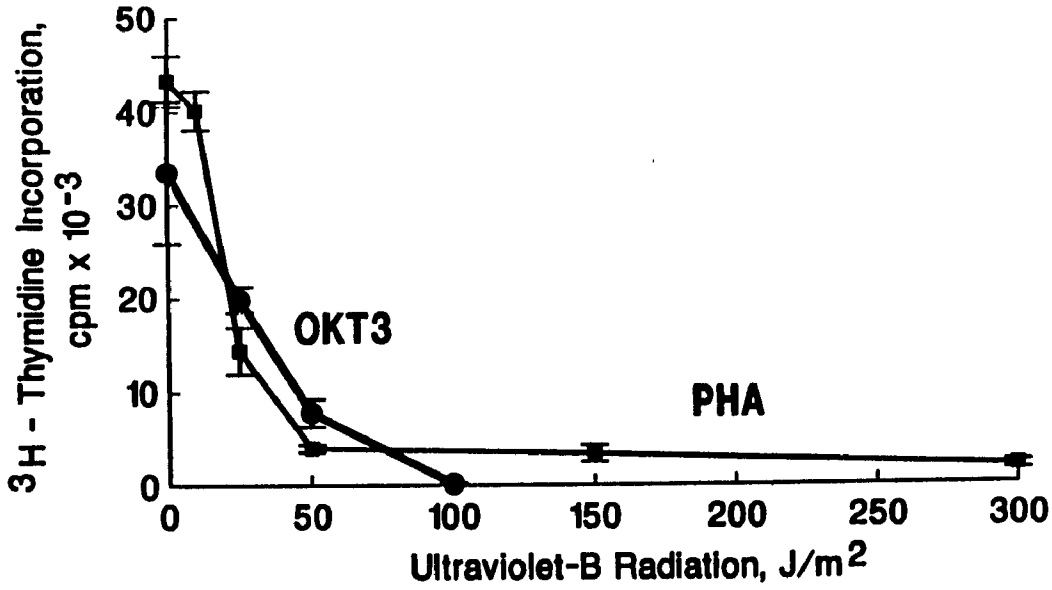

Figure 4. Dose dependent inhibition of T lymphocyte blastogenesis responses to mitogens by UV-B. Accessory cells were exposed to various doses of UV-B and then placed in culture for 3 days with purified T cells and with either PHA or OKT3 in a T lymphocyte blastogenesis assay.

response by greater than 90%. This UV-B does was almost identical to the UVB dose that inhibited the T cell response to tetanus toxoid.

We then attempted to reconstitute the mitogenic response to OKT3 with IL-1. For these experiments, recombinant human IL-1-alpha and IL-1-beta were obtained and were added to cultures containing OKT3, purified T cells, and UVB-irradiated monocytes. Despite the fact that both IL-1-alpha and IL-1-beta had significant bioactivity in the thymocyte proliferation assay, these cytokines were unable to augment the T lymphocyte response to OKT3 (Figure 5). These findings indicated that even for mitogens, IL-1 was insufficient to reconstitute the T cell response. The fact that mitogens are not processed and IL-1 could not reconstitute the response implies that UV-B exposure affected an additional, as yet undefined, signal required for T cell activation in addition to IL-1 production and antigen processing.

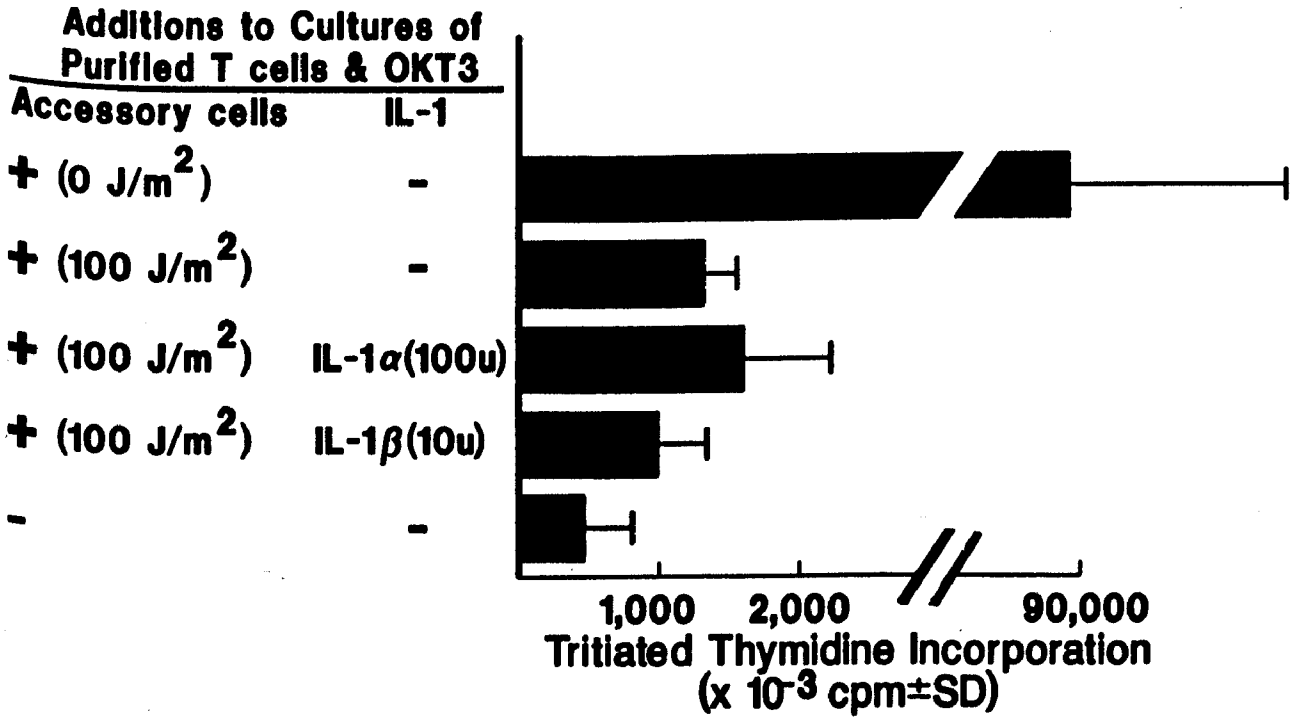

Figure 5. Incomplete reconstitution of UV-B induced inhibition of mitogen-induced T lymphocyte blastogenesis by human recombinant IL-1. Recombinant human IL-1-alpha or IL-1-beta was added to OKT3-induced T lymphocyte blastogenesis assays.

DISCUSSION

These studies have shown that in vitro exposure of human peripheral blood monocytes to UV-B radiation inhibited the accessory signals required for antigen-induced and mitogen-induced activation of T lymphocytes. Inhibition occurred at UV-B doses that were sublethal for monocytes. Inhibition of accessory function was due both to deficient IL-1 production and to impaired antigen processing by UVB-irradiated monocytes.

An additional, as yet unidentified, monocyte-derived accessory signal also appeared to be affected following UVB exposure. This was based on the finding that although IL-1 production by UVB-irradiated monocytes was diminished, the readdition of exogenous IL-1 was insufficient to reconstitute the T cell response to the mitogen OKT3. Because OKT3 is not processed by monocytes, the lack of a response cannot be attributed to a processing defect.

One possibility for this additional signal is an alteration in HLA-DR antigen expression and/or function. HLA-DR antigens are thought to function by forming complexes with processed antigenic moieties, and thus enabling the recognition of these antigenic moieties by responding T lymphocytes. Perhaps UV-B radiation reduces the number of HLA-DR antigens on the cell surface, produces alterations in the ability of UVB-irradiated HLA-DR molecules to bind processed antigen, or impairs the recognition of HLA-DR antigens by responding T cells. Alternatively, UV-B exposure may disturb membrane-associated IL-1 activity. Recent studies by Kurt-Jones et al. (1985) have shown that paraformaldehyde-fixed macrophages are capable of presenting antigen to T lymphocytes despite their inability to release IL-1. These cells express an IL-1-like substance on their cell membrane and this membrane-associated molecule may play an important role in T cell activation. Thus, an alteration in membrane-associated IL-1 could also explain why UVB-irradiated monocytes fail to function as accessory cells.

These studies have important implications with respect to stratospheric ozone depletion. The immune system plays a fundamental role in protecting individuals against noxious microorganisms and provides a surveillance mechanism against neoplastic cells as they arise. Because stratospheric ozone performs the beneficial function of filtering out harmful UV-B, destruction of this component of the atmosphere would increase UV-B at the earth's surface. The observations of this study, in which we have demonstrated a UVB-induced impairment of immune function in human monocytes, suggest that deficient cell-mediated immunity will be a major contributing factor to the postulated adverse effects that stratospheric ozone depletion has on human health. UVB-induced immune deficiencies might be expected to result in rising rates of cutaneous and possibly other malignancies, and may produce increases in the incidence of illness caused by viruses, fungi, and other microbes.

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Effects of UV-B on Infectious Disease

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INTRODUCTION

The skin is our interface with the environment and our bodies' first line of defense against infectious agents. Other papers in this volume illustrate very elegantly that UV-B irradiation has a selective, suppressive effect on immune system functioning. They show us, further, that the skin itself is an immune organ, in that certain types of lymphoid cells are found predominantly in skin (Daynes et al. this volume; De Fabo and Noonan this volume; Elmets et al. this volume). The function of this skin-associated lymphoid tissue is to respond to antigens that enter the body by way of the skin. This cutaneous immune surveillance system is vital to our ability to resist invasions by infectious agents.

Infectious diseases remain serious public health problems in the tropics and semitropics. These are the geographic areas that also receive the highest levels of solar UV-B radiation in the world (Schulze 1970). A critical question arises: What are the effects of UV-B irradiation on the body's response to infectious agents penetrating the skin?

There are few experimental data to answer this important question. Analysis is further complicated because infection and disease outcome are affected, not only by the immunogenetic background of the host, but also by other environmental factors such as humidity, temperature, vector density and efficiency, concomitant infections, and host nutrition, to name but a few. Although the issue is very complex, a growing body of evidence suggests that UV-B irradiation can affect both pathogenesis and immunity in infectious diseases involving skin.

KNOWN EFFECTS OF UV-B ON THE CUTANEOUS PHASE OF INFECTIOUS DISEASES

The effect of ultraviolet radiation on the progression of skin disease has been the subject of investigation for almost a century. In the late

1880s, the Danish physician Niels Finsen began a study of the interaction of skin with sunlight. By a series of experiments in which various wavelengths were filtered out from sunlight, he concluded that it was the ultraviolet component, which he called the "chemical rays," that caused inflammation and sunburn. In 1894, Finsen reported that the "chemical rays" of sunlight promoted scarring in smallpox, and that patients sequestered in rooms thickly hung with red curtains healed their pustules without scarring (Finsen 1901a). This illustrated that solar radiation could affect the outcome of infectious disease, and launched him on a study of ultraviolet radiation in the treatment of tuberculosis (Finsen 1901b). In 1903, Finsen received the Nobel Prize in Medicine for successful treatment of lupus vulgaris (tuberculosis of the skin) by local application of ultraviolet irradiation. Over the next four decades, ultraviolet light applied locally to skin was used to treat a variety of infectious diseases, including erysipelas, which had a 10% fatality rate until UV therapy appeared (Licht 1983). The advent of sulfones and antibiotics supplanted UV therapy, which has virtually disappeared from the infectious disease armamentarium.

As Finsen astutely observed after his discovery of the promotion of sunburn by UV, "What is more natural than that chemical rays should exert an injurious influence upon a diseased skin, when we see such severe inflammation produced by their influence upon the healthy skin?" (Finsen 1901a). The infectious diseases that are likely to be affected by UV are of two types.

TYPES OF INFECTIOUS DISEASES WITH CUTANEOUS INVOLVEMENT

Infections that have severe cutaneous pathology, damaging the skin, are listed in Table 1. The effect of ultraviolet light on the progression of disease has been documented for five of these.

But perhaps of more concern to us here are those infectious agents for which the skin-associated lymphoid tissue is the first encounter with the host's immune system (Table 2). These diseases have a primary cutaneous phase, often asymptomatic, during which protective immunity to later infections can develop. Such diseases are of particular concern, because antigens that reach the immune system via skin that has been UV-B irradiated not only escape the immune surveillance system of the skin, but also can make the host tolerant to later exposure to the same antigens (Kripke 1984; Elmets et al. 1983). For most of the diseases with a primary cutaneous phase, the development of cell-mediated immune responses is necessary for protection. Other papers in this volume show that cell-mediated immunity is more critically affected by UV-B.

MODEL FOR UV-B ACTION ON AN INFECTIOUS DISEASE: LEISHMANIASIS

As a model for the effects of UV-B on infectious disease, let us turn to leishmaniasis in the mouse. Leishmania have an initial cutaneous phase, which can be asymptomatic and precede more serious sequelae; and they also cause skin ulcers (Tables 1 and 2).

The leishmaniases are a spectrum of diseases, caused by members of the genus Leishmania, which are obligate intracellular protozoan parasites living with macrophages and monocytes. Paradoxically, for Leishmania to survive in the body, they must be ingested by macrophages, whose function normally is to

Table 1. Infectious Diseases With Severe Cutaneous Involvement

	Disease	Etiologic Agent	Classification
	Onchocerciasis	*Onchocerca volvulus*	Nematode
*	Leishmaniasis	*Leishmania* species	Protozoan
*	Lupus vulgaris	*Mycobacterium tuberculosis*	Mycobacterium
	Hansen's disease	*Mycobacterium leprae*	Mycobacterium
	Mycobacterial skin ulcers	*Mycobacterium ulcerans*	Mycobacterium
*	Erysipelas	*Streptococcus pyogenes*	Bacterium
	Measles	Measles virus	Virus
	Chickenpox	Varicella-zoster virus	Virus
*	Herpes	Herpes Simplex Type II	Virus
*	(Smallpox)	(Variola virus)	(Virus)

* Effects of the UV light on progression of disease have been documented. In early studies before ca. 1935, broad-spectrum UV light sources were shown to affect smallpox, lupus vulgaris, and erysipelas (reviewed by Licht 1983). Because such sources emit UV-A and UV-C as well as UV-B, their effects cannot be attributed to UV-B only. More recent studies on herpes and leishmaniasis used lamps emitting UV-B only.

digest invading microorganisms. The parasites are transmitted from host to host by phlebotomine sandflies, which deposit the Leishmania in the upper layer of the dermis or in the epidermis. Phlebotomine proboscides are too short to penetrate protective clothing, so that sandflies must feed on exposed skin.

Most infections with Leishmania species lead to self-healing skin ulcers, which leave the patient permanently scarred but immune to reinfection with related species of Leishmania. However, more serious sequelae are noted in some forms of leishmaniasis. In chiclero's ulcer, the entire ear pinna can be eroded away; in mucocutaneous leishmaniasis, parasites metastasize from the original skin lesion to the mucocutaneous junctions. These severe mutilating lesions do not heal spontaneously, and they occur on parts of the body that receive high amounts of UV-B in sunlight (Urbach 1969). A primary skin ulcer

Table 2. Infectious Diseases With a Primary Cutaneous Phase

Disease	Etiologic Agent	Classification
Schistosomiasis	*Schistosoma* species	Trematode
Leishmaniasis	*Leishmania* species	Protozoan
African sleeping sickness	*Trypanosoma brucei* subspecies	Protozoan
Chagas' disease	*Trypanosoma cruzi*	Protozoan
Yaws	*Treponema pertenue*	Spirochaete
Hansen's disease (?)	*Mycobacterium leprae*	Mycobacterium
Cutaneous diphtheria	*Corynebacterium diphtheriae*	Bacterium
Bubonic plague	*Yersinia pestis*	Bacterium
Anthrax	*Bacillus anthracis*	Bacterium

does not always precede the onset of the most fatal form of leishmaniasis, kala-azar, in which the Leishmania invade the entire reticuloendothelial system. Kala-azar smoulders for years in endemic foci, to erupt into fulminating epidemics in times of migration, famine, and war.

Leishmanial disease occurs on every continent except Australia and Antarctica. There are at least four different species causing human disease in the Old World, and at least three in the New World.All Leishmania species can cause skin ulcers, but only some of them can cause more severe disease in immunogenetically predisposed individuals. Yet because not all infected persons in groups at high risk for serious sequelae develop severe disease, other factors are probably involved. In countries endemic for leishmaniasis, skin is naturally exposed both to the bites of infective sandflies and to UV-B in sunlight, which is known to depress the functions of the skin-associated lymphoid tissue. So it seemed likely that early immunological events occurring in the skin could critically determine the outcome of infection with Leishmania.

In a series of experiments designed to evaluate the effects of UV-B on the development of cutaneous leishmaniasis in mice (Giannini 1986), I found that local irradiation of the injection site with low doses of UV-B around the time of initial infection indeed affected lesion development (Figure 1). The doses used were not high enough to cause sunburn, but were about the amount of UV-B in an hour's exposure to sunlight in the semitropics on a bright day at noon. Surprisingly, lesion severity was reduced in the irradiated mice (Giannini 1986). The main targets of the UV-B were host cells, and not Leishmania, because the parasites were present in the skin and *in vitro* in the

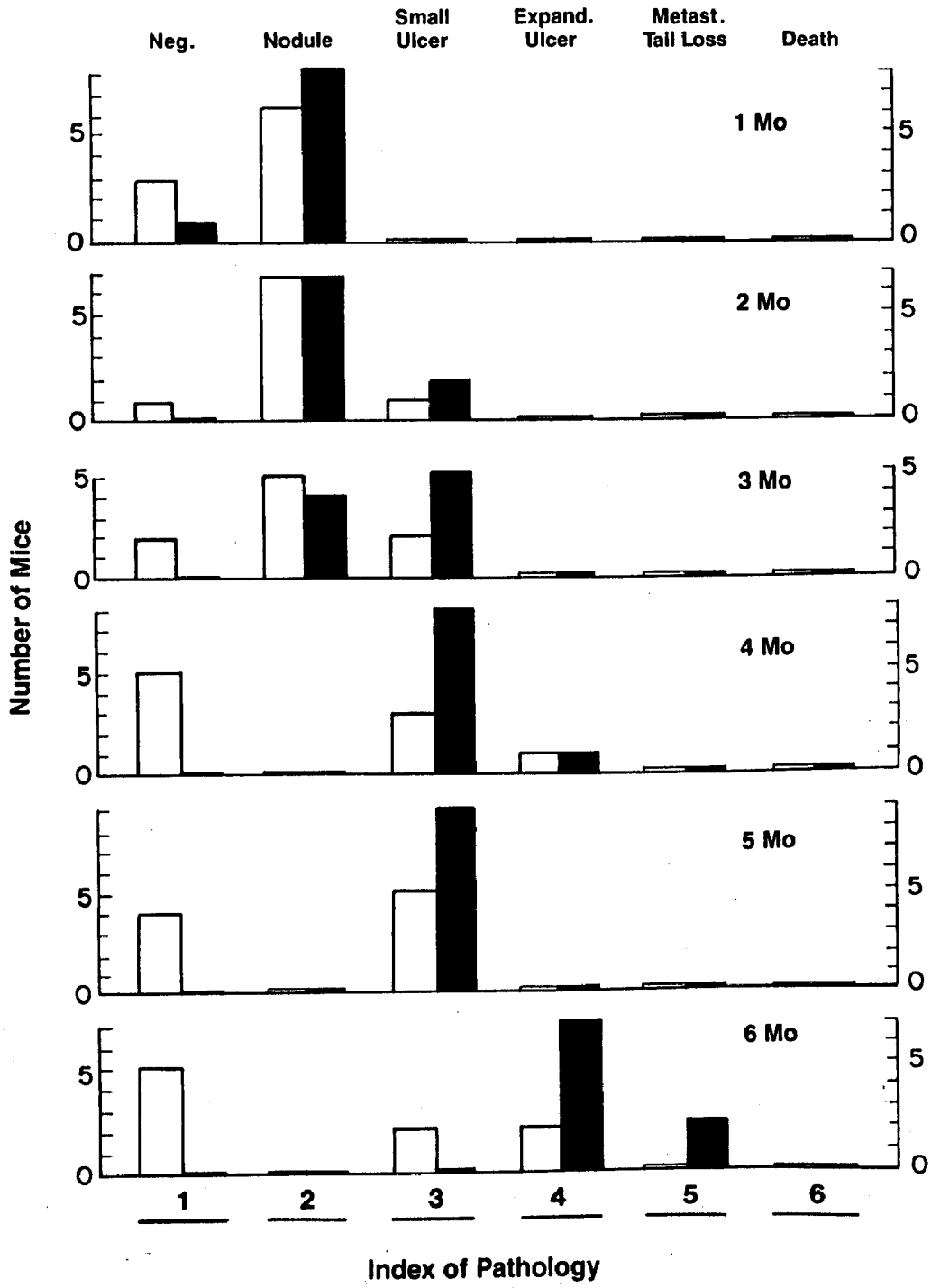

Figure 1. Effect of Local UV-B Irradiation on the Long-term Development of Cutaneous Lesions in Mice. Groups of eight or nine mice were infected intradermally in the tail with with 1 x 10^6 Leishmania major promastigotes. Irradiated mice (open bars) received 15 mJ cm^{-2} UV-B on the tails at -24 hours and +24 hours post infection, and every 48 or 72 hours thereafter for four weeks. Control mice (solid bars) were shielded. Lesion development was significantly different at six months ($p < 0.01$).

same numbers in both irradiated and control groups (Tables 3 and 4). But despite their apparently healthy skin, the irradiated mice had parasites at the injection site and in their local lymph nodes (Table 5). When they were tested for their ability to mount cell-mediated immune responses to leishmanial antigens, the UV-B irradiated mice had almost no immunity, compared with the unirradiated control mice (Table 6). Their lack of cell-mediated immunity reduced the irradiated mice's ability to control a second challenge infection in a skin site different from that of their first exposure, which had been irradiated (Figure 2). Since Leishmania may persist in the local lymph nodes, long after skin lesions have healed, and even when parasites cannot be detected in the skin (Hill, North, and Collins 1984; Titus et al. 1985; Giannini, unpublished), it is possible that depression of the host immune response by other factors such as stress or impaired nutrition could put the infected, though asymptomatic, individual at risk for disseminated disease from the parasites lingering in the lymph nodes.

A similar suppressive effect of UV-B on cell-mediated immunity to herpes simplex Type II virus has also been shown (Hayashi and Aurelian 1986). Exposure to UV-B during the primary phase of infection suppresses the development of cell-mediated immune responses to the virus (Howie, Norval, and Maingay 1986), and irradiation of healed skin lesions triggers recurrent herpes infections (Wheeler 1975; Blyth et al. 1976).

From these and other data we can conclude that local perturbations in the functions of the skin-associated lymphoid tissue during cutaneous infections can profoundly influence the immunological response to antigens of invading microbes and the subsequent development of clinical disease.

PREDICTED ALTERATIONS IN PATTERNS OF INFECTIOUS DISEASES IN HUMANS FOLLOWING INCREASED SOLAR UV-B RADIATION

If the immune functions of the skin cannot be protected from the effects of UV-B by the use of sunscreens (Menter this volume), then it may be difficult to avoid exposure to UV-B, especially in agricultural societies in the tropics and semitropics, where heat makes protective clothing impractical to wear.

Increases in the amount of solar UV-B reaching the earth's surface will likely affect the severity of infectious disease. More precise predictions are not possible, because wavelengths and dosages will critically affect the response curve. In addition, the global effects of UV-B include other environmental factors that impact on disease development, such as climate, vector density, and food availability. It is probable that some cutaneous infectious diseases will be exacerbated by increased UV-B radiation, as is the case with smallpox and herpes. In other cases, pathogenesis of cutaneous disease may be suppressed, such as in cutaneous leishmaniasis and lupus vulgaris.

Table 3. Growth and Viability of *Leishmania major* in UV-B Irradiated Macrophage-Like Cells *In Vitro*

mJ/cm^2 UV-B	Leishmania[a] per host cell	Host cells[a] per 0.06 mm^2	Leishmania[b] per 0.06 mm^2	Log_{10} viable parasites[a]
15	3.6 ± 0.4	17 ± 10[c]	61	4.5 ± 1
5	3.3 ± 1.1	50 ± 21	164	5.2 ± 1
1.5	3.3 ± 0.6	45 ± 16	150	4.3 ± 1
0.5	3.0 ± 0.5	61 ± 16	183	5.2 ± 1
none	3.5 ± 1.0	43 ± 16	150	5.0 ± 1

[a] Means ± standard deviation of four J774A.1 host macrophage-like cell cultures; numbers of viable parasites were determined by an *in vitro* assay system described in Giannini (1985); no significant differences in viability or numbers of Leishmania per host cell were seen.

[b] Calculated by multiplying the mean number of Leishmania per host cell times the mean number of host cells per 0.06 mm^2.

Table 4. UV-B Irradiation *In Vivo*[a] Does Not Reduce Viability of *Leishmania major*[b] in Skin at Injection Site

Experiment	Time Post Infection	Log viable amastigotes per 15 µl of triturated skin: 15 mJ/cm UV-B	Shielded Controls	p
1	3.5 mo.	3.3 ± 3.2	1.3 ± 1.5	n.s.
2	9 mo.	8.5 ± 2.6	8.0 ± 3.1	n.s.

[a] Groups of 4-8 B10.129 (10M) mice were irradiated with 15 mJ/cm^2 UV-B on the tails 24 hours before and 24 hours after infection with 1×10^4 (Expt. 1) or 1×10^6 (Expt. 2) *L. major* promastigotes, and subsequently irradiated every 48 or 72 hours for 4 weeks. See Giannini (1986) for further details.

[b] Determined by culturing serial tenfold dilutions (described in Giannini 1985).

Table 5. Effects of UV-B Irradiation on Lesion Development and Parasite Dissemination at 6 Months Post Infection[a] With *Leishmania Major*

	Controls	Irradiated
Lesion pathology index	4.2 + 0.4	2.1 + 1.4
Infected injection site	100%	100%
Infected local lymph nodes	100%	100%
Infected spleen	89%	67%
Infected skin distal to injection site	33%	33%

[a] Only lesion pathology index was significantly different between the two groups.

See Table 4, footnote a, for methods, and footnote b for explanation of lesion pathology index (from Giannini 1986).

Table 6. Cellular Immune Responses of UV-B Irradiated Mice[a], Infected with *Leishmania major*, After Challenge with Leishmanial Antigens[b]

Time post Infection hrs	Treatment	Footpad Thickness[c] (mm x 10^{-2}) 4 hrs	24 hrs	48
	Uninfected controls	0	8	0
2 weeks				
	Unirradiated	8[d]	25[d]	12[d]
	UV-B irradiated	0	11	0
6 weeks				
	Unirradiated	12[d]	11	18[d]
	UV-B irradiated	0	0	0

[a] 4 to 5 B10.129 (10M) mice per group were irradiated with 15 mJ/cm^{-2} on the tails 24 hours before and 24 hours after infection with 1 x 10^6 *L. major* promastigotes, and subsequently every 48 or 72 hours for 4 weeks.

[b] Mice were challenged in the left rear footpad with 10 µl containing 2 x 10^6 promastigotes, solubilized by freezing and thawing in PBS. The right rear footpad was injected with 10 µl PBS.

[c] Thickness of left footpad minus right footpad. Median values are given because lesion pathology (and therefore the mice's responses) are not normally distributed (see Figure 1).

[d] Significantly different from the primary response of uninfected controls ($p < 0.05$).

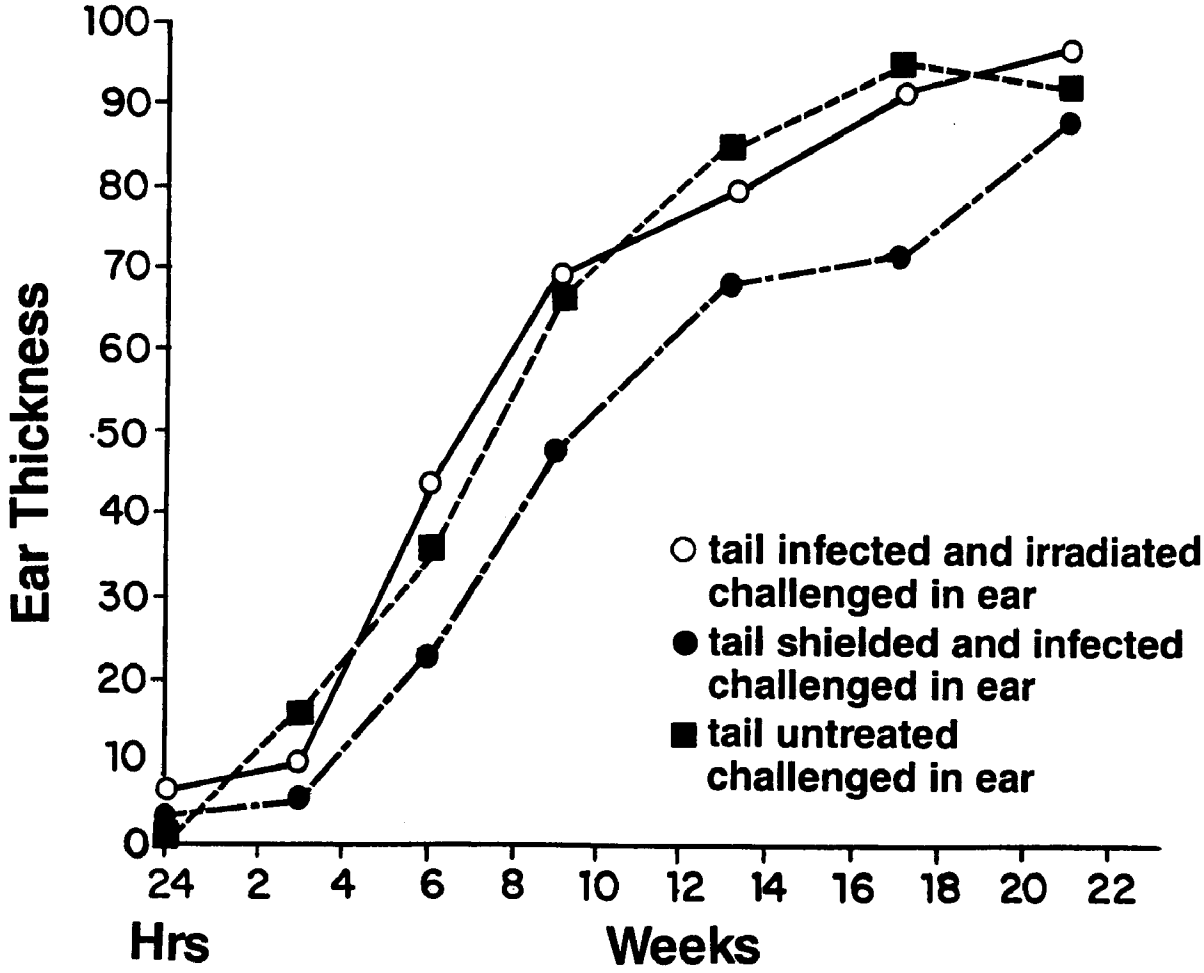

Figure 2. Abrogation of Protection to a Challenge Infection by UV-B Irradiation of the Primary Site of Infection with Leishmania major. Mice were irradiated or shielded and infected in the tail, as described in the legend to Figure 1. At two weeks after the primary infection in the tail, mice received a large challenge infection in the ear (1×10^6 parasites). Expansion of the secondary lesions in the ear in previously infected mice was compared with that in mice not previously exposed to L. major, for which the ear was the primary injection site. The previously infected, shielded controls had significantly smaller lesions in response to the challenge infection than the unexposed controls, at all times between 4 and 18 weeks, while the responses of the previously infected, irradiated controls were indistinguishable from the unexposed controls at all times.

But we do well to remember that escape from overt skin disease may have a high price, namely, forfeiture of protective immunity to microbes that escape the immune surveillance network in the skin while its functions are inactivated by UV-B.

ACKNOWLEDGMENTS

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Urocanic Acid: On Its Role in the Regulation of UVB-Induced Systemic Immune Suppression

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ABSTRACT

We have previously postulated that UVB-induced systemic immune suppression is mediated by a specific skin photoreceptor situated in the stratum corneum of mammalian skin. This photoreceptor is considered to be a biological signal transducer capable of converting UV-B radiation (290-320 nm) directly into a biochemical signal that then "switches-on" formation of antigen-specific suppressor cells by way of an "alteration" in antigen presentation.

The UVB-absorbing photoreceptor is tentatively identified as trans-urocanic acid (trans-UCA). The mode of immune regulation associated with it is suggested to be initiated by photoconversion to its cis isomer (cis UCA). Recent evidence is presented to support this concept showing that cis UCA in contrast to trans-UCA can induce an antigen-presenting "alteration" in splenic dendritic cells in the absence of any UV radiation.

A model is presented outlining a mechanism in which UCA-initiated immune suppression is involved with the prevention of autoimmune attack against "photoantigens" associated with sun-damaged skin. We propose that sunlight-induced skin tumor outgrowth may be an inadvertent consequence of this normally protective mechanism.

INTRODUCTION: UCA AND IMMUNE SUPPRESSION

Urocanic acid (UCA, de-aminated histidine) is a major UVB-absorbing compound located in the stratum corneum. The naturally occurring form is the trans isomer; it isomerizes to the cis form on absorption of UV-B radiation.

Although the existence of UCA has been known for more than 100 years (Jaffe 1974), no physiological function for it has ever been established. Furthermore, even though UCA can attenuate ultraviolet (UV) radiation striking

the skin of mammals, in effect acting as a photoprotector of DNA or as a sunscreen protecting against erythema, it may be playing a more fundamental physiological role as an immune-regulating skin photoreceptor (De Fabo and Noonan 1983). We discuss the evidence supporting this idea in this paper.

BACKGROUND

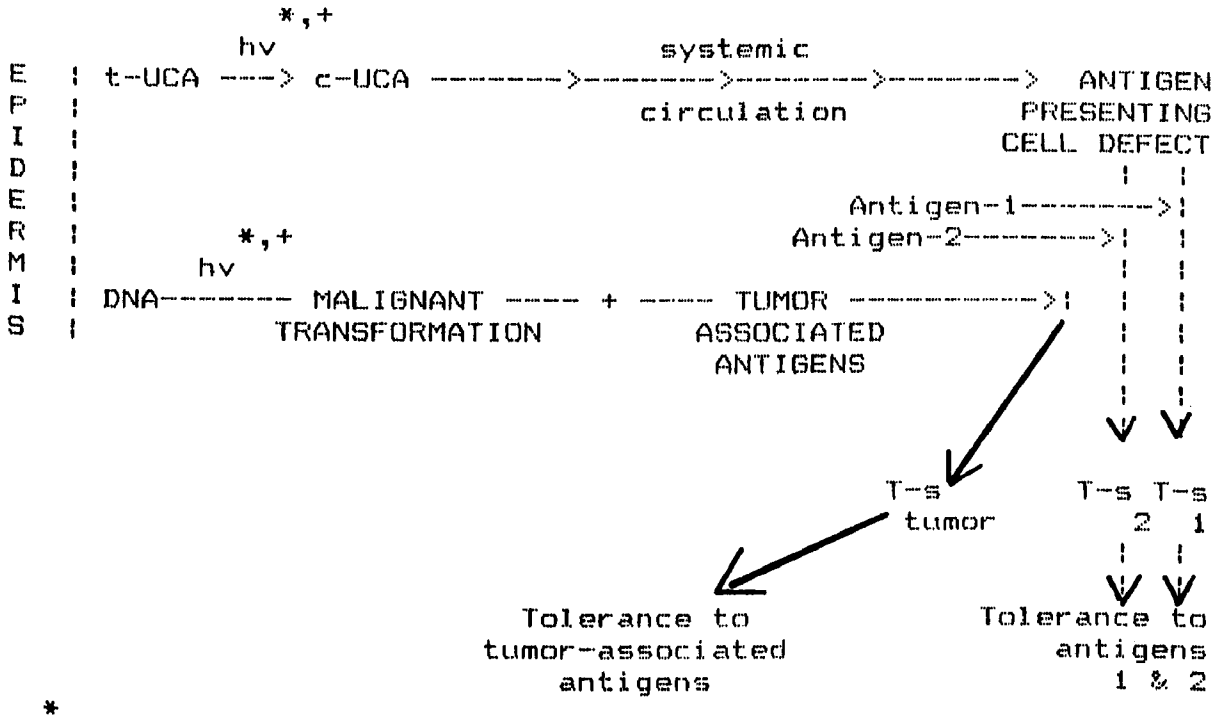
The ability of UV radiation to suppress a variety of immune responses in animals and humans has been well documented and reviewed (Kripke and Fisher 1976; Fisher and Kripke 1977; Morrison 1984; Parrish, Kripke, and Morrison 1984; Daynes and Krueger 1983). It has been shown that UV-B (290-320 nm) is responsible for suppressing the immunologic rejection response to transplanted UV-induced tumor (De Fabo and Kripke 1979, 1980).

Another major UV-B effect on immunity is its ability to suppress the delayed-type hypersensitivity (DTH) response to skin sensitizing antigens such as trinitrochlorobenzene (TNCB) in mice (Noonan, De Fabo, and Kripke, 1981a, 1981b). In this reaction mice that have been pretreated with UV radiation show a systemic suppression of the DTH response. We previously showed that it was energy primarily in the UV-B band that can diminish the DTH response in dose-dependent fashion (Noonan, De Fabo, and Kripke 1981a). The kinetics of this response was similar to the UVB-induced suppression of tumor rejection (De Fabo and Kripke 1979, 1980).

In further studies on the systemic suppression of DTH which were designed to elucidate the first light-absorbing event a relative wavelength effectiveness or "action" spectrum of this effect by UV radiation was estimated. The significant finding of this study was that radiation of wavelengths between 320 nm and 250 nm (UV-B and UV-C) exhibited differing effectiveness in suppressing the DTH response. This clear-cut differential in wavelength effectiveness suggested that a specific molecule, i.e., a "UVB-transducing" photoreceptor was absorbing this radiation and using it to "turn on" an immune suppressor response to the skin sensitizing chemical antigens. We suggested this mechanism might exist to "down regulate" the rejection response against new "photoantigens" put on skin cells by exposure to the UV-B component of sunlight. In effect this suppression would prevent uncontrolled autoimmune attack against sun-damaged cells (De Fabo and Noonan 1983). We found the shape of the action spectrum closely matched the absorption spectrum of UCA (De Fabo and Noonan 1983). Because of the close match between our in vivo derived action spectrum and the in vitro absorption spectrum of urocanic acid, we suggested that trans-UCA may be acting as the UVB-absorbing photoreceptor initiating events leading to immune suppression of DTH (De Fabo and Noonan 1983). Furthermore, because of close photobiological and immunological similarities to the suppression of tumor rejection, we suggested that perhaps this suppressive response to transplanted UV-induced skin tumors might also involve UCA (De Fabo and Noonan 1983).

UCA AND SKIN CANCER: A POSSIBLE LINK

One of the major puzzles about skin cancer is that nearly all UV-induced skin tumors in mice are highly antigenic and capable of evoking strong immunologic rejection responses by the host. Thus, it is unclear, given the strong immune response against these tumors, how they are able to grow out in the host.



*
wavelengths are identical for activating both systems (UVB/C: 320-250 nm)

\+ UVB/C flux is low for UCA activation; high for DNA transformation.

t-UCA = trans isomer; c-UCA = cis isomer

T-s 1 : Suppressor T cells specific only for antigen 1

T-s 2 : Suppressor T cells specific only for antigen 2

T-s tumor : Suppressor T cells specific only for tumor-associated antigens

Figure 1. Induction of Tolerance to Tumor-Associated Antigens. In this model a scheme is presented to include the production of a tumor cell with "tumor-associated antigens." This is formed when high doses of radiation of wavelengths 250-320 nm cause a malignant transforming event in DNA. This leads to the formation of a neoplastic cell. Associated with this neoplasm are UVB-induced antigens. We suggest that these antigens interact with the UCA-induced antigen-presenting cell alteration in a manner that eventually leads to the production of antigen-specific suppressor T cells. In the case of a tumor cell, however, the suppressor cells formed are specific for the "tumor-associated antigens." Formation of these suppressor cells leads to tolerance (acceptance) of the tumor cell instead of rejection. Tumor outgrowth is the final consequence.

One possible explanation may involve an interaction between immune suppression mediated by UCA and tumor formation mediated by UV effects on DNA (De Fabo and Noonan 1984). Figure 1 presents a scheme for this type of interaction.

As previously noted, the underlying immunologic problem in mice irradiated with UV-B appears to be an alteration or "defect" in antigen presentation (Greene et al. 1979; Noonan et al. 1981). It seems that, because of this "defect", antigen-specific suppressor cells, rather than effector cells, are formed when antigen is given to a UVB-irradiated mouse. The hypothesis we put forward is that UCA may interact with antigen-presenting cells, e.g., macrophages, skin Langerhans cells, or splenic dendritic cells in such a way as to make defective their presentation of antigen to stimulator T cells. This process we propose is initiated by photoisomerization of trans-UCA to its highly soluble cis form. Once formed, the cis isomer or a secondary intermediate would interact with antigen-presenting cells in such a way as to alter their capacity to present antigen. This "defect" in antigen presentation (or processing) would become the signal for stimulation of suppressor cells rather than effector cells. The specificity of the suppressor cells would be determined by the type of antigen presented to the "defective" antigen-presenting cell (De Fabo and Noonan 1983).

This hypothesis has received strong support from the observations that (a) injection of cis UCA into mice induces an antigen-processing alteration in splenic dendritic cells without UV radiation; injection of trans-UCA was without effect (Noonan, De Fabo, and Morrison 1986) and (b) mice genetically deficient in histidase and thus in epidermal UCA showed no suppression of the CHS response when exposed to known immunosuppressive doses of UV-B (De Fabo et al. 1983).

In conclusion, it appears that a mechanism exists in skin that can directly bring about interaction between solar UV-B and the immune system in a way previously unknown. Further investigations into such a mechanism may open up new directions for determining the causes of skin cancer and other UV-B immune-associated diseases.

SUMMARY

For many years the physiological role for UCA has remained obscure, although a relatively large amount of this substance, in the trans configuration, accumulates in mammalian epidermis. UCA has been suggested to act in the skin as a natural sunscreen. Alternatively, we have proposed a physiological role for trans-UCA as a UVB-absorbing skin photoreceptor necessary to regulate against autoimmune attack on sun-damaged skin. In this capacity, trans-UCA is converted to the cis isomer that can then initiate the production of antigen-specific suppressor cells via an alteration in antigen-presentation. Thus, during prolonged sun exposure, suppressor cells form, which are specific for "photoantigens" induced on sun-damaged skin. These suppressor cells can prevent effector cells from carrying out an autoimmune attack against these "photoantigens."

Because such a mechanism exists, UCA may be involved in the outgrowth of sun-induced skin tumors, inadvertently protecting tumor cells from attack by effector cells. That is, due to the production of these "photoantigen"-

specific suppressor cells that also recognize UV-induced antigens on tumors, tumor growth continues and the concentration of these UV-induced antigens steadily increases, keeping primed the production of specific suppressor cells (Figure 1). Without reversing this process, immune surveillance of the tumor (attack and destruction) will be prevented and tumor outgrowth will be the final outcome.

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Solar Wavelengths of Ultraviolet Light-Induced Cytoplasmic Damage

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SUMMARY

Since solar ultraviolet (UV) light is the major cause of human skin cancer, it is important to determine the UV-induced cellular lesions that contribute to the carcinogenic process. The majority of investigations have examined DNA damage resulting from exposure to short wavelength UV light. This has led to the suggestion that pyrimidine dimers are the critical lesion responsible for solar carcinogenesis. However, people are not exposed to such short wavelength UV light, because it does not penetrate the atmospheric ozone layer. There is evidence that cellular lesions other than pyrimidine dimers are involved in the carcinogenic, mutagenic, and lethal effects of UV light that reaches the earth's surface. However, alterations of non-DNA targets in mammalian cells caused by UV have received little attention. Because the cytoskeleton is an important participant in the control of normal cell growth and because epigenetic events may play a role in carcinogenesis, we have begun to explore the induction of cytoskeletal alterations induced by UV light in human skin fibroblasts. We have been particularly interested in the effects of irradiation with polychromatic UV light composed of environmentally relevant wavelengths. Our data indicate that exposure to germicidal UV-C light results in no discernible changes in the cytoskeletal organization as revealed by fluorescence microscopy. However, exposure to polychromatic UV-B and UV-A light causes severe injury to the cytoskeleton.

INTRODUCTION

Epidemiological and experimental evidence has established solar ultraviolet (UV) light as the major cause of human skin cancer. More than 90% of non-melanoma cutaneous tumors appear to result from exposure to sunlight. A great deal of effort has therefore been expended trying to elucidate the mechanisms by which UV light induces the transformation of normal cells to a malignant phenotype. The vast majority of investigations have been performed with germicidal lamps that emit primarily 254 nm light. Unfortunately, such

short wavelength UV light is environmentally irrelevant because it does not penetrate the stratospheric ozone layer. The UV light spectrum has been operationally divided into three wavebands: UV-C, 200-290 nm; UV-B, 290-320 nm; and UV-A, 320-400 nm. The solar spectrum of UV light reaching the earth's surface is fairly constant at wavelengths greater than 320 nm and drops off precipitously between 320 and 290 nm. UV-A, the least studied UV waveband, comprises the largest UV component to which people are exposed. UV-B light appears to be responsible for the induction of skin cancer. The contribution of interactions between UV-A and UV-B light towards the development of cutaneous tumors remains to be determined.

Decreases in the concentration of stratospheric ozone, resulting from the anthropogenic release of chlorofluorocarbons and other agents into the environment, will significantly increase the levels of carcinogenic UV-B light reaching the earth's surface. In a recent report from the National Research Council, it was suggested that every 1% decrease in ozone concentration will result in a 2% to 5% higher incidence of basal cell carcinomas and a 4% to 10% higher incidence of squamous cell carcinomas (National Research Council 1982). Since it appears that a significant decrease in stratospheric ozone is likely to occur during the next century, special emphasis needs to be placed on the adverse health effects of increased exposure to UV-B light. However, to attain an accurate assessment of the potential health hazards, the UV-induced lesions that contribute to the carcinogenic process must be identified. This paper explores the possibility that exposure to wavelengths of UV found in the environment alters cytoplasmic elements that are important in the regulation of cell growth.

The cytoplasm of eukaryotic cells contains an intricate network of filamentous structures that are collectively referred to as the cytoskeleton. The three major components of the cytoskeleton are microtubules, microfilaments, and intermediate filaments (Alberts et al. 1983). Microtubules are hollow structures consisting primarily of tubulin and have an outer diameter of 22-25 nm. They appear to originate at microtubule organizing centers close to the nuclear membrane and extend throughout the cytoplasm. Microfilaments, composed primarily of F-actin, are thinner filamentous structures having an average diameter of 6 nm. In well flattened cultured cells, microfilaments are often found in bundles, referred to as stress fibers. The diameter of intermediate filaments, approximately 10 nm, falls between those of microtubules and microfilaments. Immunological and biochemical studies have demonstrated that the protein composition of intermediate filaments is very heterogeneous. Intermediate filaments have therefore been grouped into five subclasses: keratin (epithelium), vimentin (mesenchyme), desmin (muscle), glial fibrillary acidic protein (glial cells), and neurofilament proteins (neurons). Using fluorescence microscopy, we have investigated alterations of cytoskeletal components induced by exposure to UV-C light or polychromatic UV-B and UV-A light in a human skin fibroblast cell strain.

MATERIALS AND METHODS

Cells

AG 1522, a human skin fibrobast cell strain, was obtained from the Human Genetic Mutant Cell Repository, Institute for Medical Research (Camden, NJ). Cells were grown in Eagle's minimal essential medium supplemented with 10% fetal calf serum, 0.9 g/l D-glucose, 0.66 mg/l sodium pyruvate, 110 μ/ml penicillin and 110 μg/ml streptomycin sulfate. Cultures were incubated at 37°C in an atmosphere of 95% air and 5% CO_2.

UV Light

General Electric G8T5 germicidal lamps, Westinghouse FS40 lamps, and Sylvania FR40T12 lamps were our sources of UV-C, sunlamp, and UV-A light, respectively. Polychromatic UV-B and UV-A light sources were selected because they more closely simulate the type of UV light to which we are exposed at the earth's surface. Furthermore, it has been demonstrated that the biological effects of polychromatic light frequently do not comprise a simple composite of the effects induced by monochromatic light corrected for the appropriate relative intensities (Suzuki et al. 1981; Wells and Han 1984). The UV-C lamps emit greater than 95% of their energy at 254 nm. The emission spectra of the Westinghouse FS40 and Sylvania FR40T12 lamps, filtered through plastic petri dish covers, are shown in Figure 1. The sunlamps transmit approximately equal amounts of light in the UV-B and UV-A wavebands with a peak emission in the UV-B region between 310 nm and 315 nm. More than 98% of the light from the UV-A lamps (maximum emission at 350-355 nm) is in the UV-A waveband. UV-C dose rates were determined with an International Light (Newburyport, MA) IL254 germicidal photometer. Sunlamp and UV-A dose rates were determined with an International Light IL443 photometer with appropriate filters for the individual light sources. Cultures exposed to UV-C light were irradiated without their covers at a dose rate of 0.4 $J/m^2/sec$, in the presence of Hank's buffered salt solution supplemented with 15 mm Hepes (HBSS). Cultures exposed to sunlamp (approximately 4.5 $J/m^2/sec$) or UV-A light (approximately 30 $J/m^2/sec$) were irradiated, in the presence of HBSS, through their plastic covers in order to filter out wavelengths shorter than 290 nm. Sunlamp- and UV-A treated cultures were maintained in a room temperature water bath during irradiation. In order to protect cultures from ambient UV light, only yellow light was used in the laboratory during the course of our experiments.

Cytoskeleton Preparation

Microtubules and microfilaments were examined using modifications of procedures previously described (Chou and Shaw 1984; Wang et al. 1982). Cells grown on glass coverslips were washed with a microtubule stabilizing buffer, pH 6.9 (PM2G buffer), that contains 0.1 M Pipes, 1 mM $MgSO_4$, 2 mM EGTA, and 2 M glycerol. This buffer condition effectively preserves microtubules as well as microfilaments and intermediate filaments. The cells were fixed for 30 minutes with 3.7% formaldehyde, freshly prepared in PM2G buffer, followed by incubation in phosphate buffer solution, pH 7.4 (PBS), for 5 minutes. They were then treated with 0.1 M Glycine in PBS for 5 minutes to quench formaldehyde, and extracted with 0.3% Nonidet P-40 (NP40) in PBS for 10 minutes. All operations up to this step were conducted with room temperature reagents.

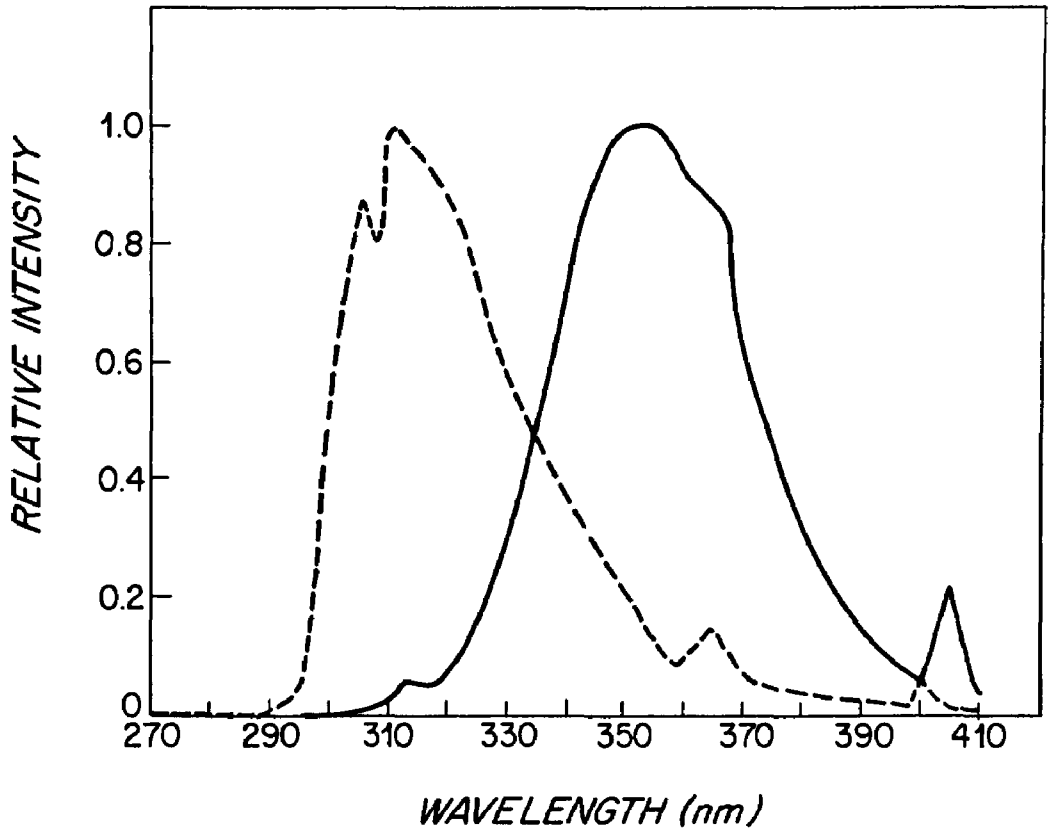

Figure 1. Emission Spectra of Westinghouse FS40 (sunlamp) and Sylvania FR40T12 (UV-A) Lamps.

The fixed cytoskeletons on coverslips were incubated at 37°C for 30 minutes with a mixture of rabbit anti-tubulin antibodies and NBD-phallacidin to double label microtubules and microfilaments, respectively. NBD-phallacidin, a fluorescent reagent, specifically labels F-actin filaments of cultured cells, resulting in a higher degree of resolution than obtained with indirect labelling procedures using two antibodies. After the initial labelling period, coverslips were thoroughly washed with PBS and treated for 30 minutes with goat anti-rabbit antibodies conjugated to rhodamine. After the 30-minute exposure to rhodamine-conjugated antibodies, the coverslips were again thoroughly washed with PBS and finally with double-distilled water to remove residual PBS. They were then mounted on glass slides with a drop of Gelvatol and allowed to harden at 4°C overnight before examination with a Nikon fluorescence microscope. Using coded coverslips, the percent of cells with intact microtubules and/or microfilaments was determined by examining 200 cells in randomly selected microscopic fields at each UV dose. The person viewing the cells was not familiar with the code used to identify the coverslips. Photographs of representative cells were taken using Kodak Tri-X film (ASA 400) and developed by "pushing" to an effective rating of ASA 1600.

RESULTS

Initial comparisons between cells irradiated with UV-C and sunlamps indicated that even at extremely toxic UV-C doses, the microtubules and microfilaments of AG1522 cells remain intact. However, we consistently observed disruption of microtubular structures in cells irradiated with sunlamps. In order to quantify this phenomenon, we have performed dose-

response experiments in which we estimated the percent of cells with intact microtubules (Table 1). Coded coverslips were used to avoid counting bias. Because the efficiency of inducing erythema, cellular inactivation, mutagenesis, transformation, and other biological effects usually decreases as the wavelength of light increases, the progressively higher doses at longer wavelengths of light were selected. As seen in Table 1, intact microtubules were found in cells irradiated with UV-C doses as high as 100 J/m^2. Exposure to sunlamps resulted in the disruption of microtubules, decreases in intact microtubules usually being observed after exposure to 3000 J/m^2. Experiments with UV-A light indicated that it also causes a dose-dependent loss of organized microtubules. No perceptible alterations of microfilaments occurred in cells irradiated with UV-C, sunlamps, or UV-A. We have previously found that the doses required to reduce survival of AG1522 cells to 10% (as measured by colony forming ability) are approximately 8.2 J/m^2 for UV-C, 1180 J/m^2 for sunlamp, and 157 kJ/m^2 for UV-A light (Zamansky 1986). The disruption of microtubules therefore appears to be induced by sunlamp and UV-A exposures, which result in less cell death than after 100 J/m^2 UV-C, a dose at which no effect occurs.

Table 1. Disruption of Microtubules by UV-C, Sunlamp, or UV-A Light

UV-C		Sun Lamp		UV-A	
Dose (J/m^2)	Intact MT (%)*	Dose (J/m^2)	Intact MT (%)*	Dose (kJ/m^2)	Intact MT (%)
0	96.5 ± 0.5	0	96.7 ± 0.9	0	95.8 ± 1.2
10	96.0 ± 1.0	750	94.3 ± 0.9	50	83.3 ± 8.3
20	96.0 ± 0.5	1500	89.7 ± 1.3	100	38.3 ± 3.0
40	94.5 ± 0.8	3000	54.7 ± 15.2	150	26.5 ± 3.0
75	94.8 ± 2.3	5000	37.3 ± 2.8		
100	91.5 ± 2.5	7500	19.7 ± 9.2		

* The percent of cells with intact microtubules is presented as the mean ± 1 standard error of 2 (UV-C), 3 (sun lamp) or 2 (UV-A) experiments. 200 cells were examined in randomly selected microscopic fields of coded coverslips in each experiment.

The microscopic appearance of microtubules and microfilaments in control and irradiated cells is shown in Figures 2A-2F. As expected, the microtubules of unirradiated AG1522 cells (Figure 2A) appear to emanate from perinuclear microtubule organizing centers and extend throughout the cytoplasm. The intricate network of microtubules is also present in cells irradiated with UV-C (Figure 2C). However, distinct microtubules are often completely absent in the cytoplasm of cells irradiated with sunlamps (Figure 2E). Although remnants of the microtubule organizing center may still be present, the majority of anti-tubulin-stained cytoplasm takes on a fine powdery appearance. The extent to which this occurs is dependent upon the UV dose. Thus, microtubules appear to be disassembled or fragmented. UV-A irradiated

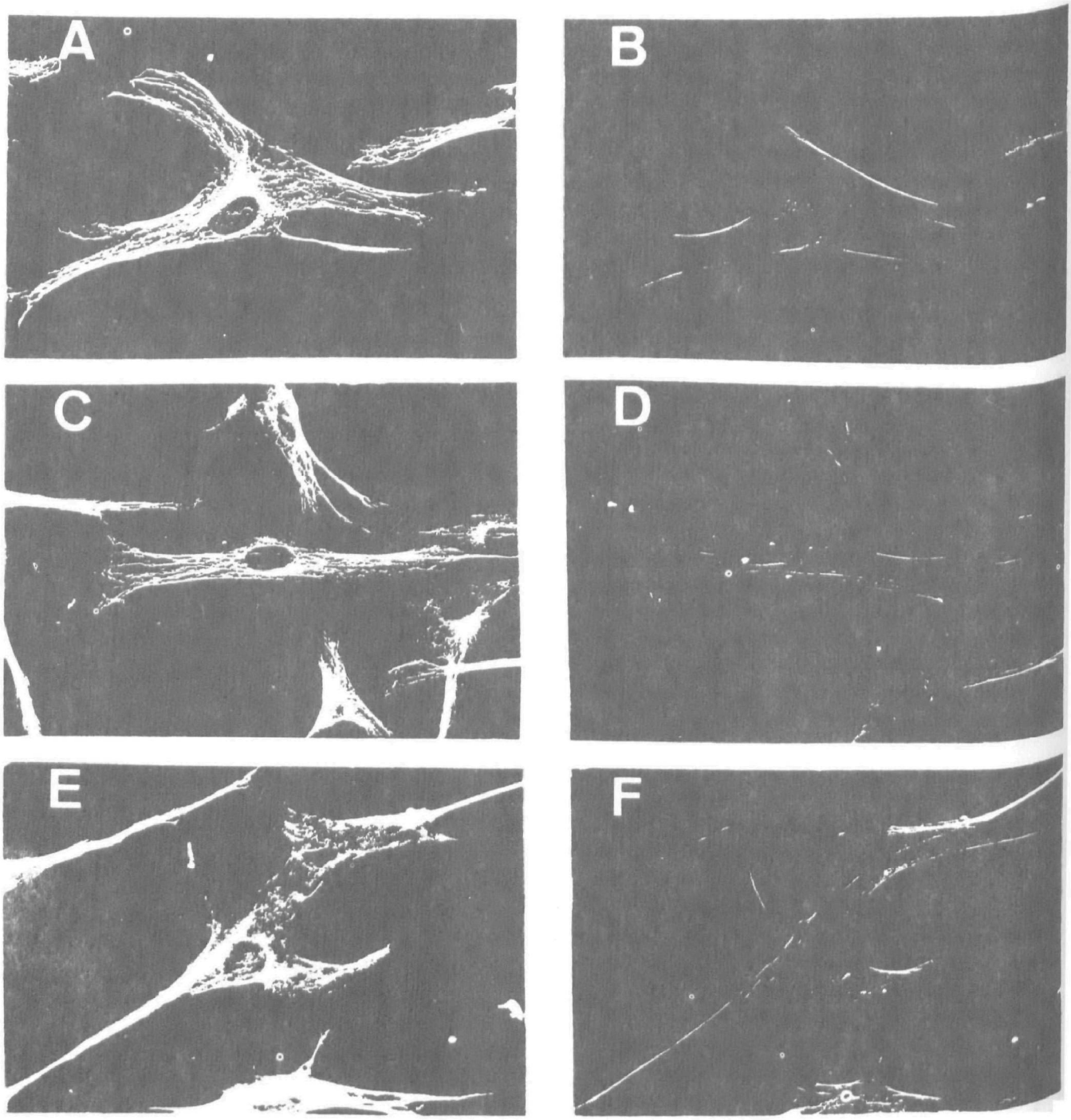

Figure 2. Microscopic Appearance of Microtubules (A,C,E) and Microfilaments (B,D,F) in AG1522 Cells. No UV light (A and B), UV-C, 100 J/m^2 (C and D); sunlamp, 5000 J/m^2 (E and F).

cells (not shown) have the same powdery appearance as sunlamp-treated cells. The microfilaments of the cells depicted in Figures 2A, 2C, and 2E are shown in Figures 2B, 2D, and 2F, respectively. No changes were observed in the microfilaments, which remain quite apparent as distinct actin filament bundles stretching across the length of the cells.

DISCUSSION

DNA is usually considered the critical site of UV-induced cellular damage. Experiments utilizing short wavelength UV light have yielded an abundance of information concerning the induction of pyrimidine dimers, and the mechanisms by which dimers are removed have become the most understood DNA repair pathways. However, the types and relative frequencies of UV-induced lesions vary greatly for the three wavebands of the UV spectrum (Cerutti and Netrawali 1979; Tyrrell 1982; Han, Peak, and Peak 1984). Most action spectra for cellular inactivation correlate well with the absorption spectrum of DNA and with the formation of pyrimidine dimers out to 313 nm (Rothman and Setlow 1979; Kantor, Sutherland, and Setlow 1980; Jacobson, Krell, and Dempsey 1981; Doninger et al. 1981; Tyrrell, Wertelli, and Moraes 1984) supporting the role of DNA as the critical target for the lethal effect. The action spectra for mutation, transformation, cellular capacity for viral growth, reactivation of UV-damaged virus, and the induction of virus synthesis in transformed cells also correspond closely to the absorption spectrum of DNA at wavelengths as long as 313 nm (Jacobson, Krell, and Dempsey 1981; Doninger 1981; Coohill and Jacobson 1981). These findings and the observation that patients with xeroderma pigmentosum (XP) exhibit cutaneous hypersensitivity to sunlight, the development of multiple malignant tumors in sun-exposed areas, and an inability to properly repair pyrimidine dimers (Cleaver and Bootsma 1975; Robbins et al. 1978) have led to the suggestion that these lesions are responsible for solar carcinogenesis. However, evidence has begun to accumulate that lesions other than pyrimidine dimers may be involved in the carcinogenic, mutagenic, and lethal effects of solar UV light (Zelle et al. 1980; Smith and Paterson 1981, 1982; Suzuki et al. 1981; Zbinden and Cerutti 1981; Botcherby et al. 1984; Wells and Han 1984; Tyrrell 1984).

We have previously reported that the relative sensitivity of human skin fibroblasts to UV-C, sunlamp or polychromatic UV-A light depends on the composition of the light to which they are exposed (Zamansky et al. 1985; Zamansky 1986). Our findings suggest that the inability of XP cells to properly remove pyrimidine dimers is of less importance to cells exposed to polychromatic, long wavelength light and that no lesion commonly induced by our three UV light sources is likely to be solely responsible for cellular inactivation. This agrees well with a recent study by Keyse et al. (1983) in which monochromatic UV light was used (1983). Additional support for the importance of non-dimer lesions has been obtained in studies demonstrating that certain cell strains from patients with Bloom's syndrome, ataxia telangiectasia, and actinic reticuloid are hypersensitive to long wavelength UV light, but not to 254 nm light (Smith and Paterson 1981; Zbinden and Cerutti 1981 Botcherby et al. 1984). Furthermore, a lack of correlation between the induction of pyrimidine dimers and cellular inactivation, mutagenesis, and transformation has been noted in human and rodent cells exposed to UV light composed of wavelengths greater than 313 nm (Zelle et al. 1980; Smith and Paterson 1981; Suzuki et al. 1981; Tyrrell 1984).

The transformation of normal cells to a cancerous phenotype is a complex process that has most often been described as involving two stages: initiation and promotion (Diamond, O'Brien, and Baird 1980; Farber and Cameron 1980). Current experimental evidence suggests that initiation is caused by genetic damage and that promotion results from epigenetic events. Because exposure to promoters is not required for tumor induction by "complete" carcinogens such as UV light, most investigations into the carcinogenic effects of UV light have emphasized its genotoxic capability. Is it likely that UV-induced alterations of non-DNA targets may contribute to the establishment of human skin cancer? It has been known for many years that solar wavelengths of UV light induce biologically important lesions in non-DNA targets in bacteria. Several effects due to damage of bacterial membranes have been studied. Moss and Smith (1981) have demonstrated that membrane damage contributes to lethality under certain growth conditions. Others have reported the inactivation of membrane transport systems and the inhibition of oxidative phosphorylation (Kashket and Brodie 1962; Robb and Peak 1979; Brodie, Sutherland, and Lee 1979; Sharma and Jagger 1981). Absorption of UV-A light by 4-thiouridine, an unusual base found in bacterial transfer RNAs, results in growth inhibition caused by a cessation of RNA synthesis (Ramabhadran and Jagger 1976; Favre and Thomas 1981). It has also been shown that UV-A light inhibits bacterial DNA repair enzymes (Tyrrell 1976). Very little is known about the induction of alterations in non-DNA targets in mammalian cells, though recent studies have demonstrated that cell membranes can be damaged by broad spectrum UV-A and UV-B light (Aberer et al. 1981; DeLeo et al. 1985).

The results presented above represent the first demonstration of UV-induced disruption of cytoskeletal elements in cultured cells. In addition, our data indicate that exposure to germicidal, UV-C light or to environmentally relevant wavelengths of UV-B and UV-A light has extremely different effects on the cytoskeleton. Since the cytoskeleton is an important participant in the control of normal cell growth and since epigenetic events probably play a role in carcinogenesis, such alterations of the cytoskeleton may be an important consequence of natural exposure to UV light. The individual components of the cytoskeleton are structurally associated with each other as well as with the cellular membrane and nuclear matrix (Brinkley 1981; Schliwa, van Blerkom, and Pryzwansky 1981; Singer et al. 1981; Ben-Ze'ev 1985). It has therefore been suggested that the cytoskeleton may serve as a critical means of transmitting external signals to the nucleus. The molecular mechanisms by which it performs this function are not understood. The complex organization of the cytoskeleton participates in the regulation of cellular shape and motility, the spatial arrangement of organelles and secretory processes. Because cell shape is an important regulator of cell growth, alterations of the cytoskeleton disrupt normal shape-related regulatory signals (Folkman and Moscona 1981; Penman et al. 1981). The structural state of the cytoskeleton also appears to contribute to the control of DNA synthesis (Friedkin and Rozengurt 1981; Otto 1982). Agents causing the disassembly of microtubules enhance the initiation of DNA synthesis in cells exposed to peptide growth factors, though this response may be dependent upon the cell type or culture conditions. The close association between polyribosomal mRNA and cytoskeletal components has led to the suggestion that the cytoskeleton may also contribute to the regulation of protein synthesis (Nielsen, Goelz, and Trachsel 1983). Because there is extensive interaction between cytoskeletal elements, agents that affect one component may influence the regulatory capacity of others. It is thus reasonable to expect that

perturbations of the normal assemblage of the cytoskeleton induced by UV light could result in a variety of functional consequences. Indeed, cytoskeletal abnormalities have now been associated with several pathologic phenomena including the malignant transformation of cells (Lockwood, Trivelte, and Pendergast 1981; Penman et al. 1981; Rungger-Brandle and Gabbiani 1983; Ben-Ze'ev 1985). With these considerations in mind, it is also intriguing to note that tumor promoters have recently been found to cause structural changes in the three major cytoskeletal components (Weber, Wehland, and Herzog 1976; Rifkin, Crowe, and Pollack 1979; Seif 1980; Schliwa et al. 1984; Fey and Penman 1984).

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Sunscreens Do Not Abrogate UV-Induced Suppression of Contact Hypersensitivity

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ABSTRACT

Contact hypersensitivity (CHS) in mice can be induced by cutaneous sensitization followed by elicitation via ear-painting with trinitrochlorobenzene (TNCB). This CHS reaction is systemic and can be suppressed by exposure of mice to suberythemogenic doses of 280-315 nm radiation. In this paper, we investigate whether a commercially available water-resistant sunscreen (SPF-6) containing padimate O (UV-B absorber) and oxybenzone (UV-A absorber) was effective in preventing systemic suppression of CHS induced by either FS36 sunlamp exposure or solar simulating radiation. We observed that this sunscreen preparation could not prevent the immunologic suppression of contact hypersensitivity by UV radiation. These results indicate that application of sunscreen does not retard the development of suppression of CHS following repeated UV exposure under conditions where erythema is not clinically observed. Thus, erythema may not be a good end point for assessing systemic immune suppression and its consequences.

INTRODUCTION

Exposure of mice to ultraviolet (UV) radiation systemically suppresses their ability to reject highly antigenic UV-induced skin cancers (Fisher 1977; Fisher and Kripke 1977). This state of unresponsiveness is mediated immunologically and is due in part to the presence of antigen-specific suppressor T lymphocytes (Fisher 1977, 1978; Fisher and Kripke 1977, 1978, 1982; Spellman and Daynes 1977). A second UV-induced immunosuppressive defect has also been described in which a single exposure of the dorsal skin of mice to UV radiation was sufficient to suppress contact hypersensitivity (CHS) to 2-chloro-1,3,5-trinitrobenzene (TNCB) applied to the abdominal surface (Noonan et al. 1981). The cellular basis for the suppression of contact hypersensitivity appears to involve a UV-induced alteration in the functional activity of antigen-presenting cells (Noonan et al. 1981; Greene et al. 1979).

The photobiologic and immunologic similarities of these two UV-induced systemic immunosuppressive events suggest that they may share common steps (Noonan et al. 1981; De Fabo and Kripke 1979; Noonan, De Fabo, and Kripke 1981a, b), which appear to be: (a) an alteration in the presentation of certain antigens, particularly those encountered via the cutaneous route (Noonan et al. 1981) and (b) the induction of T suppressor cells as a consequence of altered antigen presentation (Greene et al. 1979). The main difference between both forms of systemic immunologic suppression is that the antigen that induces the suppressor cells is applied exogenously in the case of contact hypersensitivity, but must be formed endogenously by UV exposure of the skin in the induction of suppressor T cells that regulate tumor rejection. Another important difference between the two systems is the actual dose of UV needed to induce 50% suppression. For CHS, the dose is approximately 13 times less than that needed to induce the tumor susceptibility in 50% of the UV-irradiated animals (Noonan, De Fabo, and Kripke 1981b).

Sunscreens, the majority of which use para-aminobenzoic acid (PABA) or one of its derivatives and/or a benzophenone derivative as their active ingredients, protect against a number of UV radiation effects. The ability of topically applied sunscreens to reduce erythema and skin damage caused by chronic UV exposure, expressed as a "sun protective factor" (SPF), is well documented (Pathak 1969; Willis and Kligman 1970; Kligman, Akin, and Kligman 1980, 1982). In addition, sunscreens have been shown to protect against both the cocarcinogenic as well as the carcinogenic effect of UV radiation (Snyder and May 1975; Kligman, Akin, and Kligman 1980; Wulf et al. 1982; Stern, Weinstein and Baker 1986). To date, however, very few studies have evaluated the effect of these sunscreen agents on the more subtle immunoregulatory effects now known to be produced by UV-B irradiation. One such study by Gurish et al. (1981) found that pretreatment of mice with a PABA sunscreen completely prevented the UVB-induced histologic changes in exposed skin and rendered the mice tumors susceptible, although this susceptibility could not be adoptively transferred to normal untreated animals by lymphoid cell injection. A later study (Morison 1984) with a similar sunscreen but with higher doses of UV-B radiation showed not only induction of the tumor-susceptible state, but also that this state could be transferred to syngeneic animals by lymphoid cells.

In this study, we report the results of experiments designed to evaluate the potential protective effect of a commercial sunscreen preparation on the immunologic suppression of contact hypersensitivity by UV radiation. We observed that this sunscreen preparation with PABA and oxybenzone as its active ingredients was incapable of preventing the immunologic suppression of contact hypersensitivity by UV radiation.

MATERIALS AND METHODS

Animals

Inbred albino hairless mice which have been bred in our laboratories from the inbred HRA/Skh strain were obtained from Stanley Mann of Philadelphia. The hairless mice were from 8 to 16 weeks old at the start of an experiment, but in any one experiment, the age of the animals did not vary by more than 1 week. The animals had free access to Purina Lab Chow and water and were

housed in rooms where ambient lighting was automatically regulated on a 12-hour light/dark cycle.

Contact Sensitization

Each experimental group utilized 5-10 hairless mice. Contact hypersensitivity was induced by the method of Asherson and Ptak (1968). The abdomen was painted with 100 µl (microliters) of a 5% solution of trinitrochlorobenzene (TNCB) in acetone. The mice were tested for the development of contact hypersensitivity five days later by applying 5 µl of a 1% TNCB in acetone to both surfaces of each ear. Ear thickness was measured with an engineer's micrometer (Model No. 7309, Mitutoyo, Japan) before and 24 hours after application of the challenge dose. The ear swelling obtained was compared to the ear swelling of control mice that were challenged, but not sensitized. The statistical significance of the differences in ear swelling between groups was evaluated using the Student's T-test.

Sunscreen Preparation

A commercial sunscreen (SPF-6) contained padimate O and oxybenzone as active ingredients. The sunscreen was applied uniformly to the dorsal skin of the mice at a final dose of 2 $\mu l/cm^2$, 20 minutes prior to UV exposure.

Energy Sources and Measurements

Two energy sources were used during the experiments. One source was a 1.6 kW xenon arc solar-simulating lamp which has been previously described (Willis, Menter, and Whyte 1981). Energy output from this lamp system was measured by a calibrated Eppley Thermopile in conjunction with a Keithley millimicrovoltmeter (Model 149). The total radiation was determined to be 26.2 mW/cm^2, 86 cm (11.5 in) from the exit port. The second source was a bank of three FS36T12-UV-B-VHO lamps (Light Sources) housed in an Ultralite bench top irradiator [BT 3-36 VHO(UV-B)]. Energy output from this system was measured as above 19 cm (7.5 in) from the source and the total output was determined to be 1.4 mW/cm^2.

Minimal Erythema Dose (MED) Determination

For each UV energy source, five hairless mice were irradiated at 25% dose increments. Erythemal responses were noted 24 hours after irradiation. The MED was taken as the exposure required to elicit barely visible minimal redness. Each set of experiments was repeated three times. SPF values were assessed for a sunscreen preparation as the ratio of MED with sunscreen to MED without sunscreen.

Effect of UV Exposure on the Development of CHS

To assess the depression of CHS by UV treatment, mice were sensitized three days after the last UV treatment and challenged five days later. The net ear swelling (ear swelling of sensitized and challenged mice minus the ear swelling of unsensitized, but challenged mice) was determined for unirradiated and UV-treated mice. The percentage of control response was determined by the following formula: A-B/C-D X 100. The letters represent the ear swelling in: (A) mice exposed to UV and sensitized with TNCB; (B) mice exposed to UV,

but not sensitized; (C) unirradiated and sensitized mice; and (D) unirradiated and unsensitized.

RESULTS

MED Determination

The doses required to elicit minimal erythema responses for solar-simulating radiation (SSR) and FS36 sunlamps are given in Table 1. Minimal erythemal dose determination for three FS36 sunlamps was determined to be 38.5 mJ/cm^2 while the total MED dose for SSR was 8 J/cm^2. To test the MED of a commercially available sunscreen (SPF-6), sunscreen was applied to the dorsal surface of inbred hairless mice at a final dose of 2 $\mu l/cm^2$, 20 minutes prior to UV exposure. For both radiation sources, the commercially available sunscreen was determined to have a MED of 6.0 ± 0.5.

Topical Application of Sunscreen and UV-Induced Suppression of Contact Hypersensitivity

In the first set of experiments, two groups of ten mice were pretreated with sunscreen; one group served as an unirradiated control, group 2, while the other group, group 4, was irradiated with a bank of three FS36 sunlamps. During the treatment regimen, no erythematous response was observed in the group receiving the sunscreen preparation. Table 2 shows the results obtained from mice given sunscreen and treated with UV-B radiation. Sunscreen application had no effect on the contact hypersensitive response, as seen by the similarity in groups 1 and 2. The amount of suppression with (44%) and without (50%) topically applied sunscreen was similar.

In the second part of the experiment (Table 3), the sunscreen preparation was topically applied to a group of ten mice receiving SSR radiation (group 4), and another group of ten mice, group 2, which served as a control. The results show that topical application of sunscreen had no effect on the ability of mice to mount a CHS response, groups 1 and 2. The presence of the sunscreen had no effect on suppression of contact hypersensitivity by SSR. The amount of suppression with applied sunscreen was 45% (group 4) while suppression from SSR alone (group 3) was 40%. As with the previous experiment, no erythematous response was observed in the group receiving the sunscreen preparation.

To determine if the previously observed suppression was due to heat alone, two groups of mice were exposed to SSR radiation through a Schott WG 385 filter, a cut off filter with 50% transmission at 385 nm (Table 4, groups 2 and 3). Group 3 received topical sunscreen application while group 2 was treated with SSR alone. The total UV dose of 36 J/cm^2 was unable to suppress the contact hypersensitivity response when filtered through a Schott WG 385 filter. However, in the absence of the Schott filter in both the SSR group alone (group 4) and the group receiving topical sunscreen plus SSR (group 5), suppression of contact hypersensitivity was observed.

DISCUSSION

Treatment of hairless mice with a commercial sunscreen (SPF-6) which contained padimate 0 and oxybenzone as the active ingredients did not

Table 1. Minimal Erythemal Dose Determination for UV Sources in Presence and Absence of Applied Sunscreen

Sunscreen[a]	UV Source	Total UV Dose (J/cm^2)	MEDs	Rated SPF[b]
-	SSR[c]	8	1	
-	FS36[d]	0.0385	1	
+	SSR	44-52[e]	5.5-6.5[e]	6
+	FS36	0.21-0.25[e]	5.5-6.5[e]	6

a. Obtained from a commercial source. Active ingredients were Padimate 0 and Oxybenzone.

b. Manufacturer's rating.

c. Solar simulating radiation from a 1.6 kW xenon arc lamp.

d. 3 FS36T12-UVB-lamps housed in an Ultralite bench top irradiator.

e. Range of 3 determinations.

Table 2. Effect of Padimate O and Oxybenzone on Suppression of Contact Hypersensitivity by UV-B Radiation[a]

Group	Treatment	Total UV dose (mJ/cm^2)	Ear swelling[b] ($cm \times 10^{-3}$)		Net ear swelling	Percent suppression	P[c]
			+TNCB	-TNCB			
1	Nil	0.0	44.3 ± 2.3	8.5 ± 1.3	35.8	--	
2	Sunscreen (SS)[d]	0.0	43.2 ± 2.2	8.2 ± 1.2	35.0	--	
3	UVB	173.3[e]	28.3 ± 2.3	8.3 ± 1.4	20.0	44	<.001
4	UVB + SS	173.3[e]	26.3 ± 3.8	8.4 ± 1.5	17.9	50	<.001

a FS36 Sunlamp - see text

b Mean (± SD) of 10 mice challenged 24 hours earlier with TNCB on the ears.

c Probability of no difference from group 1 (no treatment).

d Commercially available sunscreen, SPF 6.

e Five equal treatments of 34.7 J/cm^2 were given on consecutive days. Mice were sensitized 3 days after after final exposure and challenged 5 days after sensitization.

Table 3. Effect of Padimate O and Oxybenzone on Suppression of Contact Hypersensitivity by Solar-Simulating Radiation (SSR)

Group	Treatment	Total UV dose[c] (J/cm^2)	Ear swelling[b] ($cm \times 10^{-3}$)		Net ear swelling	Percent suppression	P[d]
			+TNCB	-TNCB			
1	Nil	0.0	44.3 ± 2.3	8.5 ± 1.3	35.8	--	
2	Sunscreen (SS)[e]	0.0	43.2 ± 2.2	8.2 ± 1.2	35.0	--	
3	SSR	36.0	30.1 ± 2.3	8.5 ± 1.5	21.5	40	<.001
4	SSR + SS	36.0	27.7 ± 2.5	8.1 ± 1.6	19.6	45	<.001

a Xenon arc, 1.6 kW (see text).

b Mean (± SD) of 10 mice challenged 24 hours earlier with TNCB on the ears.

c Five equal treatments of 7.2 J/cm^2 given on consecutive days. Following the last exposure, the mice were sensitized 3 days later. Five days after sensitization, the mice were challenged.

d Probability of no difference from group 1 receiving no treatment.

e Commercially available sunscreen with an SPF of 6.

Table 4. Effect of a Schott WG 385 Filter on the Suppression of Contact Hypersensitivity

Group	Treatment	Total[b] UV dose (mJ/cm^2)	Ear swelling[a] ($cm \times 10^{-3}$)		Net ear swelling	Percent suppression	P[c]
			+TNCB	-TNCB			
1	Nil	0.0	33.8 ± 3.49	6.2 ± 1.1	27.5	--	
2	Schott WG385 + SSR	36	37.2 ± 3.19	6.9 ± 1.3	30.3	--	
3	Schott WG385 + SSR & SS[d]	36	35.4 ± 2.6	6.4 ± 1.6	29.0	--	
4	SSR	36	23.1 ± 2.6	6.2 ± 1.2	16.9	39	<.001
5	SSR + SS	36	22.9 ± 3.2	6.1 ± 1.3	16.8	40	<.001

a Mean (± SD) of 10 mice challenged 24 hours earlier with TNCB on the ears.

b Five equal treatments of 7.2 J/cm^2 given on consecutive days. Following the last exposure, the mice were sensitized 3 days later. Five days after sensitization, the mice were challenged.

c Probability of no difference from group 1 receiving no treatment.

d Commercially available sunscreen with an SPF of 6.

prevent systemic immunologic suppression of contact hypersensitivity following exposure with either SSR or FS36 sunlamps. These results differ from those of Morison (1984), who found that pretreatment of C3H mice with PABA sunscreen (SPF-8) only induced 25% suppression of the contact hypersensitivity response following UV-B exposure, which he determined not to be significant. We used different mice, albino hairless, and a sunscreen with an SPF-6 rating. In addition, our total dose of UV was lower than his by a factor of 10. These differences alone could account for the discrepancy between Morison's and our results.

In Morison's study, the feet and nose were neither covered nor treated with sunscreen. Hence, the UV-induced suppression he observed in C3H mice could have been due to irradiated areas not treated with sunscreen. In our experiments, the head and neck were covered with a shield and the back, feet, and tail treated with sunscreen. Thus, our results are difficult to explain by exposure of non-sunscreen treated sites.

If mice are irradiated with polychromatic UV radiation, which was insufficient to elicit an erythema "sunburn" response, then UV-A rather than UV-B might be responsible for the induction of immune suppression observed in our experiments. However, because previous studies have shown that wavelengths shorter than 320 nm are responsible for systemic immunologic suppression (Noonan et al. 1981a; De Fabo and Kripke 1980), other alternatives are considered. The finding that the sunscreen did not abrogate the effects of UV radiation on contact hypersensitivity might be explained by assuming that sunscreen agents act as photosensitizers initiating a photochemical reaction in the skin that could then lead to the suppression of contact hypersensitivity (Gurish et al. 1981). In support of this theory, Hodges et al. (1977) found increased genetic damage when E. coli were irradiated in the presence of PABA. Another explanation is that the amount of energy not absorbed by the sunscreen agent is sufficient to induce the suppression of contact hypersensitivity. The location of the sunscreen in the skin may be very important in this regard. Willis and Kligman (1970) demonstrated that significant photoprotection from PABA persisted despite repeated stripping of the stratum corneum. These results suggest that sunscreens may "pool" beneath the stratus corneum. Recently, De Fabo and Noonan (1983) suggested that a photoreceptor, urocanic acid, resides close to the surface of the skin in the stratum corneum. This receptor mediates the initial event in systemic immunologic suppression by UV radiation. Our results are consistent with their interpretation: for if sunscreens pool beneath the stratum corneum, this would allow exposure of the photoreceptor, which is in the stratum corneum and above the sunscreen, so that subsequent events associated with systemic immunologic suppression could take place.

PABA absorbs radiation between 270 and 320 nm very efficiently, but not wavelengths below 270 nm. The experiment using the FS36 lamps could be explained by radiation exposure below 270 nm because no filter was used to cut out these wavelengths. However, the experiment that used SSR, which emits essentially no irradiation less than 290 nm, cannot be explained as due to UV-C radiation not absorbed by the sunscreen.

Because sunscreens are known to protect against both skin damage and the carcinogenic effects of UV radiation, our results seem to suggest a separation of UV-induced carcinogenic effects from the systemic immunologic effects. Our

results also suggest that if these results can be generalized to humans, the use of sunscreens may not provide the protection needed to abrogate the immunologic effects of UV radiation. Thus, even sunscreen users would be at increased risk for skin cancer, because altered antigen presentation would already have been established and the formation of suppressor T cells would appear earlier in these individuals than in those persons who have never had any sun exposure.

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Sunlight and Malignant Melanoma in Western Australia

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INTRODUCTION

The incidence (frequency of occurrence of new cases per unit of population) of malignant melanoma (MM) of the skin is increasing about 5% per year in most white populations (Muir and Nectoux 1982). Lancaster (1956) was the first to suggest that this cancer may be substantially due to ultraviolet radiation from the sun. He observed a threefold variation in mortality from melanoma between the populations of Queensland in the north of Australia (high mortality) and Tasmania in the south (low mortality). The populations of the other states were distributed between them approximately in relation to the latitudes of their main population centers. Similarly, Lancaster noted that European populations residing in the comparatively sunny climates of Australia, South Africa, and California exhibited higher mortality from MM than in the European countries from which they originated.

A number of observations are apparently inconsistent with a simple causal relationship between sun exposure and MM. Other skin cancers, which are generally believed to be caused mainly by sunlight, are more common in men than women (perhaps because men often work outside), increase exponentially in incidence with age (the pattern expected from lifelong exposure to an agent that can initiate cancer), and occur most commonly on the more exposed head, neck, and hands. In contrast, MM occurs as often in women as in men, shows a relative peak in incidence in middle life, and is most common on the back in men and legs in women (Holman et al. 1980). There are also a number of geographical areas in which the incidence of MM does not increase with increasing proximity to the equator. These areas include the Australian states of Western Australia and Queensland, which cover a wide range of latitudes, and Europe where MM incidence decreases with increasing latitude to about 50°N and then increases with increasing latitude (Armstrong 1984). These geographical inconsistencies, however, may be caused by a failure to adequately consider skin pigmentation and climatic factors that modify exposure and sensitivity to sunlight. More significant is the relationship between the incidence of MM

and occupation and socioeconomic status. Whereas other skin cancers are more common in outdoor than indoor workers, as would be expected if they are caused by exposure to the sun, the opposite is true for MM. Similarly, incidence of other skin cancers tends to increase with decreasing socioeconomic status (perhaps because low status jobs tend more often to be outside) while the opposite is true for MM (Holman et al. 1980).

These observations led to the "intermittent exposure hypothesis" for the relationship of sunlight to MM. Briefly, this hypothesis states that:

- Incidence of MM is determined as much (or more) by the pattern of sun exposure as by the total accumulated "dose" of sun exposure.

- Infrequent (intermittent) exposure of untanned skin to high doses of sunlight is particularly effective in increasing incidence of MM. Thus, incidence rises initially as frequency of exposure increases but may fall as exposure becomes more continuous.

A simple rationalization for this complex, postulated exposure-response pattern is that developing and maintaining a suntan protects one from the carcinogenic effects of continuing sun exposure. With infrequent sun exposure, a tan is not maintained (except in those with high natural skin pigmentation or who tan very easily), and the melanocytes are substantially unprotected from solar UV on each occasion of exposure.

The Western Australian Lions Melanoma Research Project, carried out in Western Australia in 1980 and 1981, was designed to test the intermittent exposure hypothesis.

METHODS OF DATA COLLECTION AND ANALYSIS

The methods have been fully described elsewhere (Holman and Armstrong 1984a). In brief, 511 patients with histologically confirmed MM were studied. They were 76% of a total of 670 cases less than 80 years of age and diagnosed in accessible regions of Western Australia in a period of 675 days beginning January 1, 1980. Clinical details were obtained from the doctors who treated the patients and the histopathological diagnosis was reviewed and confirmed by a panel of pathologists.

Five hundred eleven control subjects, each matched to one of the melanoma patients by age, sex, and area of residence, were also studied. They were selected at random from the Australian Commonwealth Electoral Roll (electoral registration is compulsory in Australia) or, if the MM patient was less than 18 years of age, from the student roll of the area public school. The final series of 511 control subjects was 69% of those approached.

The patients with MM and the control subjects were approached in identical fashion and asked to participate in an interview on "environment, lifestyle, and health," which lasted from one to two hours. The interviews were conducted in the subjects' homes (occasionally workplaces) by trained nurse interviewers who were not told whether the person that they interviewed was a MM patient or a control subject. The interview covered demographic, constitutional and genetic factors, sun exposure, hormone use, diet, and some other variables. Objective measurements were made of skin, eye and hair color,

weight, height, amount of body hair, number of raised moles (pigmented naevi) on the arms and degree of sun damage to the skin on the back of one hand.

It is possible, by comparing the data obtained from the patients with MM (often called "cases") and the control subjects, to estimate the extent to which exposure to specific levels or categories of particular exposure variables increases the incidence of MM above the incidence in some arbitrarily chosen reference group (usually those not exposed or those in the lowest exposure category). The statistic calculated is the incidence rate ratio or relative risk (as estimated by the exposure odds ratio), abbreviated hereafter as RR. Because it is a ratio, values of the RR above 1.0 for a particular category of exposure imply that the incidence of MM is increased in that category in comparison with the incidence in the reference category. RRs were calculated by the methods recommended for matched case-control studies by Breslow and Day (1980). For each, a 95% confidence interval (CI) was also calculated. Given that samples of both MM patients and controls were studied, each of the statistics calculated has sampling variability. The CI is the interval in which it is 95% likely that the true value of the RR for the population as a whole lies.

When interpreting an RR for a particular exposure category, it is necessary to consider the possibility that the observed association is influenced by some "confounding" variable that is related to both the exposure variable for which the RR has been calculated and to MM. For example, people with highly sun-sensitive skins may tend to expose themselves less to the sun than those with not-so-sensitive skins. If sun sensitivity is associated with an increased incidence of MM, this could reduce the strength of any association between sun exposure and MM unless the confounding effects of sun sensitivity are controlled when examining the effects of sun exposure. This control was achieved by use of conditional logistic regression analysis (Breslow and Day 1980) and adjusted RRs were calculated, where relevant, free of the effects of specific confounding variables.

RESULTS

Pigmentary Characteristics and Sensitivity of the Skin to the Sun

The RRs for categories of skin color, hair color, and eye color are summarized in Table 1. The skin color measurement was a reflectance measurement; thus low values represent dark skin. It was made on the skin of the upper inner arm to avoid, as far as possible, pigmentation due to sun exposure.

Incidence of MM increased in ordered categories of each of these variables with increase in the characteristics that are usually thought of as being associated with sun sensitivity. Thus the highest incidence of MM was in those with light skin, red hair, and blue eyes. A "P value" of <0.05 means that the probability that the pattern observed in the RRs was due solely to chance (sampling variability) was less than 5% (i.e., 1 in 20). For skin color and hair color it was very much less than 5%.

Table 1. Associations Between Pigmentary Characteristics and Incidence of Malignant Melanoma in Western Australia, 1980-81

Characteristic	Relative Risk	95% Confidence Interval	
Skin color (reflectance)			
<47%	1.00		
47-55%	1.05	0.69	1.59
56-64%	1.59	0.95	2.66
65%+	3.07*	1.47	6.39
Hair color			
Black or dark brown	1.00		
Light brown	1.45	1.08	1.94
Fair or blond	1.89	1.29	2.77
Red	2.33*	1.26	4.30
Eye color			
Brown	1.00		
Hazel	1.49	1.01	2.18
Green	1.57	0.97	2.53
Grey	1.33	0.71	2.46
Blue	1.61*	1.16	2.21

* P value for trend in each case <0.05.
Source: Holman and Armstrong (1984a)

Sensitivity of the skin to the sun was ascertained by asking two questions:

If your skin was exposed to strong sunlight for the first time in summer for one hour, would you...
(1) Get a severe sunburn with blistering?
(2) Have a painful sunburn for a few days followed by peeling?
(3) Get mildly burnt followed by some degree of tanning?
(4) Go brown without any sunburn?

After repeated and prolonged exposure to sunlight would your skin become...
(1) Very brown and deeply tanned?
(2) Moderately tanned?
(3) Only mildly tanned due to a tendency to peel?
(4) Only freckled or no suntan at all?

Relative risks of MM for these categories of sun sensitivity are shown in Table 2. Both acute and chronic skin response to sunlight were strongly

Table 2. Associations Between Sensitivity of the Skin to Sunlight and Incidence of Malignant Melanoma in Western Australia, 1980-81

Characteristic	Relative Risk	95% Confidence Interval	
Acute skin reaction to sunlight			
No sunburn	1.00		
Mild sunburn	2.63	1.63	4.26
Painful sunburn	2.63	1.62	4.26
Blisters	3.39*	1.90	6.03
Chronic skin reaction to sunlight			
Deep tan	1.00		
Moderate tan	1.45	1.08	1.94
Mild tan	2.27	1.58	3.26
No tan	3.53	1.82	6.84

* P value for trend in each case <0.001.
Source: Holman and Armstrong (1984a)

related to incidence of MM with the highest incidence being in those with greatest sensitivity to the sun.

Of all the pigmentary characteristics and measures of skin response to sunlight, chronic skin response to sunlight was the strongest predictor of the risk of MM. When these variables were included together in a logistic regression model, acute skin response to sunlight and hair color, together with chronic skin response to sunlight, were significantly correlated with incidence of MM. It is at least plausible to suggest that skin response to sunlight is the important predictor of risk of MM and that hair color appeared to be independently predictive only because skin response was measured with some error.

Ethnic Origin

MM is known to be rare in pigmented races (Crombie 1979). Like the association of MM with response of the skin to sunlight in white races, this observation suggests that sunlight may be a cause of MM. In the Western Australian study, subjects were classified by the ethnic origin of their grandparents (if they had two or more grandparents of the same ethnic origin) into one of the following categories: Celtic (Irish, Scottish, or Welsh), English, Australian (mainly Celtic or English, there were no Australian aborigines in the study), Southern European, Northern European, African, or Asian. RRs for MM by ethnic origin are given in Table 3. The RRs have been adjusted for possible confounding effects of age at arrival in Australia

Table 3. Associations Between Ethnic Origin and Incidence of Malignant Melanoma in Western Australia, 1980-81

Ethnic Origin	Relative Risk	95% Confidence Interval	
Celtic	1.18*	0.82	1.70
English	1.03	0.71	1.48
"Australian"	0.97	0.66	1.43
Northern European	0.57	0.31	1.06
Southern European	0.39	0.17	0.89
African or Asian	0.42	0.05	3.34

* RRs are adjusted for age at arrival in Australia and each other ethnic origin category.

Source: Holman and Armstrong (1984a)

(many of the non-Australian subjects were migrants and therefore had a low incidence of melanoma regardless of their ethnic origin; see below) and represent the effects of each ethnic group independently of all the others.

There were few subjects in the study who belonged to ethnic groups (e.g., born in Africa or Asia) that might reasonably have been expected to have had pigmented skin. The lowest risk, however, was in those of Southern European ethnic origin whose skins are generally darker than those of people originating elsewhere in Europe. The pattern is therefore consistent with a protective effect of ethnically determined skin pigmentation against MM.

Birthplace, Age at Arrival, and Duration of Residence in Australia

Most Australians of European origin who were born outside Australia have migrated to Australia from a region of lower exposure to the sun. Thus, if sunlight is a cause of MM, they would be expected to have lower incidence rates of MM than native-born Australians. This has been observed to be the case in descriptive studies (Armstrong et al. 1982). In the 1980-81 case-control study, incidence of melanoma increased with increasing duration of residence in Australia and fell with increasing age at arrival in Australia. Since age at arrival and duration of residence are correlated one with the other, both were included in a logistic regression analysis to see which, if only one, was independently related to incidence of MM. The results of this analysis are shown in Table 4. After adjustment for duration of residence, incidence still fell with increasing age at arrival while adjustment for age at arrival removed the observed effect of duration of residence. Thus, if sun exposure is responsible for the high incidence of MM in native-born Australians relative to that in migrants to Australia, it appears that exposure early in life is necessary to have this effect.

Table 4. Associations Between Incidence of Malignant Melanoma and Age at Arrival in Australia and Duration of Residence in Australia, Each Adjusted for the Effects of the Other, Western Australia, 1980-81

Characteristic	Relative Risk	95% Confidence Interval	
Age at arrival			
Birth	1.00		
0-9 years	0.89	0.44	1.80
10-29 years	0.34	0.16	0.72
30+ years	0.30*	0.08	1.13
Duration of residence			
0-24 years	1.00		
25-39 years	0.80	0.41	1.56
40-59 years	0.93	0.26	3.25
60+ years	1.02	0.20	5.08

* P value for trend <0.001.
Source: Holman and Armstrong (1984b)

Mean Annual Hours of Bright Sunlight

To obtain a measure of the potential for exposure to the sun at all their places of residence, Holman and Armstrong (1984b) calculated the mean annual hours of bright sunlight (as given on climatology maps) averaged over all places of residence (as obtained in a residence history) and weighted by the duration of residence. This measure did not account for the time that each subject spent in the sun. The analysis was restricted to native-born Australians to separate the effects of residential sunlight from those of place of birth. The results are shown in Table 5. Incidence of MM nearly doubled between those with less than 2600 mean annual hours of bright sunlight at places of residence and those with more than 2800 hours. Migrants to Australia had about half the incidence of MM as native-born Australians with less than 2600 annual hours of bright sunlight on average. When mean annual hours of bright sunlight were controlled in a logistic regression analysis, the mean latitude of residence showed no association with risk of melanoma. This suggests that the effect of latitude on incidence of MM can be explained by the effect of sunlight.

Table 5. Association Between Mean Annual Hours of Bright Sunlight at Places of Residence of Native-Born Australians and Incidence of Malignant Melanoma in Western Australia, 1980-81

Mean Annual Hours of Bright Sunlight	Relative Risk	95% Confidence Interval	
Native-born Australians			
<2600 hours	1.00		
2600-2799 hours	1.34	0.96	1.86
2800+ hours	1.92*	1.16	3.18
Migrants (all hours)	0.51	0.35	0.74

* P value for trend 0.003.
Source: Holman and Armstrong (1984b)

Other Objective Measures of Total Accumulated Exposure to the Sun

The degree of damage caused to skin on the back of the hand by exposure to the sun was measured by means of cutaneous microtopography (Holman et al. 1984). A silicone mold is made of the skin markings on the back of the hand, examined under a dissecting microscope, and graded on a scale from 1 to 6, 1 indicating the least solar damage and 6 the most. The degree of skin damage was taken to be an indicator of total accumulated exposure to the sun. A history of past non-melanocytic skin cancer was also obtained. Because non-melanocytic skin cancers are believed to be predominately sun-induced they were considered to indicate individuals who had been heavily exposed to the sun.

The relationships of MM incidence with cutaneous microtopograph grade and past history of skin cancer are shown in Table 6. Incidence of MM increased with increasing severity of solar damage to the skin such that the incidence in those with grade 6 damage was nearly three times that in those with only grades 1-3 damage. Similarly, the incidence of MM was higher in those with a past history of non-melanocytic skin cancer than in those without. Adjustment of the latter association for possible confounding effects of acute and chronic reactions of the skin to sunlight, hair color, and numbers of European, African, and Asian grandparents reduced the RR from 3.71 to 2.87 (CI 1.64-5.04).

Table 6. Associations Between Incidence of Malignant Melanoma and Degree of Sun Damage to the Skin of the Back of the Hand and Past History of Non-Melanocytic Skin Cancer, Western Australia, 1980-81

Characteristic	Relative Risk	95% Confidence Interval	
Sun damage to the skin of the back of the hand (cutaneous microtopography grade)			
Grades 1-3	1.00		
Grade 4	1.64	0.97	2.78
Grade 5	1.76	0.97	3.19
Grade 6	2.68*	1.44	4.98
Past history of non-melanocytic skin cancer			
No	1.00		
Yes	3.71*	2.11	6.57

* P value for trend in each case <0.01
Source: Holman and Armstrong (1984b)

Pattern of Sun Exposure

The evidence on the associations between incidence of MM and average hours of bright sunlight at places of residence, sun-induced skin damage, and past history of non-melanocytic skin cancer strongly supports the role of sun exposure in the causation of the disease. These measures of sun exposure, however, are essentially measures of total accumulated exposure over a lifetime and reveal nothing about the pattern of exposure. A detailed history of sun-exposure habits was taken from subjects in the Western Australian study to provide evidence relevant to the intermittent exposure hypothesis. They were asked to provide estimates of the time spent outdoors in both summer and winter on typical working and non-working days for all periods of employment throughout their working lifetimes (Mondays through Fridays were treated as typical working days for students, housewives, and retired people). Details were also sought regarding specific outdoor pursuits and clothing habits when outdoors. All the RRs presented are adjusted for the potential confounding effects of acute and chronic skin reaction to sunlight, hair color, ethnic origin, and age at arrival in Australia.

Table 7 summarizes results for average estimated outdoor time per week over the lifespan (since leaving school) and the proportion of the total outdoor time that was recreational (a measure of intermittency of the exposure) between 10 and 24 years of age.

Table 7. Associations Between Incidence of Malignant Melanoma and Average Average Estimated Outdoor Time in Summer Over the Lifespan and the Proportion of the Total Outdoor Time During the Summer at Ages 10-24 Years That Was Recreational, Western Australia, 1980-81

Characteristic	Relative Risk	95% Confidence Interval	
Average total outdoor time			
0-10 hours/week	1.00	-	-
11-15 hours/week	0.85	0.55	1.31
16-22 hours/week	0.78	0.52	1.19
23+ hours/week	0.70	0.43	1.12
Proportion (%) of outdoor time that was recreational (Ages 10-24 years)			
0-29	1.00	-	-
30-39	1.14	0.71	1.84
40-59	1.16	0.76	1.77
60+	1.28	0.85	1.94

Source: Holman, Armstrong, and Heenan 1986

The trend in the RRs for total outdoor time showed the anomalous pattern that has been observed in descriptive data: incidence of MM fell rather than rose with increasing time spent out of doors. This trend, however, could have been due to chance as the P value was quite high (0.13). Both of the components of outdoor time (outdoor time at work and time in outdoor recreation) showed similar downward trends (Holman, Armstrong, and Heenan 1986) but as the proportion of the outdoor time that was recreational increased, the incidence of MM tended to rise (Table 7). The P value for this trend, however, was also rather high (0.25). The recreational exposure proportion was examined for the period of life from 10 to 24 years of age because this was the period in which the negative gradient with total outdoor time was most evident.

Because the intermittent exposure hypothesis is thought to relate most strongly to superficial spreading melanoma (SSM) (Holman, Armstrong, and Heenan 1983), the most common of the four histological types of MM, the analyses of Table 7 were also carried out for this particular type of MM. SSM was more strongly related negatively to average total outdoor time per week during the summer (P=0.09) and positively to the proportion of outdoor time that was recreational (P=0.15) than was MM as a whole.

Incidence of SSM was also strongly related to frequency of participation in some but not all outdoor recreations that involve substantial sun exposure (Table 8). It is particularly interesting that a relationship between frequency of sunbathing and incidence of SSM was evident when only SSM of the trunk was analyzed (the trunk is presumably the body site subject to the most

Table 8. Associations Between Frequency of Particular Outdoor Recreations in Summer and Incidence of Superficial Spreading Melanoma in Western Australia, 1980-81

Activity	Relative Risk	95% Confidence Interval	
Boating			
Never	1.00		
Less than once a week	1.06	0.54	2.09
Once or more a week	2.43*	1.10	5.39
Fishing			
Never	1.00		
Less than once a week	1.03	0.63	1.68
Once or more a week	2.72	1.15	6.43
Swimming			
Never	1.00		
Less than once a week	1.30	0.76	2.20
Once or more a week	1.14	0.72	1.82
Sunbathing (Ages 15-24 years only)			
Never	1.00		
Less than once a week	1.26	0.78	2.05
Once or more a week	1.32	0.80	2.17
(Ages 15-24 years and SSM of trunk only)			
Never	1.00		
Less than once a week	1.20	0.51	2.81
Once or more a week	2.55*	1.05	6.19

* P value for trend in each case <0.05.
\+ Detailed questions asked only for this period of life.
Source: Holman, Armstrong, and Heenan (1986)

intense intermittent exposure with sunbathing). MM other than SSM did not show strong associations with any of these recreational exposures.

Clothing may modify the relationship between sun exposure and incidence of MM, as Table 9 shows. Incidence of both melanoma and SSM is examined in relation to the person's clothing habit during outdoor work in summer at the primary site of the MM. The incidence of both was substantially higher in those who sometimes or usually exposed the primary site than in those who usually kept it covered. For all MM the RR was highest in those who sometimes exposed the site, an observation that is consistent with the intermittent exposure hypothesis. This was not the case, however, for SSM. SSM of the trunk in women was very strongly related to the type of bathing suit that had been worn in summer between 15 and 24 years of age. Relative to an RR of 1.00 in those who had worn a one-piece suit with a high back-line, the RR was 4.04 (CI 0.65-25.2) in those who had worn a one-piece suit with a low back-line, and 13.0 (CI 1.95-83.9; P value for trend 0.005) in those who had worn a two-piece suit or no bathing suit at all.

Table 9. Associations Between Clothing Habit at the Primary Site of Malignant Melanoma During Outdoor Work in Summer and Incidence of Malignant Melanoma in Western Australia, 1980-81

Clothing Habit	Relative Risk	95% Confidence Interval	
All Melanomas			
Usually covered	1.00		
Sometimes exposed	2.49	1.58	3.94
Usually exposed	2.08*	1.27	3.40
Superficial Spreading Melanoma Only			
Usually covered	1.00		
Sometimes exposed	2.16	1.14	4.10
Usually exposed	2.43*	1.18	4.97

* P value for trend in each case <0.01.
Source: Holman, Armstrong, and Heenan (1986)

DISCUSSION

The reduction of MM incidence by high natural skin pigmentation and its increased incidence in skin that is highly sensitive to the sun are well known. These observations have been confirmed by the Western Australian study. Indirectly, they implicate sunlight in the etiology of MM. This study has also provided evidence that incidence of MM increases with total accumulated exposure to the sun. Incidence was lower in migrants to Australia than in native-born Australians, was positively associated with mean annual hours

of bright sunlight averaged over all places of residence, and increased in those with sun-damaged skin and a past history of non-melanocytic skin cancer (generally accepted as being due to exposure to the sun).

Superimposed on this background, there was some evidence that intermittency of sun exposure may be particularly important in causing MM. Incidence rose slightly with increases in the proportion of total exposure that was recreational, but certain outdoor recreations, often involving intense sun exposure, appeared to be particularly strongly related to it, for example, boating, fishing, and sunbathing for MM of the trunk. When clothing habits were taken into account, there was strong association between MM and unclothed exposure of the primary site of the tumor while at work. Thus the anomalous relationship between MM incidence and outdoor work overall may be due, in part at least, to a tendency of those who work outdoors to be more careful in protecting themselves from the sun than those who expose themselves only recreationally.

If the intermittent exposure hypothesis for the relationship of MM to sunlight is correct, it has important implications for prevention. Figure 1 shows a concept of the exposure-response relationship for SSM and sunlight under the intermittent exposure hypothesis. While this concept applies to lesions that have their origin in SSM, as they are probably the majority of MM, it may be considered to apply to MM as a whole. On average (curve C) incidence of SSM first rises as frequency of exposure increases and then falls as some critical exposure frequency is passed. Thus, on average, whether or not reduction in sun exposure will be a "good thing" (in terms of prevention of MM) will depend on where a population lies on this exposure-response curve. The position will probably depend both on the genetic composition of the population and the available sunlight (expected absorbed dose per unit of time of exposure to the sun).

At the individual level, the shape of the exposure response curve will probably be determined by the pigmentary response of the skin to sunlight. Those with a poor pigment response (curve B) may experience progressively increasing incidence of SSM, whatever the frequency (and dose) of sun exposure, because a protective tan is never obtained. Others who tan readily (curve A) may show little rise in incidence of SSM at all with increasing sun exposure before incidence falls back to background levels with further sun exposure. While the former may be best advised to protect themselves against the sun at all times; the latter, if exposed more than minimally, should perhaps continue sun exposure to ensure that they stay as far as possible to the right of the peak. Until these concepts are clarified or discarded, caution should be exercised in making recommendations about sun exposure--at least with reference to prevention of MM.

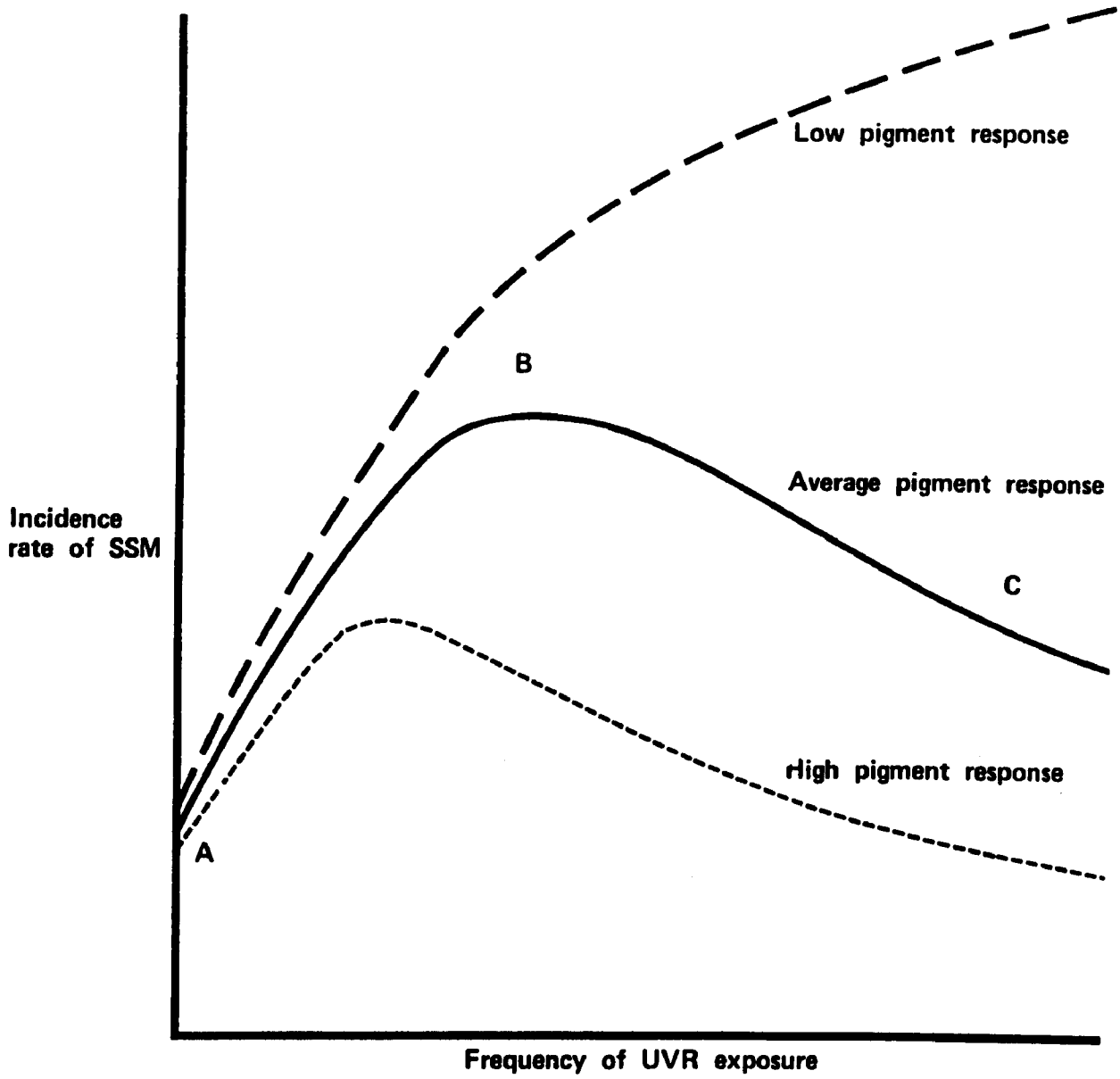

Figure 1. A Concept of the Exposure Response Relationship for Superficial Spreading Melanoma And Sunlight

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Radiometry of Solar UV-B

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ABSTRACT

Measurements have been conducted using several detectors: polysulfone film, a Robertson-Berger (R-B) meter, an EG&G Spectroradiometer, and an IL phototherapy radiometer. The purpose of the study was to assess the solar UVB in Kuwait and to arrive at correlations among these detectors, which are widely used internationally. These relationships have not previously appeared in literature.

Polysulfone-based measurements were carried out over a two-year period at solar maximum, as a function of the time of day and the angle of the detector with the horizontal. The R-B meter measurements were conducted over one year with a half-hourly record. Spectroradiometric measurements were conducted over a two-month period and simultaneously with the other two in order to establish correlations. The International Light instrument was operated over a two-month period simultaneously with the polysulfone measurements.

We find that although none of the integrating detectors adequately represents the sensitivity of human skin (erythema effectiveness) to ultraviolet B radiation (UV-B), they can provide an estimate of the incident UV-B. Based on the polysulfone measurements, we have arrived at an empirical expression that predicts (within 10%) the polysulfone reading for any half-hour interval of the year. We find further that the R-B meter measurements are very strongly affected by high temperatures (>45°C). The need therefore remains for a simple recording detector that is not affected by environmental conditions.

INTRODUCTION

In looking through the literature for solar middle UV-B measurements, as related to the effect of UV-B on human skin (Ambach and Rehwald 1983; Bener 1969; Berger and Urbach 1982; Blumthaler et al. 1985a, b; Diffey, Larko, and

Swanbeck 1982; Kollias and Baqer 1984; Mosely, Davison, and Mackie 1983; Rosenthal, Safran, and Taylor 1985; Qayyum and Davis 1984; Young et al. 1982), one finds that the following detectors have been used predominately: R-B meter (sunburning meter), Eppley Labs UV-B radiometer, International Light phototherapy radiometer, and polysulfone film.

Of these, the Eppley Labs radiometer was not available in the market when our study started in the fall of 1983. There is a wealth of data on the R-B instrument and a rapidly accumulating set of data on the polysulfone film detectors because they are inexpensive and easy to use. The International Light instrument is available at many phototherapy centers, and therefore it is tempting to use it outdoors to estimate the dose that the patients receive after treatment. Spectroradiometric measurements are difficult to find as they require expensive instruments and competent operators.

A.E.S. Green and his group (1976-82) have carried out a number of investigations both theoretically and experimentally to characterize the solar UV-B flux at the earth's surface.

The major aim of this study was to determine the correlation among the various integrating detectors and then how they relate to the sensitivity of human skin. By setting all measurements on a common denominator we can make use of assessments made in various parts of the world with different detectors.

MATERIALS AND METHODS

Polysulfone Measurements

The films (40 μm in thickness and 12 x 12 inches in area) were cut and mounted in 35 mm slide mounts and assessed before and after exposure by measuring their absorbance at 330 nm (Davis and Gardiner 1982). The change in absorbance due to the exposure to solar UV-B was converted to an equivalent 305 nm UV-B dose in J/cm^2. The films showed good stability with temperature and humidity; however, their absorbance post exposure changed rather rapidly with time, especially over the first two days of post irradiation. As a consequence, all films were assessed as quickly as possible after being exposed to solar UV-B. Measurements were carried out (8/83-10/85) at solar maximum, as a function of the time of day, and as a function of the detector orientation with the horizontal.

Robertson-Berger Meter (Sunburning Meter) Measurements

This is an integrating detector that gives a half-hourly printout of the measured UV-B radiant energy in counts, where 440 counts equal one "sunburning unit." Measurements were conducted from 11/84-11/85 at a location very near to the polysulfone detector holders. This detector had a very large temperature coefficient when the full range of temperatures attainable in Kuwait was realized (14% per 10°C). The dry bulb temperature in an exposed thermometer ranges from 10° to 75°C. This error becomes very noticeable in the summer months when the apparent maximum radiation recorded always occurred before solar maximum. Because we had no way of knowing the internal temperature of the detector, we decided not to correct the data and not to use them other than to obtain an estimate of the way the UV-B was varying over the day and the months of the year.

EG&G Spectroradiometer (Model 550) Measurements

This instrument was interfaced to a HP-85 microcomputer, and thus a spectral scan could be conducted in less than 30 seconds and recorded for further analysis. Measurements were carried out at solar noon from 3/85 to 5/85. We could not continue beyond May as the ambient temperature was above 40°C in the shade and the instruments started overheating in the sun. Measurements were carried out at the same location as the polysulfone and the R-B meter and at the same time.

International Light 442 Radiometer With a SEE1240 Detector Measurements

The sensitivity of this instrument did not prove to be adequate, and we therefore installed an analogue output that could be read on a portable voltmeter, Fluke 77. Measurements were carried out simultaneously with polysulfone films in March and April 1986.

RESULTS AND DISCUSSION

Although it has a diffuser at its input, the spectroradiometer has a receiving angle of ±5°. This means that our measurements record only the direct component of the solar radiation. The principal use of these measurements has been to correlate these with the ones conducted by the polysulfone and by the R-B detectors. The correlation among the three instruments can be seen in Figure 1. The readings seem to relate well to each other for clear days, i.e., when the readings are at their maximum values, and they do not appear to relate as well when the measurements were attenuated either due to clouds or rising dust. We selected the days that were clear, or reasonably clear to establish a relationship between the integrated value of irradiance over the wavelengths 280-325 nm and over 1/2 hour with the values obtained from the other detectors. (See Table 1.)

Table 1. Spectroradiometric, Polysulfone, and Robertson-Berger Meter Readings for Selected "Clear" Days

Date <d-mo-yr>	Irrad. 1/2 hr. <J/cm^2>	Polysulfone <J/cm^2>	R-B meter SBU
12.3.85	0.1488	0.3010	0.9540
26.3.85	0.2435	0.3710	0.3450
30.3.85	0.2320	0.3280	0.2600
01.4.85	0.1733	0.3380	0.8920
03.4.85	0.1506	0.3130	1.0950
07.4.85	0.2424	0.3940	1.4000
09.4.85	0.2671	0.4180	1.4600
13.4.85	0.2300	0.4320	1.3650
15.4.85	0.1893	0.3940	1.2700
29.4.85	0.1866	0.3890	1.3050

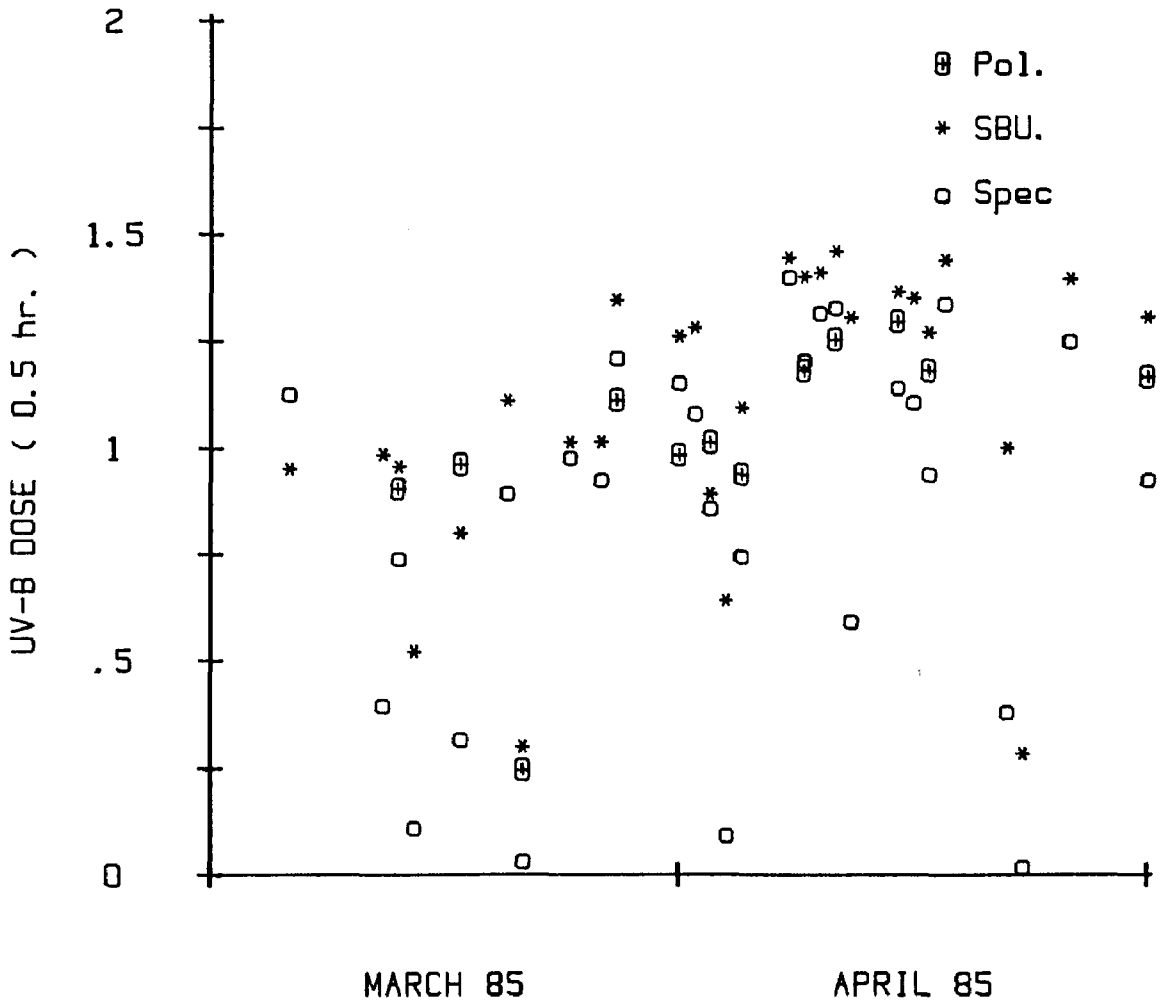

Figure 1. The half-hour integrated readings for the polysulfone film detectors, the R-B meter, and the spectroradiometer, for the months of March and April 1985. The polysulfone readings have been multiplied by 3 and the spectroradiometer by 50 so that all the points fall within one range.

The polysulfone reading is in J/cm^2 of equivalent monochromatic 305 nm radiation and for this reason the first two columns in Table 1 are different. The irradiance was first integrated over the UV-B wavelengths and was then converted to J/cm^2 by multiplying the final result by 1,800, i.e., the number of seconds in 1/2 hour. The R-B meter was integrating over 1/2 hour also.

The relationship between total irradiance for 1/2 hour and the polysulfone detector readings was established by assuming a linear relationship between the two. The equation $y = A + B x$ was fitted with the value of coefficient $A = 0$. The value of the slope (B) was calculated from the line joining the centroid of all the points to the origin (x = total irradiance per 1/2 hour, y = polysulfone reading). The centroid was found to be (0.206, 0.368). Thus the value of coefficient B was calculated to be $B = y/x = 1.78$. Hence the relation between total irradiance per 1/2 hour and the polysulfone readings can be represented by: Polys. reading (1/2 hour) = 1.78 * Total irrad.(1/2 hour) where both readings are given in J/cm^2.

The relationship between total irradiance and the R-B meter was also found in the same manner as above. The centroid was located at (0.206, 1.235), yielding a slope of B = 5.98. Thus:

R-B meter (SBU) = 5.98 * total Irrad.(1/2 hour) (J/cm^2).

For the International Light instrument we obtained the following relationship:

0.145 * polysulfone (J/cm^2) = Intern. Light (J/cm^2).

Both detectors overestimate the actual UV-B; although both the polysulfone and the R-B meter detectors show a cosine response with the angle of incidence of the radiation on the detector surface, they do not discriminate between the direct and diffuse components. On the other hand, the spectroradiometer gives readings that are closely associated with the direct component. Checking our results on the dependence of the solar UV-B on the detector orientation with the horizontal, we find that the diffuse component (90° reading) is approximately 50% of the total; therefore the polysulfone correlation with the spectroradiometer is approximately correct. The R-B meter correlation is high; however, there is no effort made by the producers of this instrument to correlate the readings to the solar insolation; their interest is to correlate it to the Minimum Erythema Dose.

Figure 2 presents the polysulfone measurements at solar maximum carried out over a period of two and one-half years. Given this large volume of data, it seemed natural to attempt to model the solar UV-B to predict, based on a model, the solar UV-B insolation. When we considered Figure 2 it became clear that the solar UV-B insolations follow a sinusoidal variation with time of the year. The question then was whether we fit the average of each month or the outline of the curve? We decided to fit the outline of the curve from the top, i.e., we selected the points that represent readings taken on "clear days." The judgement "clear day" was made on our own observations together with those of the Meteorological Department of the Civil Aviation Directorate of the State of Kuwait. We then selected the mathematical expression that we felt best represented the results and chose, as a start, to fit the daily totals of solar UV-B. The following expression was chosen:

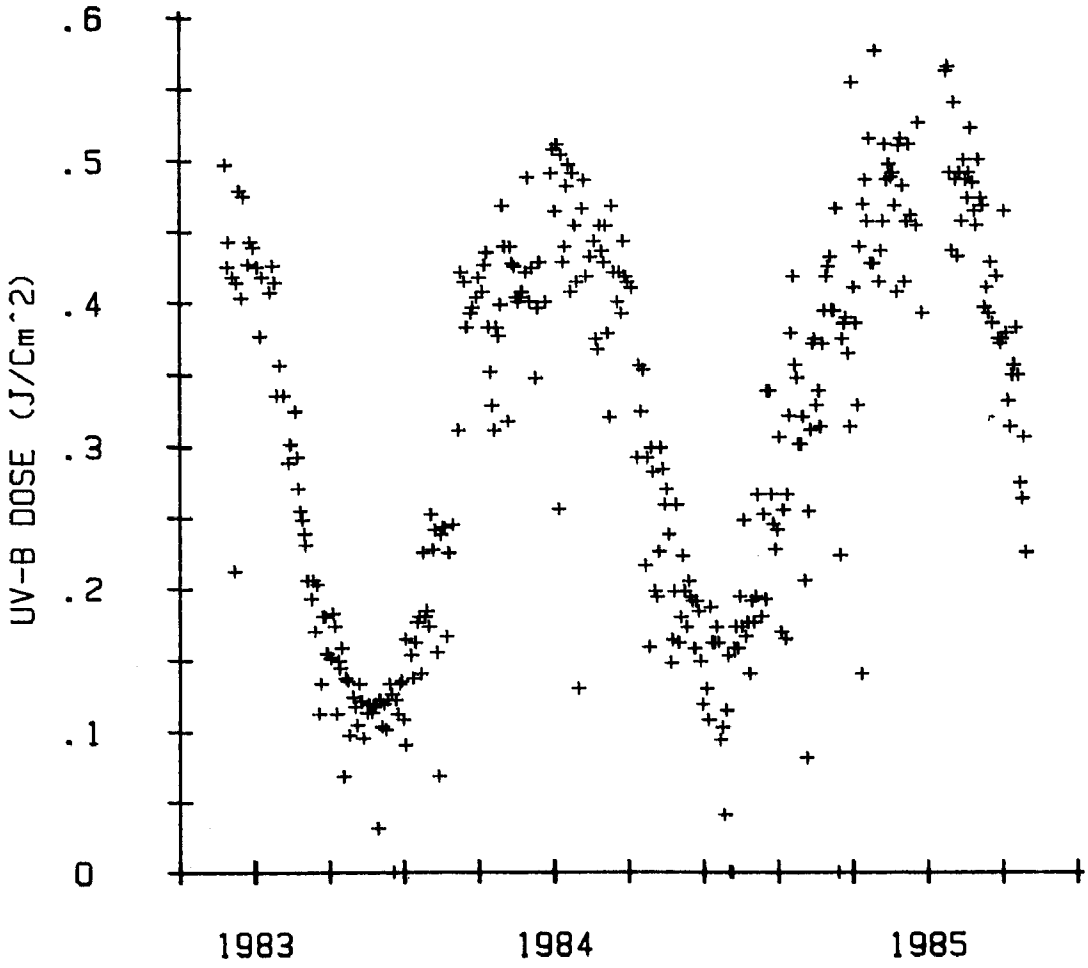

Figure 2. UV-B Insolation Measured From 11:30 to 12:00 Noon With Polysulfone Film Versus the Time of Year.

$$\text{Total UV-B (per day)} = A + B \sin(t),$$

where t represents an angle that corresponds to the day of the year; A is the DC level, i.e., the average over the year, total daily UV-B insolation; and B is the AC amplitude, i.e., the amplitude of variation of the solar insolation above and below the average. It is interesting to note at this point that A multiplied by 365 gives us the total UV-B insolation for the year. The angle t was chosen so that 0° corresponded to March 21 (the spring equinox), 90° to June 21, 180° to September 21 and 270° to December 21. The angle was calculated using the following expression:

$$t = 30\,(M-1) + D - 81,$$

where M is the month of the year (January = 1, February = 2, etc.), and D is the day of the month.

A graph of the total daily UV-B dose versus sin(t) shows how well the two variables are correlated (see Figure 3). A good straight line fit to the experimental points follows. The regression analysis yields $R^2 = 0.983$ (correlation coefficient R = 0.991). The equation predicting the amount of daily UV-B insolation then becomes:

$$\text{Total UV-B (per day)} = 3.71 + 2.42 \sin[2{=}30(M-1){+}D{=}81],$$

where M is the month of the year and D the day of the month.

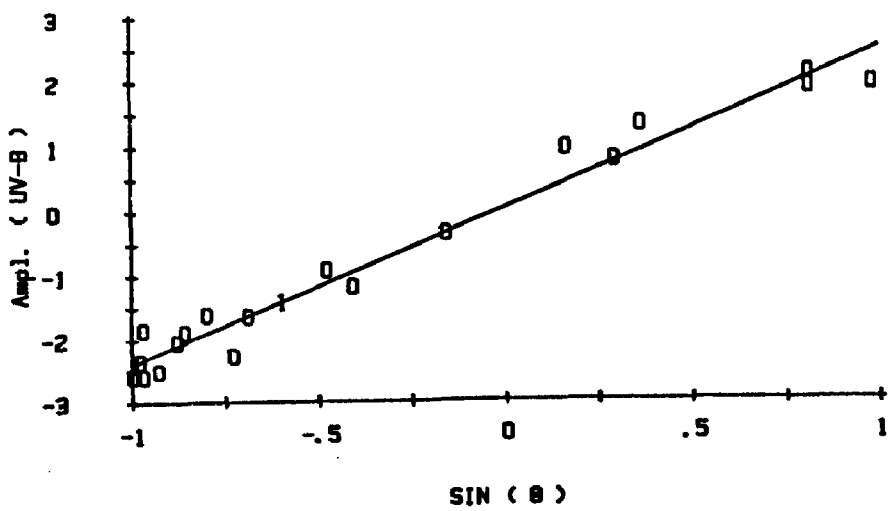

Figure 3. Total Daily UV-B Minus the Yearly Average Versus Sin(t), Where t=0 for 3/21, t=90 for 6/21, etc.

Because the derived relation worked so well, we proceeded to fit the UV-B insolation as a function of the time of day (i.e., the diurnal variation). To proceed, we needed to describe mathematically the daylight hours, i.e., the time interval (sunset-sunrise). This was calculated from data obtained from

the Meteorological Department and was plotted against sin(t) where t is the same angle as in the previous discussion. It was noticed that the midpoint between sunrise and sunset <i.e., midday> on the average falls at 11.714 hour Kuwait local time (11 hours, 42 minutes, 50.4 seconds). The variations in the midpoint values are between 11.52 hour and 11.96 hour (Kuwait time), with standard deviation = 0.191.

On the other hand, the maxima of the daily UV-B curves have an average value of 11.81 hour (11 hours, 48 minutes, 28.8 seconds Kuwait time), the standard deviation being 0.462. It is interesting to note that both the midpoint of the sunrise-sunset interval and the UV-B maxima on the diurnal measurements are behind 12 noon. The first falls 0.286 hour (17 minutes, 9.6 seconds) before noon and the second 0.192 hour (11 minutes, 31.2 seconds) before noon. For calculating the angle t, we decided to take 11.75 hours (11 hours, 45 minutes Kuwait local time) as the solar noon; that is to say that t = 90° at 11.75 hours. This result is not surprising: Kuwait city lies at longitude 48°, i.e., 3° short of the hour meridians that occur every 15°. We therefore should expect solar noon to occur 0.2 hours before Kuwait local time, that is 11 hours 48 minutes. This result coincides with the one obtained from the diurnal measurements.

To fit the polysulfone-based UV-B measurements every 1/2 hour, we selected the measurements made on reasonably clear days. These are like the curves on Figure 4. We determined that the best mathematical expression to give a reasonable fit to the data over the data collecting period was:

$$\text{UV-B dose (1/2 hour)} = C + D \sin^2 (q),$$

where C and D are arbitrary constants and q is an angle corresponding to the time of day expressed as the ratio of the interval from sunrise to the hour in question divided by the sunrise sunset interval times 180°.

The coefficient of determination R^2, for most of the readings, was better than 0.9. The average value of R = 0.91 for the days considered in this sample leads to a correlation coefficient of 0.95 with a standard deviation of 0.094. The coefficient C should be equal to zero and the value calculated is -0.006 with a standard deviation of 0.0008, which is very small compared to the UV-B doses. The value of coefficient D depends on the time of the year and it should be equal to the value of maximum UV-B half-hourly dose for the day.

We thus arrived at the following expression for the half-hourly dose of solar UV-B:

$$\text{UV-B (per 1/2 hour)} = (F * \sin^2[\{180/G\}*\{h+m/60\}-\{2124/G-90\}]) - 0.0063,$$

where

F = 0.3346 + 0.1898 *sin[30 *(M-1) + d-81];
G = 12.17 + 1.81 * sin[30*(M-1) + d-81];
h = hours (Kuwait local time);
m = minutes (Kuwait local time);
M = month of the year (Jan.=1, Feb.=2 etc.);
d = day of the month (1,2,3, etc.).

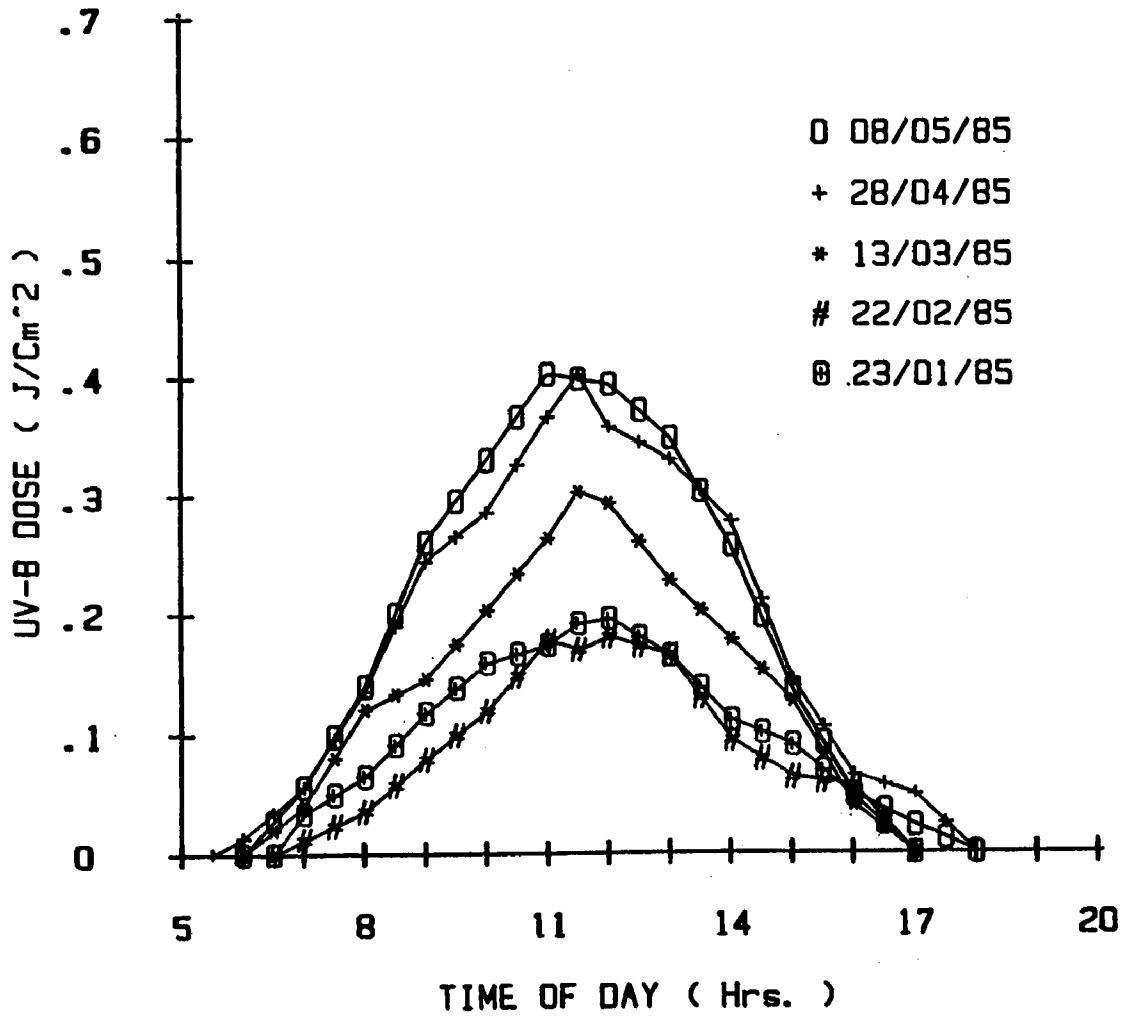

Figure 4. The Diurnal Variation of the Solar UV-B as Measured by the Polysulfone Films

Table 2. Comparison Between Calculated and Measured UV-B Insolation

Date day-mo-yr	Time hr-min	Measured UV-B <J/cm^2>	Calculated UV-B <J/cm^2>
26.10.83	08.45	0.0963	0.093
10.15	0.191	0.183	
12.15	0.248	0.218	
15.15	0.045	0.064	
16.45	0.00	0.0003	
05.01.84	08.15	0.020	0.030
10.15	0.085	0.115	
12.15	0.109	0.141	
15.15	0.023	0.030	
16.45	0.00	0.00	
16.05.84	07.15	0.081	0.120
09.45	0.327	0.385	
11.45	0.439	0.482	
15.15	0.173	0.228	
17.15	0.020	0.036	
21.11.84	07.45	0.023	0.018
10.15	0.144	0.134	
11.45	0.148	0.165	
14.15	0.092	0.088	
16.15	0.00	0.003	
13.03.85	08.15	0.120	0.104
10.15	0.202	0.253	
12.15	0.292	0.292	
15.15	0.126	0.104	
16.45	0.020	0.012	

For any particular month, day, hour, or minute, the variables F and G are first calculated and their values are then introduced in the UV-B equation. The above equation is not as complicated as it seems. It simply represents the product of the equations mentioned in the previous discussion, where the term F gives the variation of the UV-B with the time of year, and G describes the variation of the sunrise-sunset time interval with the time of year. On Table 2 we present a comparison of the calculated versus the measured values of solar UV-B at 1/2-hour intervals. We have tested the validity of the above derived expression by calculating the deviation of the observed from the calculated values for two years. From this calcuation we obtained a curve identical to the one for the variations of the ozone layer with the time of year for our latitude.

FINDINGS

Spectral Sensitivities of Detectors

The relative spectral sensitivities of all the detectors considered in this study are presented in Figure 5 (Davis, Deane, and Diffey 1976; Berger and Urbach 1982). It can be noticed that they are all different from that of human skin--at least as far as erythema induction is concerned. Furthermore, because of the shape of the curves and the fact that the spectral composition of the solar UV-B varies with the time of year, the relationships we developed for interrelating the measurements of different detectors are fairly precise only for the months during which we measured them and for clear weather. We have good reasons to expect that the atmospheric conditions at a given time will cause unequal attenuation of the various UVB spectral components (Bener 1969).

Amount of Solar UV-B in Kuwait

Based on our measurements, an empirical equation has been developed that can be used to predict fairly accurately the amount of solar UV-B in Kuwait, for any day of the year and for any time during the specified day based on the polysulfone measurements. Thus, the answer that we obtain is the reading that we would make if we exposed a polysulfone film for half an hour to the sun on a clear day. Corrections for clouds, humidity, dust, etc. have not been developed as yet.

Relationship of Detector Readings

A relationship has been established between the polysulfone readings and the R-B readings by correlating both of them to spectroradiometric readings. The relationship is: 3.36 x (polysulfone, $J/cm^2/0.5$ hr.) = 1 SBU.

Furthermore the relationship between each of the polysulfone and R-B meter and the total solar irradiance for 1/2 hour has also been established:

- Polys. reading (1/2 hour) = 1.78 x Total irrad. (1/2 hour)
- R-B reading (SBU) = 5.98 x Total Irrad. (1/2 hour) (J/cm^2).

Annual UV-B Insulation

The annual total UV-B insolation has been measured to be $1/42 \times 10^3$ J/cm^2 or 4.107×10^3 SBU.

Using these, we can estimate the conversion factor between polysulfone and SBUs. By dividing, we obtain a factor of 2.9, which is close to the above given value. Scotto, Rears, and Fraumenti (1982) have proposed the following equation for estimating the annual solar UV-B insolation in SBUs as a function of latitude and altitude:

$$\text{UV-B insolation (SBU)} = (1/440) \times [1{,}500{,}000 - 50{,}000 \times (L - 37.9) + 105 \times (A - 1{,}5000)] \times (\text{Cnts})$$

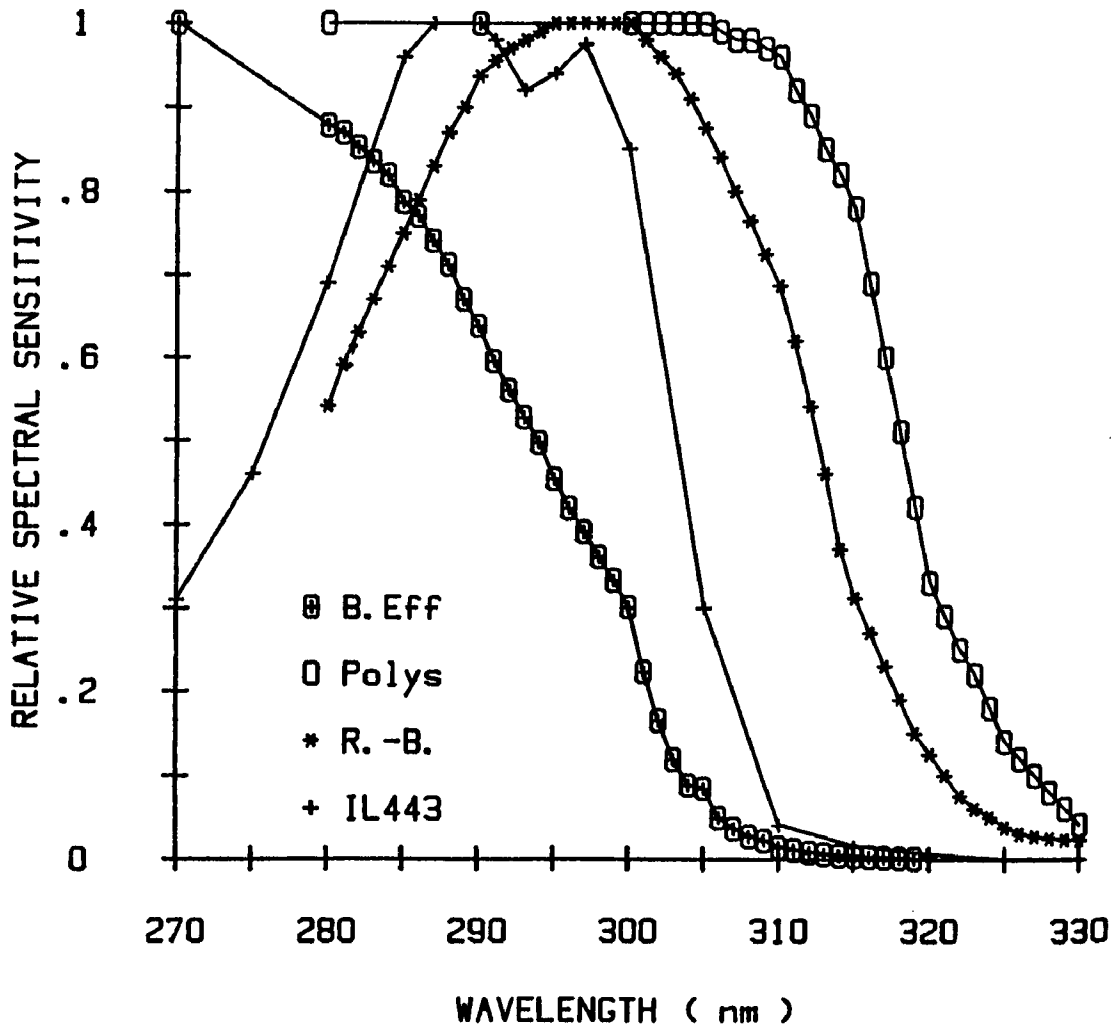

Figure 5. Spectral Sensitivity of Human Skin, Polysulfone Film, R-B Meter, I-L Meter (all curves are normalized to 1)

This equation gives for Kuwait (29.5° latitude): 4.01×10^3 SBUs which is quite close to the measured value.

Robertson-Berger Meter Measurement Adjustment

The R-B meter was found to have a very large temperature coefficient and therefore a very large correction has been made on the values obtained. Berger and Urbach (1982) have reported R-B meter based data from Tallahassee, Florida, and El Paso, Texas, which have latitudes very close to that of Kuwait. The above equation (Scotto 1982) thus gives the following values:

- ELP (calc) = 4.02×10^3 SBU
- KWT (calc) = 4.01×10^3 SBU
- ELP <meas> = 4.889×10^3 SBU
- KWT <meas> = 4.107×10^3 SBU.

While the calculated values for the two cities are essentially identical, we find a large difference in the measured values for ELP and KWT. If we correct the KWT readings for temperature, normalizing to 43.5°C, we obtain KWT (temp. adjusted) = 5.18×10^3 SBU, which is much closer to the ELP value considering the rough correction. It is thus our contention that the international data reported so far in climates where the maximum reading of an exposed dry bulb thermometer reaches over 50°C are in significant error. The R-B meter when used in warm climates needs to be modified to account for temperature or not be used. The number of counts is smaller at elevated temperatures than at normal temperatures.

CONCLUSION

In closing we summarize a useful relation. We have found that there exists a simple relationship between the total daily UV-B measures and the amount measured between 11:30 and 12:00 noon. For polysulfone readings, if we multiply the 11:30 to 12:00 reading by a factor of 10, we obtain a close approximation to the daily dose. The maximum deviation is in the months of June and July when the factor should be 12. For the R-B meter the relationship is similar. For March 12 to September 21 the factor is 12 and for September 21 to March 21 the factor should be 10. The answer is always within 10%. It is thus possible, if one is short handed and is only interested in rough estimates, to measure of the solar UV-B insolation from 11:30 to 12:00 and then to calculate the daily total.

ACKNOWLEDGMENT

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The Role of Native Pigment in Providing Protection Against UV-B Damage in Humans

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ABSTRACT

This paper assesses the average amount of pigment in a sample of the population of Kuwait and compares it with the sensitivity of the population to artificially produced UV-B, as well as monochromatic bands of UV-B, to arrive at an estimate of the protection afforded by the native pigment. The pigment level of the population sampled was 2.18 on a scale from 0 to 9, while the level in an equivalent sample of northern Europeans would be 0.6-0.8. In these measurements we estimate the pigment level (melanin concentration) assuming it resides in the epidermis. This implies that the concentration of melanin in the local population is on the order of 3.5 times larger, while the amount of light that enters the dermis after the above absorption is approximately 20 times smaller. The correlation found between the pigment level and the minimum dose for a UVB-induced erythema was very weak, with an average value less than one half the value of a northern European sample. The responses to monochromatic bands of UV-B indicate that the action spectrum of our population is different from that reported by WHO (1982) for a standard population sample, especially at 295 nm.

We conclude that the pigment plays two roles in photoprotection. First, it absorbs light, and the suppression of the erythema effectiveness of the radiation is proportional to the absorbance. Second, it modulates the amount of UV-B that is delivered to the dermis and it appears that carcinogenesis is proportional to this intensity.

INTRODUCTION

Numerous researchers have concluded that people who are more pigmented are less susceptible to UV-B (Urbach 1982; Pathak and Fitzpatrick 1974; Hawk and Parrish 1982). This implies that pigmented people are at a lower risk of developing an erythema reaction and are also at a lower risk for tumor production. Human skin has been classified in six types according to the way

it responds to sunlight (Pathak, Fitzpatrick, and Parrish 1982), i.e., always burn easily-tan little or none, usually burn easily-tan minimally, burn moderately and average tan. Such classification is based on clinical history and no mention is made of native pigment. We are looking for an objective criterion that would allow assessment of the risk factor. Native pigment is an obvious candidate.

Amblard et al. (1982) show that native pigment and eye color exhibit a good correlation with the erythema threshold level (minimal erythema dose, MED). A recent study (Shono et al. 1985) suggests that there is a strong correlation between native pigment and erythema reaction with a small statistical sample.

In Kuwait we find the native pigment to be a weak indicator of a person's anticipated response to UV-B. That is to say, given two persons of equal pigment level we can only predict their MED within a factor of two of the measured value--not a precise estimate. Native pigment is a parameter that is perceived by the eye, i.e., using visible radiation. In general, it is not a good practice to deduce the absorbance of any material in the ultraviolet simply by recording its absorbance in the visible, unless, of course, one has identified the compound by its visible spectrum. The major absorber in the skin is melanin, which resides mainly in the epidermis in melanocytes and keratinocytes. It absorbs strongly in the visible and even more strongly in the ultraviolet (UV). The absorption by intact epidermis (Kaidbey et al. 1979) shows a linear relation with wavelength down to about 320 nm; for wavelengths shorter than 320 nm the absorbance increases rapidly. Over the UV-B range the absorbance by the epidermis shows an increase by a factor of two (approximately).

A method has been developed for assessing the melanin concentration in human skin (Kollias and Baqer 1985; 1986). We have used this method to assess the pigment level in a small sample of the population of Kuwait. We then tested the sensitivity of local skin to polychromatic and monochromatic UV-B radiation. Using the statistics available through the Kuwait Cancer Registry, we assessed the UV-B risk and the role that native pigment plays in the erythemogenesis and carcinogenesis.

MATERIALS AND METHODS

Measurement of the Pigment Level of a Small Sample of the Population of Kuwait

Over the last two years, we have carried out measurements of skin pigment on 314 volunteers. The measurement was non-invasive and only took 1.5 minutes to complete. The probe that comes in contact with the skin was temperature regulated to cause a minimum of discomfort to the individual volunteer; over 250 of these were patients. The volunteers came from all walks of life and were randomly selected. The measurements on the patients were always taken on uninvolved areas of the skin. We did make measurements on involved areas but those were excluded from this study. We made certain that none of the volunteers were under any medication which could possibly have an effect on their pigmentation. The healthy volunteers were the doctors, nurses, technicians, and personnel of the Skin Department of Al-Sabah Hospital as well as some students from Kuwait University. All volunteers were informed of the nature of the experiment and their consent was obtained.

Measurement of the Minimum Erythema Dose to Artificially Produced UV-B Radiation of Human Skin

These measurements were conducted on 26 psoriatic patients over the course of one year. These patients were about to begin treatment with UV-B radiation, which constitutes a very effective line of treatment for psoriasis. Each patient was phototested to determine the minimum UV-B dose necessary to elicit an erythema reaction. Before any radiation was applied, we made sure that all the parameters were recorded and pigment index was measured. The pigment level was evaluated on an area of normal skin. The information obtained in these measurements was of interest to the physician in prescribing the indicated dose of UV-B for the treatment.

The patients were given doses of 50, 100, 150, and 200 mJ/cm^2 on four areas of the back that were 2.5 cm in diameter. Patients with very light complexion were initially given doses of 20, 40, 60, and 80 mJ/cm^2. The irradiated sites were observed 24 hours after irradiation and the minimum dose required to elicit an erythema reaction was recorded. If no reaction occurred, the test was repeated with four higher doses. If, on the other hand, all areas showed erythema, then the test was repeated with four lower doses. A Waldman Model 80001K upright UVB-UVA unit was used for the irradiations. The patient received radiation in a standing position at a power of 0.4 mW/cm^2 so that the desired doses could be delivered within a few minutes at minimum discomfort to the patient. During the phototest the patients' face and body were covered by a protective robe which allowed only the four areas on the back of the patient to be irradiated. When the dose level was reached for the first areas, the cubicle door was opened and that area was covered. This procedure was repeated until all spots were covered. The UV-B producing lamps in the apparatus were Sylvania 75/85W/UV21 fluorescent lamps.

All the tests and measurements carried out in this section were supervised by Dr. V. Heigy of the Department of Dermatology of Al-Sabah Hospital. All patients were informed of the nature of the tests and the reasons for them; their consent was obtained before the measurements were made.

Measurement of the Erythema Effectiveness of Three Selected Wavelengths of UV-B

In this series of measurements we used 16 healthy volunteers who had skin types common to the area of Kuwait. The wavelengths used were 295, 305, and 315 ±5 nm. The irradiation sites were on the upper back of each individual and we made sure that we stayed away from the middle of the back since the thickness of tissue under the skin as well as the blood supply differs from the rest of the back. The volunteers were in a sitting position during the test. At each wavelength six spots were irradiated starting with a dose that was considered to be suberythemogenic, progressing to higher doses at 20% increments. Thus, the three wavelength tests generated three rows of spots 6 mm in diameter on the back of each volunteer. We further made sure that none of the subjects had exposed their backs to the sun in the two to three months immediately before the test.

The instrument used to carry out these irradiations was an Applied Photophysics "Clinical Photoirradiator" model UV-90, which is capable of providing a monochromatic output in the UV-B wavelengths used in this study. The output power of this instrument was 2.1 mW/cm^2 at 295 nm, 2.4 mW/cm^2 at 305 nm, and 2.8 mW/cm^2 at 315 nm. The range of doses used was from 12 to 150 mJ/cm^2 at 295 nm, 33 to 270 mJ/cm^2 at 305 nm, and 330 to 2700 mJ/cm^2 at 315 nm. Because of the high doses necessary to elicit an erythema reaction at the longest wavelength the total time for the test to be completed was approximately 1.5 hours. This narrowed the number of people who were willing to volunteer.

Each volunteer had to return to the Phototherapy Unit of Al-Sabah Hospital eight hours and 24 hours after irradiation to assess their skin reaction. The bandpass of the irradiation was ±5 nm for all the wavelengths. All the tests carried out in this section were supervised by Dr. Yousef Malallah of the Department of Dermatology of Al-Sabah Hospital, who worked very closely with the investigators during these measurements.

Skin Cancer Statistics

The skin cancer statistics were provided by the Kuwait Cancer Registry and included data collected from hospitals where biopsies were obtained. Cases of patients who sought treatment overseas do not appear in these statistics.

RESULTS

Measurement of the Pigment Level of a Small Sample of the Population of Kuwait

The results of these measurements are displayed in Figure 1. The mean is 2.18 ± 0.08 with a standard deviation of 1.40. It could be argued that this is neither a random sample of the population nor a sufficiently large one. These data are not presented as a true average but rather as an unbiased indication of the pigment level of the population.

Measurement of the Minimum Erythema Dose to Artificially Produced UV-B Radiation of Human Skin

In the tests conducted we found erythemogenic doses to be from 50 to 350 mJ/cm^2, while the pigmentation index varied from 0.5 to 5.2. The correlation between the pigmentation index and the log of the minimum erythema dose for all the volunteers is displayed in Figure 2. The correlation is not a strong one; however, it is significant. The bandpass of the irradiation was ±5 nm for all the wavelengths.

Although we feel that the above results are reliable, we by no means wish to indicate that they are truly representative of what the MED of the Kuwait population would be. Such a conclusion could only be arrived at through a much more thorough study in which we would need to include a well-represented and large sample of the population of Kuwait.

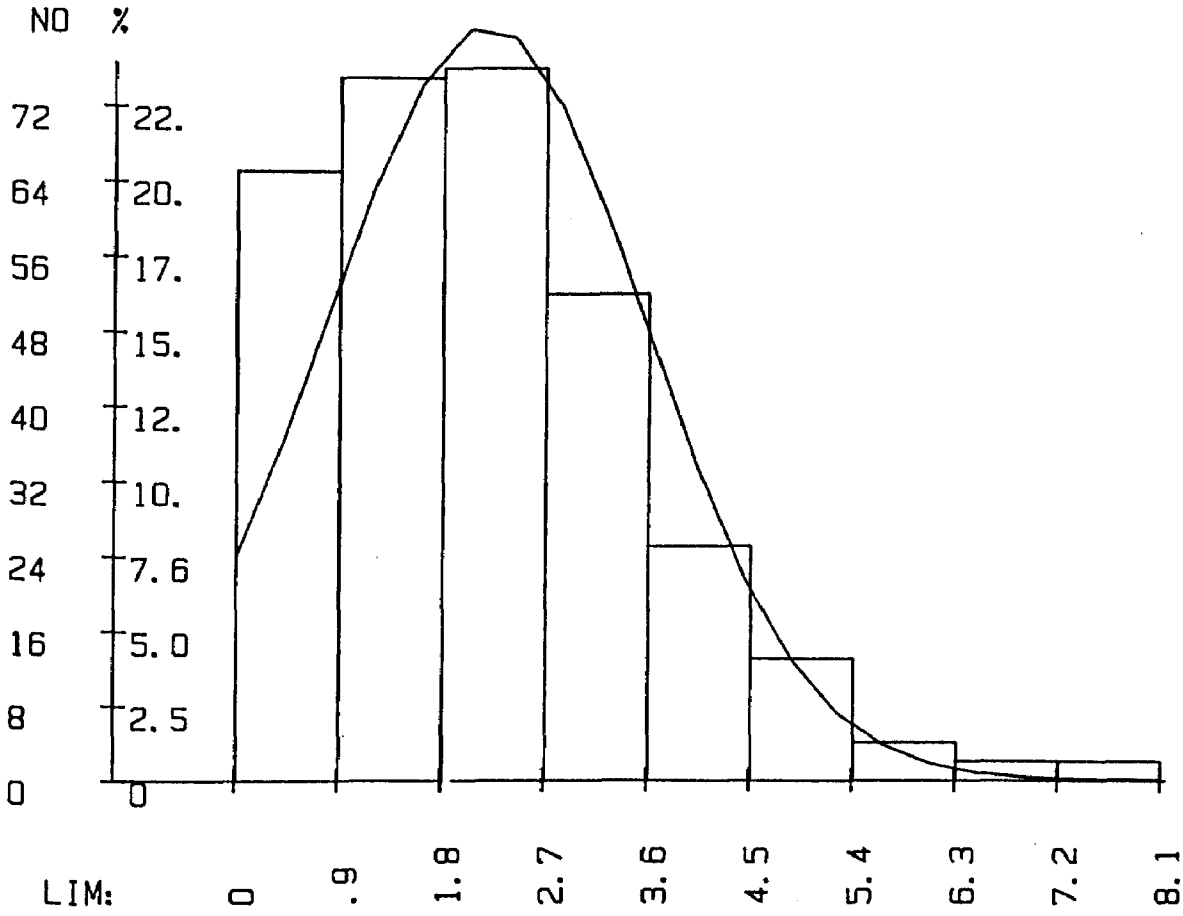

Figure 1. Pigment Level Histogram for 314 Volunteers

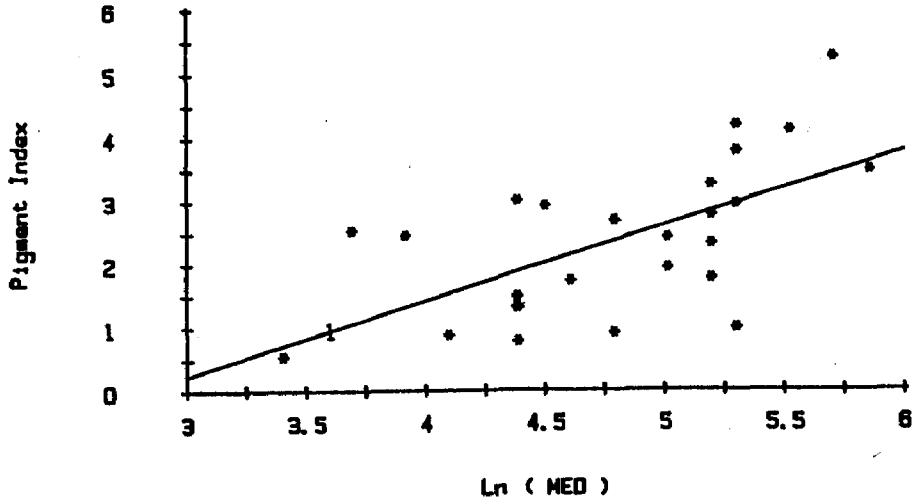

Figure 2. Correlation Between the Pigment Level and the Minimum Erythema Dose for 28 Psoriatic Volunteer/Patients.

Measurement of the Erythema Effectiveness of Three Selected Wavelengths of UV-B

The results of these tests are listed below:

1) 295 nm ---------- 7 hr erythema ---------- 56 mJ/cm^2
In (MED) = 4.0 ± 0.2
---------- 24 hr erythema ---------- 56 mJ/cm^2
In (MED) = 4.0 ± 0.2

2) 305 nm ---------- 7 hr erythema ---------- 82 mJ/cm^2
In (MED) = 4.4 ± 0.1
---------- 24 hr erythema ---------- 100 mJ/cm^2
In (MED) = 4.6 ± 0.1

3) 315 nm ---------- 7 hr erythema ---------- 820 mJ/cm^2
In (MED) = 6.7 ± 0.1
---------- 24 hr erythema ---------- 1000 mJ/cm^2
In (MED) = 6.9 ± 0.1

Skin Cancer Statistics

These statistics are presented in Table 1. They were supplied to us by the Kuwait Cancer Registry and they include only cases that were biopsied in the hospitals of Kuwait. The first numerical column indicates the total number of citizens of the Arabian Gulf countries and the second column shows the grand total. Of the 125 cases in the first reporting period, 94 were on exposed areas of the skin; this constitutes 75% of the total. Of the 78 cases in the second period, 58 were on exposed areas, also constituting 75% of the total.

DISCUSSION

Skin Cancer

Skin cancer will occur with repeated suberythemogenic doses of UV-B (Van der Leun 1984); this is a situation that prevails with our population. The data of the Kuwait Cancer Registry indicate that the most frequent forms of neoplasm are the basal cell carcinoma, with 43% of all cases reported between 1974 and 1980, and squamous cell carcinoma with 42%. For Gulf Cooperation Council nationals, 48% were squamous cell carcinomas and 37% basal cell carcinomas. The remainder of the cases were of various types of skin malignancies (see Table 1). What we consider very interesting and alarming is that skin cancer exists in this area even though people do not "burn," and that 75% of the neoplasms are on exposed areas of the skin. As the population of Kuwait exposes only the hands and the face to the sun, a reasonable estimate of the exposed area of the skin of the population would be approximately 10%. Thus 75% of the neoplasms occur on 10% of the skin. Therefore, it is reasonable to conclude that solar UV-B does contribute to the occurrence of skin cancer in the Kuwaiti population, as in other populations.

Table 1. Data on Skin Cancer Provided to Us by the Kuwait Cancer Registry

W.H.O. Diagnosis Index	74-78 Gulf	74-78 Tot.	79-80 Gulf Tot.
Squamous cell carcinoma	37	37	11.15
Malignant lymphoma	0	0	0..1
Spindle cell melanoma	0	0	1..1
Mycosis fungoides	0	0	2..3
Basal cell carcinoma	19	50	18.37
Fibrous histiocytoma, malignant	0	0	1..1
Lymphosarcoma	0	0	1..1
No microsc. confirm., Clinic. malig.	0	0	2..5
Dermatofibrosarcoma	0	0	1..1
Kaposi's sarcoma	1	1	0..4
Epithelioma, malignant	0	0	0..1
Malignant lymphoma	0	0	1..1
Sweat gland adenocarcinoma	0	0	1..1
Metastatic signet ring cell carcinoma	0	0	1..1
Basosquamous carcinoma	0	1	1..1
Other	1	2	0..0
Total	58	125	42.78

Scotto, Fears, and Fraumenti (1982) report that the annual age-adjusted rates of skin cancer in New Mexico are ten times higher for "Anglos" than for "Hispanics." Therefore, we would expect a reduced rate for darker pigmented persons. Furthermore, Scotto and Fraumenti (1982) report that the age-adjusted rate for skin cancer for American whites was 232.6 per 100,000 while for blacks it was 3.4 per 100,000. For all other cancers the rate for whites was 318.9 and for blacks 347.3 per 100,000; in other words, the latter was not as significantly different as the former. However, it has been found that experimental animals "...kept in a heated environment rapidly developed more UV-induced tumors than mice living in a temperate environment" and that "...wind and increased humidity caused increased acute UV-induced damage and acceleration of tumor formation." It is therefore likely that these environmental factors could outweigh the natural protection that darker people have for UV-induced neoplasms. Kagetsu et al. (1985) report that UV-A-induced erythema is definitely enhanced by increased skin surface temperature in humans. It is clear that more work needs to be done to evaluate the additional risk by these climatic conditions (that is, heat and humidity) as well as the genetic protection, if any, associated with skin type.

Skin Parameters

The measurements on the "pigmentation index" showed a value of 2.18 ± 0.08 for a population of 314 volunteers. This is not a large enough statistical sample but it is large enough to provide us with an estimate of where we are. Similar measurements do not exist for any other population yet since the technique for carrying out these measurements was just published in January 1986 (Kollias and Baqer 1986). Based on current experience, we would guess that the pigmentation index for English people would be approximately 0.8 ± 0.2.

If we assume that the remitted intensity from white Caucasian skin is approximately 20% of the incident intensity (Kollias and Baqer 1985), then because the Beer Lambert law can be considered valid for intact epidermis (Bruls et al. 1984) we have the following equation:

$$I / I_0 = \exp(-0.8 \times A) = 0.20 \text{ for white skin and}$$
$$I / I_0 = \exp(-2.2 \times A) \text{ for Kuwaiti skin}$$

where A is an arbitrary parameter representing the product of the thickness of the absorber and the factor that relates the pigmentation index with the absorption coefficient times the concentration. Solving the first equation for A, we obtain A = 2.0. Substituting this value of A into the second equation, we obtain a remitted intensity approximately 1% of the incident intensity. This simple calculation implies that the intensity that arrives at the dermis of a white-skinned Caucasian is approximately 20 times larger than that of a typical inhabitant of Kuwait.

The validity of this calculation hinges on the validity of the assumption that the remitted light is attenuated in the epidermis and that UV-B absorbance by the epidermis is linearly related to the visible absorbance.

If the incident intensity is attenuated so strongly by the resident pigment in the epidermis, then we should be able to predict the dose at which erythema will be induced by artificially produced UV-B in accordance with the

concentration of pigment. These measurements on 26 psoriatic patients do not substantiate this assumption. In Figure 2 we see a gentle and general correlation between the pigment level and the minimum erythema dose. [The reason that it is plotted against the logarithm of the MED is because it has been determined that the MED dose for a population does not form a normal distribution while the logarithm of the MED does form a normal curve (Mackenzie 1983; Amblard et al. 1982)].

It is known that patients tend to have a higher sensitivity to UV-B than healthy volunteers. From these data we can conclude that the native pigment does not offer a great deal of photoprotection against UV-induced erythema, although it obviously absorbs strongly the incident UV-B. The mechanism for erythema production seems to be mediated through a photoproduct that is produced in the upper epidermis. We are currently experimenting in order to establish a correlation between the native pigment and some measurable attenuating factor.

Because artificial sources tend to have a spectral output that is usually different from that of the sun, we tested for the sensitivity of human skin (local) to selected wavelengths in the UV-B range. The wavelengths selected were 295, 305, 315 ± 5 nm. In these measurements no effort was made to correlate the MED and the pigment level; rather phototests were carried out on 16 volunteers with pigment level similar to the average determined previously. The results obtained were compared with those of the photobiology unit of Dundee, Scotland (Mackenzie 1983).

The log (MED) ratio for 295 nm was 1.6 times larger for the Kuwait subjects. The ratio for 305 nm was 1.1. The ratio for 315 nm was 0.99. It follows that the difference in the MED is maximum for the 295 band and is almost the same for the other two bands. The waveband for which the MED's ratio is the largest is the one that is the weakest in the solar insolation. We can thus conclude that the erythemal effectiveness of monochromatic UV-B wavelengths is similar at the long wavelength end of the populations of Kuwait and Scotland with the difference becoming maximum for the 295 nm band.

Since the erythema effectiveness maximum factor of five is different at the shortest wavelength [a factor of 1.6 for Log (MED)], it cannot possibly account for the factor of one hundred difference for the carcinogenic effect of UV-B on deeply pigmented versus white individuals. These observations provide further evidence that the erythema effectiveness of UV-B wavelengths is not necessarily the same as the effectiveness for carcinogenesis or for photokeratosis.

There is no doubt that the population of Kuwait is naturally protected from the intense rays of the sun more than Caucasian people. The question is, are they adequately protected from their severe UV-B environment? The absence of severe photodermatoses as well as the rarity with which one observes severe sunburns is adequate testimony to some protection. The skin of our population is more pigmented than that of northern Europe and the sensitivity of the local skin to artificially produced solar-simulated radiation is at least half that of Caucasians (Y. Malallah, private communication). The results presented in the previous section relate to the log (dose) and consequently they appear small. The actual energy dose is 12.5 mJ/cm^2 for Dundee and 54

mJ/cm^2 for Kuwait for 295 nm to produce an erythema reaction. At 305 nm and at 315 nm the differences remain small even on a linear scale.

Skin cancer incidence according to the statistics that are available to us is at the level of 2.6 per 100,000 for Kuwaiti males and 1.3 per 100,000 for Kuwaiti females for the 1979-81 period. It is interesting that the levels in the previous reporting period were much lower, i.e., 1.1 per 100,000 for Kuwaiti males and 1.0 per 100,000 for Kuwaiti females. This trend indicates either a significant increase in skin cancer incidence or a significant increase in the reporting of skin cancer cases to the Registry. Another factor that is completely beyond the control of the experimenters and is difficult to account for is the number of suspected skin cancer patients who seek treatment overseas. These cases are missing from the local statistics and could make the numbers smaller than they actually are. As the majority of skin neoplasms appear in exposed areas it would not be surprising if a good number of people seek plastic or reconstructive surgery elsewhere.

Our conclusion from these results is that melanin in the epidermis plays two roles: one in the case of carcinogenesis, and another in the case of UV-B-induced erythema. It appears that in the case of carcinogenesis, melanin (the primary pigment in human skin) acts as an absorber, attenuating the incident intensity. Thus, tumor initiation appears to be proportional to the amount of UV-B that arrives to the basal cell layer. In the case of UV-B-induced erythema the correlation between the concentration of melanin and the MED dose is very weak, which implies that there must be some other mediator for the production of erythema. The role of melanin in this case would possibly be as a scavenger of free radicals generated by the UV-B radiation in the epidermis. The radicals that are not compensated would then migrate into the dermis through the basal cell layer and generate the erythema reaction. Thus, the concentration of melanin will be a determining factor in the erythema. In a paper that we are currently submitting for publication we discuss the different forms that melanin molecules can take up in the skin and the different functions that these molecules might perform.

CONCLUSIONS

The estimated average pigmentation index of the population of Kuwait is 2.2, while that of European Caucasians is estimated to be less than 1.0 and assumed to be 0.8. The pigmentation index is a parameter that varies from 0 to 9 (there are darker people but we have not measured their pigmentation yet), and is directly related to the absorbance of the epidermal melanin.

A weak relationship exists between the minimum erythema dose of UV-B and the pigment level. Considering the average values of MED and pigment level we are led to the conclusion that the relative protection that the pigment renders is related to the absorbance or pigment level; i.e., as the pigment level increases by a factor of 2+ the MED decreases by a factor of 2+. It is unfortunate that for these preliminary results we had to use data obtained from psoriasis patients as the data are probably slightly biased to a lower MED value. MED values determined for normal Kuwaiti subjects would render more definite answers.

Variation in the sensitivity of Kuwaiti skin and that of Caucasians is demonstrated with smaller wavelengths of solar UV-B. At 295 nm, sensitivity

varies by a factor of four while at longer wavelengths it is not very different. This is similar to results described in Nakayama et al. (1974). This means that to properly evaluate the UV-B risk, we need to establish the spectral relative sensitivity (action spectrum) of Kuwaiti skin to UV-B. This means that the Effective Spectral Irradiance is not the same as that of Caucasian skin just attenuated by an appropriate factor. It is essential that the action spectrum for UV-B wavelengths of Kuwaiti nationals and typical expatriates be determined.

Analysis of the cancer statistics shows that skin cancer in the Kuwaiti population relates strongly with UV-B exposure. It should be noted that 75% of the skin neoplasms reported between 1974 and 1980 occurred on exposed areas of the skin, i.e., on 10% of the skin. This is an unmistakable and alarming signal. Statistical evaluation of the results is not complete because we are in the process of obtaining more information from the Kuwait Cancer Registry.

ACKNOWLEDGMENTS

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Ozone Modification: Importance for Developing Countries in the Tropical/ Equatorial Region

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The adverse biological and environmental effects due to any inadvertent ozone modification have received considerable scientific attention but have been largely concerned with ultraviolet radiation damage to human skin in specific relation to protection of deficient white skin. In this respect, the ozone problem somehow has not appeared to be of serious interest to the people of developing countries living in the tropical/equatorial geographical belt. However, in view of the manifold increase in the ultraviolet radiation received at the lower latitudes compared to the mid and high latitudes, it seems reasonable that the ozone layer issue should be of serious interest in the tropics. In fact, in these regions, more serious kinds of solar UV radiation-induced health effects such as cataracts, viral infections, and immune system damage may be connected to the "ozone-UV" issue. These aspects need to be studied under harsher tropical conditions, but the understanding that has been achieved so far (for mid-latitude conditions) can provide a valuable base from which the specific situation for tropical countries can be tackled as an extension.

INTRODUCTION

For several decades, atmospheric ozone, especially the stratospheric layer, has been known for its important protective role against the incoming solar ultraviolet radiation. In general, there is a natural balance between the ozone's production and its destruction with a net ozone surplus. Under the natural equilibrium, there are small dosages of solar ultraviolet radiation to which populations, living organisms, plants, and aquatic systems have generally become adapted. The harmful effects are thus generally very small except perhaps in the tropical/equatorial region. The surface dosage of natural ultraviolet radiation increases by many folds in going from high/mid latitudes to the equator. Because people living in the high dosage tropical belt have a darker skin tan which provides greater protection to the skin against ultraviolet radiation, this high UV radiation situation has not been

considered particularly seriously in terms of adverse health and environmental effects that might occur in these regions.

The ozone layer suddenly came into scientific prominence about 12 years ago when it was realized that certain human activities may lead to significant ozone depletion from the naturally balanced level, thereby increasing the surface dosage of ultraviolet radiation leading to increased skin cancer and many other adverse biological, environmental, and climatic effects. Since any such ozone destruction would spread out globally, the net UV radiation effect would be most serious in the equatorial and tropical belt when already the ozone column thickness is minimum and ultraviolet radiation penetration is maximum. But perhaps even a more fundamental issue is to ascertain whether the already high UV radiation dosages received at the lower latitudes are contributing to tropical diseases and medical problems. Whether the 5 to 10 times higher dosages (in absolute terms) that would be received in the low latitude region in comparison to the higher latitudes (if a certain fraction of ozone column is reduced) would bring the radiation level above a certain threshold is another aspect relevant to the tropics. In other words, the "ozone modification" issue is of serious importance to the low latitude countries. The whole issue of ozone layer protection is thus indeed of global dimension. Yet, there is very little apparent involvement of scientists from the developing countries in the "ozone layer-ultraviolet radiation" work. While financial constraints may account for some of this non-involvement, lack of proper understanding of the effects at the public, scientific, and political levels is also a serious factor. Nevertheless, in the coming years, it should be appropriate to examine the "ozone layer-UV-B" matter in the specific context of tropical countries.

TYPICAL CONDITIONS

In order to examine the relevance of the ozone layer issue for the developing countries in the equatorial/tropical region, it would be helpful to summarize the atmospheric ozone and solar ultraviolet radiation influx globally. The seasonal distribution of vertical ozone column for different latitudes is shown in Figure 1. It is clear that at the lower latitudes, not only the ozone column thickness is significantly small but it also does not vary much seasonally. The solar ultraviolet radiation penetration is thus maximum at the lower latitudes throughout the year. The overall effect of the low ozone content coupled with smaller seasonal change in the solar declination results in the latitudinal distribution of annual erythemal dosage (incoming radiation weighted according to skin erythemal action spectrum) as shown in Figure 2. This diagram illustrates the manifold increase in the damaging UV-B dosage from high latitudes to the equator. Some towns are also marked on the curve against their respective latitudes. Figure 2 is based on calculated data for clear sky conditions (Mattingly 1976; Ilyas 1979).

THE EQUATORIAL DATA FROM PENANG

In the equatorial/tropical region, there is a general lack of observational data pertaining to the atmospheric ozone and solar UV radiation. With this consideration in mind, about 10 years ago we initiated a comprehensive program of measurements involving ozone soundings, erythemal UV-B, UV-A, total solar radiation, surface ozone, and a series of relevant meteorological parameters at our equatorial place, Penang (5.5°N). This is perhaps one of the

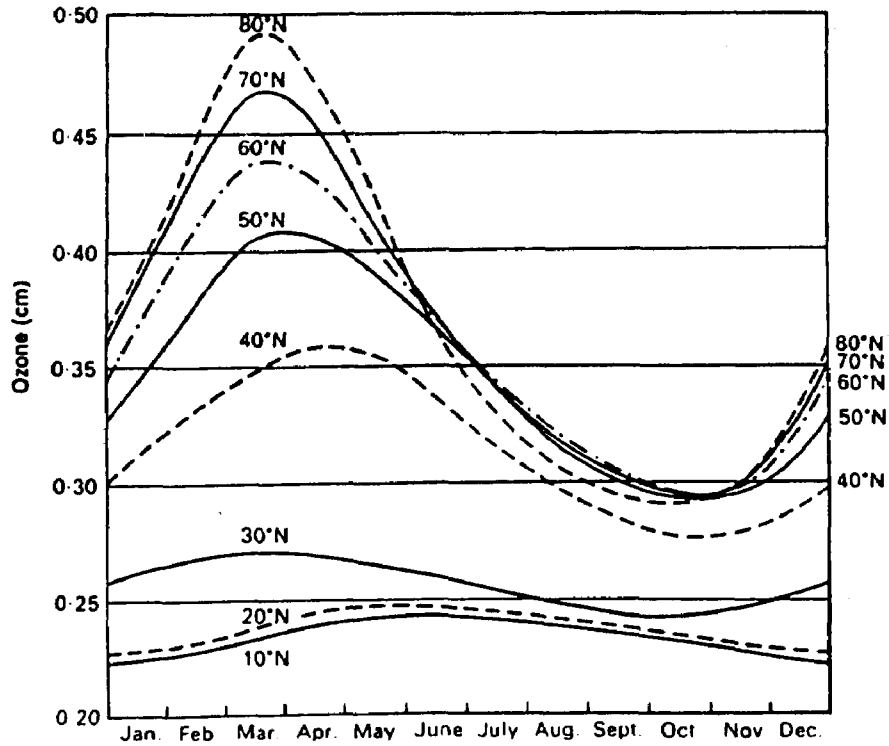

Figure 1. Annual Variation of Total Ozone for Each 10° of N Latitude

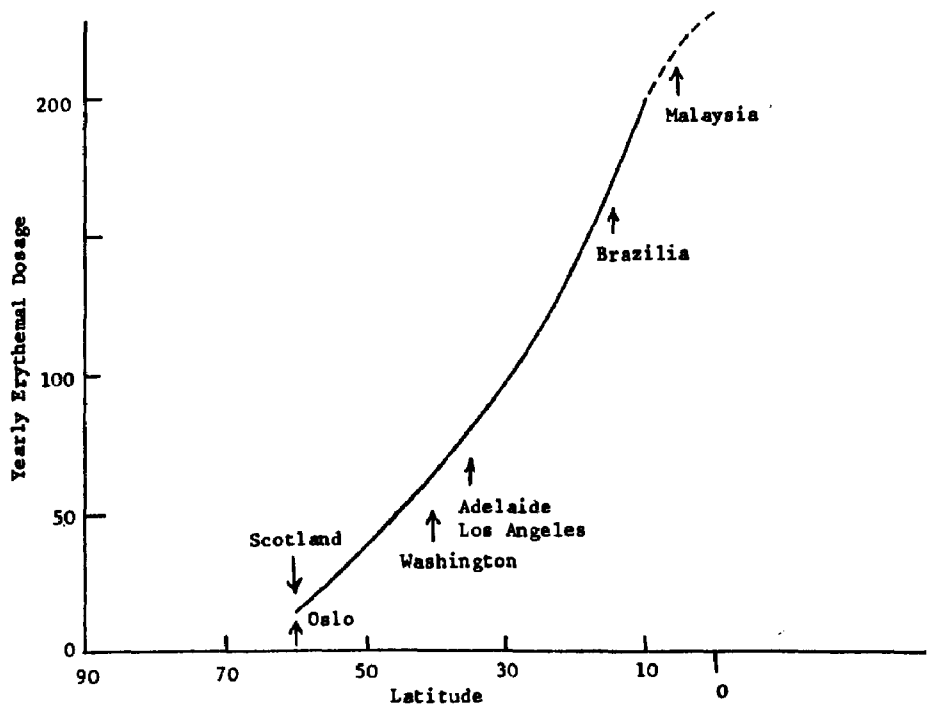

Figure 2. Variation of erythemal dosage (joules/cm^2) with latitude for clear days. Also indicated are the dosages for specified places as compared to an equatorial location (Malaysia).

most rare comprehensive programs in the equatorial/tropical belt. Some of the initial results from this study have become available and more refined data should follow (Ilyas 1984a, 1984b; Ilyas and Barton 1983).

One of our immediate interests from this study is to see if the observational data obtained at Penang can be used in a general way for the equatorial/lower tropical region. For this, the most important and directly usable data in the context of ozone layer effects study are the directly involved ultraviolet radiation data. The input radiation flux does not change much over the entire equatorial/lower tropical belt. It would, however, be modified differently at different places depending upon the sky conditions (cloud cover). The long-term cloud cover data at Penang indicate the cloud cover to be close to 85% (Ilyas et al. 1981) which reduces the incoming UV-B radiation to about half. This is consistent with the theoretical relationship of cloud cover effect on ultraviolet radiation (Johnson et al. 1976). In a more comprehensive study of cloud cover effect, we used 5-year-long observed UV-A data (Ilyas et al. 1986), together with the calculated data for clear sky conditions (Johnson et al. 1976). The observed data were found to be in excellent agreement with the computed data modified for the average cloud cover (Ilyas 1986). This is shown in Figure 3.

The excellent agreement between observational data and calculated data indicates that the cloud cover effect for other locations may be easily incorporated into the clear sky calculations of ultraviolet radiation. The radiation conditions for evaluating the adverse biological/medical and environmental effects can thus be ascertained. In any case, because of the relatively high cloud cover conditions at Penang (5°N), the observed UV flux represents a lower limit of UV dosage that would be received anywhere in the entire equatorial belt. A summary of the erythemal UV-B dosage together with some meteorological conditions at Penang is presented in Figure 4. The information in Figures 3 and 4 thus represents lower tropical model conditions which must be simulated for the photobiological effect studies for this region.

UV RADIATION EFFECTS IN THE TROPICAL REGION

Figures 1-4 provide a good summary of low ozone content and high ultraviolet flux conditions prevailing at the lower latitudes against the high ozone content and low ultraviolet flux at mid and high latitudes. Besides, the high (air) temperatures and humidities in the tropics prevail throughout the year and may couple together with the UV radiation in producing more severe biological and physiological effects. Lack of education and awareness of UV-induced damages, skin's false sense of protection, increased outdoor occupations including young school children being exposed to relatively large amounts of ultraviolet radiation (under hot and humid conditions) due to inadequate clothing and/or outdoor work (like P.E. lessons and games) at wrong times of the day may all add up to the seriousness of the situation. Unfortunately, there is not much basic data available let alone studies of adverse radiation effects under specific conditions. Although skin cancer and other related problems, hitherto the prime concern in Western populations, may not be of sufficient importance in the tropics, more serious effects on humans such as viral infections (herpes, hepatitis), eye damage (cataracts), damage to the immune system, and life expectancy (perhaps we don't record many skin cancer cases in the tropical countries because most of such people don't live

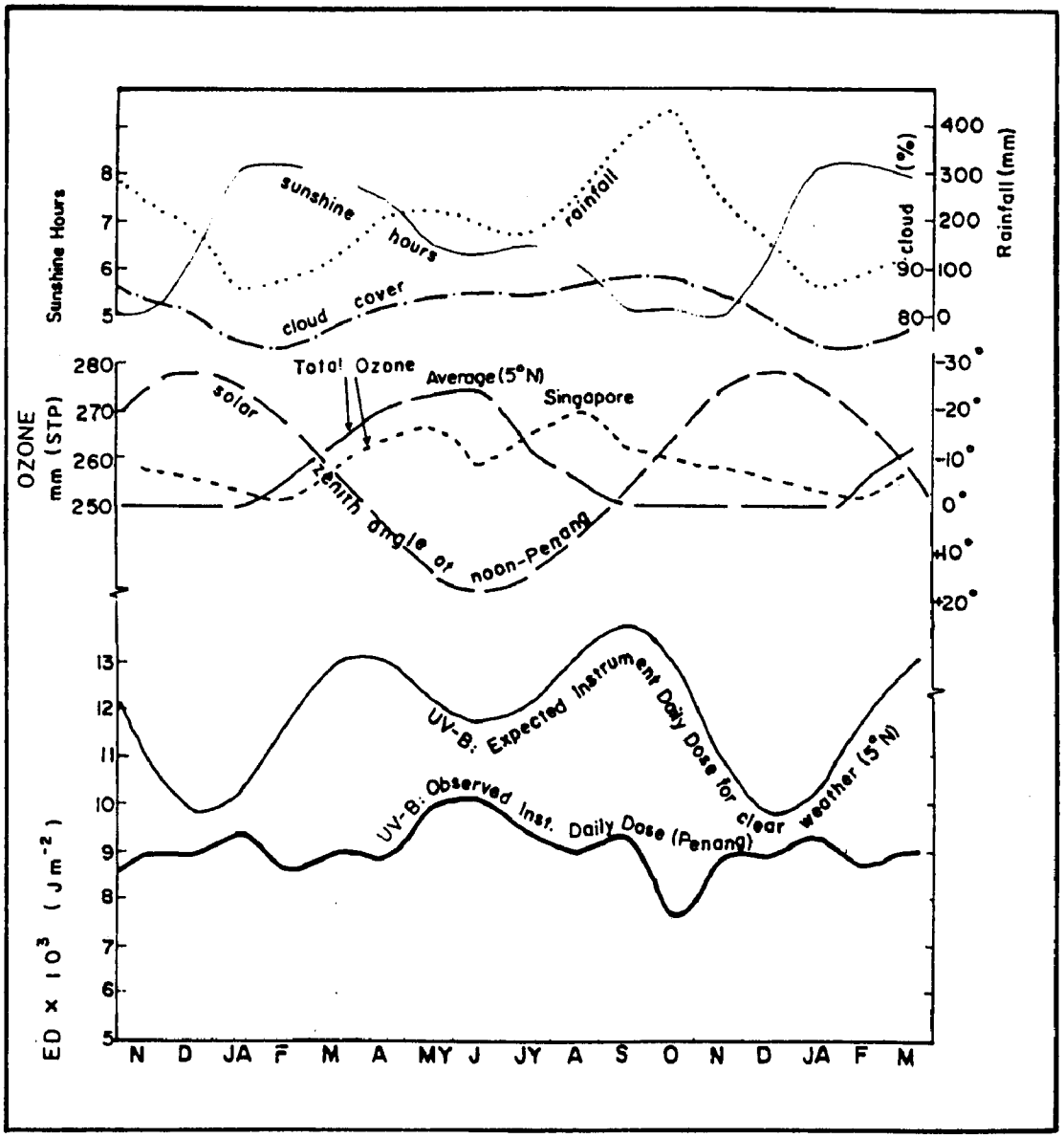

Figure 3. Annual variation of the average daily total erythemal dosage (ED) (instrument response) as measured at Penang together with the clear weather dosage (calculated) and the related observational climatic parameters for the station and the solar position [minimum (noon time) zenith angle].

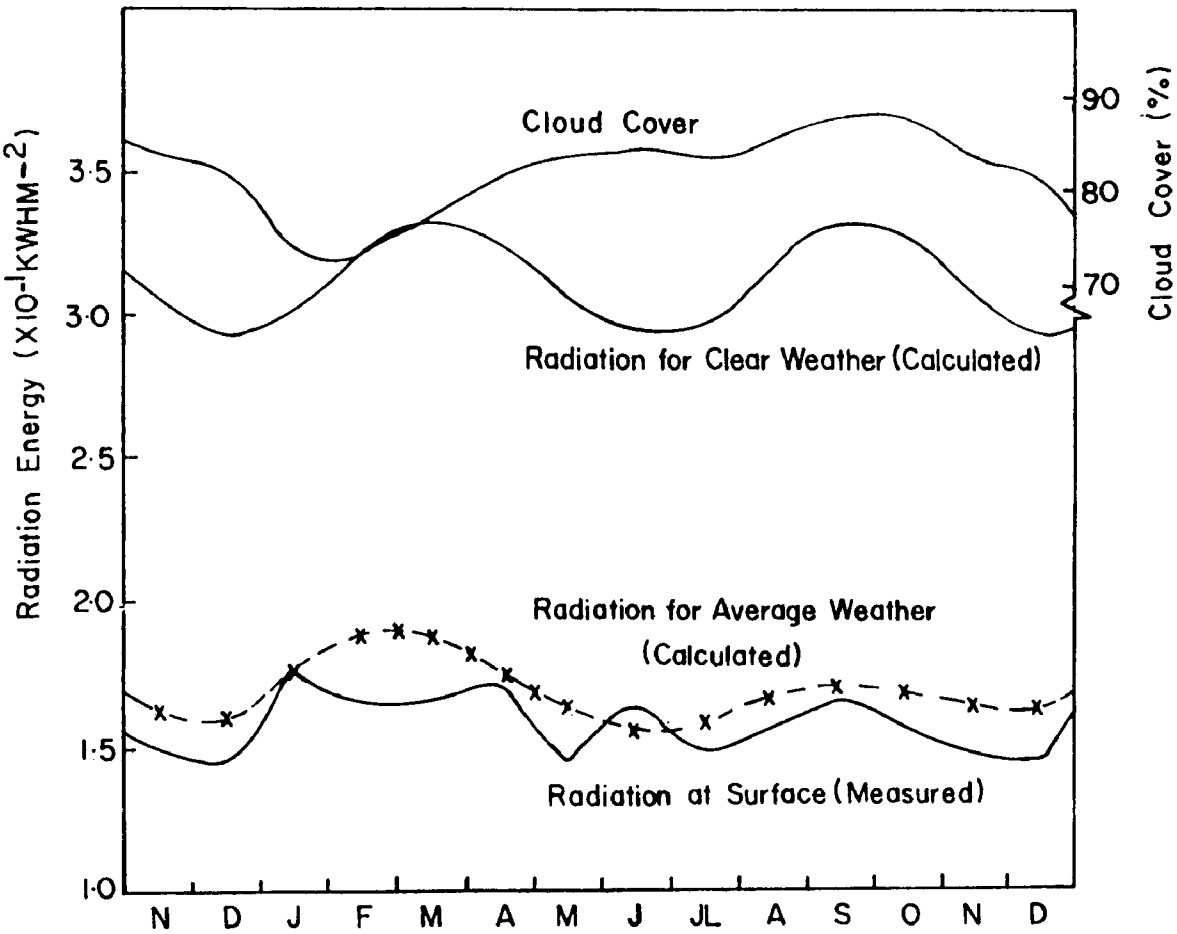

Figure 4. A comparison of the calculated UV-A dosages outside the atmosphere and at the surface (after accounting for cloud cover effect) with the measured data at our station (Penang).

long enough for the effects to show up) are important avenues for future work. Also, what adverse effects the very high dosages may have on tropical plants and aquatic organisms and the exposed materials would also need to be studied. Finally, whether any further increase in UV radiation, as a result of ozone reduction due to human activities, would affect any threshold limit--to which the tropical systems may have become adapted--would be important to examine. Side by side, populations would need to be educated on these effects and some simple protection methods, whereas scientists in these regions should be increasingly involved in this program with the realization of local relevance and overall importance. The exercise of protecting humans globally by protecting the ozone layer would then become a very involved matter. An organization such as UNEP is well suited to make a move in this direction.

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The Tan of Ultraviolet-B Summer

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During my studies of the environmental parameters of ozone depletion, one idea often comes to mind: What is going to happen with the surface of our planet? As this approach was too prosaic for this solar and galactic beauty of which we are all proud, I transformed the question into a more poetic one: What is going to happen to its tan? I hope you will agree that the criteria that evolve from this approach are more appropriate because of the abundant chemical make-up of our planet and the ultraviolet tanning the planet is starting to acquire in the new "anthropogeochemical" era.

I beg your pardon for this novelistic digression and let me approach the problem as we usually do--more prosaically.

I agree with Watson, Hansen, Hoffman, and others that we have barely scratched the surface of the problem and that many unanswered questions have to be approached. I also agree that we possess enough knowledge to qualify the risk. The differences in quantifications all fall within a range that guarantees danger. The nature of the processes, including phenomena of momentum and inertia, makes it possible to conclude that we are in the incubation phase of the disease. The "infection" is here, the process is irreversible, the disaster is imminent. The problem is how to cope with it.

I think that we can cope with the disease of the planet. The guarantee is in the fact that there is no disagreement in qualification of the changes in anthropogeochemistry. I could not avoid the implications of the facts with which we are confronted. These facts suggest one general title that can be given to all particular parameters of this environmental and climate complex discussed in all papers: "disastrous chemical summer." All our estimates of warming, ice melting, flooding, rising sea levels, inundation, epidemics, ultraviolet aids, deaths, droughts and hunger, temperature rising, etc. suggest this integrating qualitative statement: chemical summer with all its anthropogeophysical and anthropogeobiological events. Whether it is going to be called ultraviolet summer, chemical summer, ozone summer is a question of

convention. It seems to me that whether cool or hot, it still can be called burning ultraviolet summer. We all know that ultraviolet radiation is burning, although invisible and without heat.

This excellent example of a man-made hazard that can trigger natural hazards of incomparable energy and destructive force should remind us that the disastrous chemical summer can be as disastrous as "nuclear winter." The nuclear season can be prevented by avoiding nuclear warfare. But the summer produced by the greenhouse effect and ozone depletion surely has already begun. The ozone shield has already been transformed into a less protective curtain.

Cosmonauts tell me that they often admire this beautiful shield of life on earth. They look at it and through it as if they are looking through a protective window. Let us help them to always look through this protective window and not through the holes in it. Otherwise, instead of us they will see only burns and scratches on the face of what was once the galactic beauty Mother Earth.

AQUATIC SYSTEMS

Effects of Enhanced UV-B Radiation on the Survival of Micro-Organisms

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Microorganisms play an important role in both aquatic and terrestrial ecosystems on our planet since they represent the basic level in a complicated food chain on which the lives of heterotrophic organisms such as animals depend. In addition, plants rely heavily on the activity of microorganisms such as the fixation of atmospheric nitrogen, which is also an important economical factor.

Many microorganisms utilize external signals for orientation and behavior in their microenvironment to find a suitable niche for survival and growth of a population. Not only is light the single most important factor for orientation in photosynthetic organisms, which naturally depend on the availability of light for energy fixation, but it is also critical for non-photosynthetic microorganisms.

Microorganisms have developed a number of different strategies to orient with respect to light including phototaxis, which is a directed movement towards or away from a source of light; photokinesis, which describes the dependence of the speed of movement on the intensity of light; and photophobic responses, which are elicited by sudden changes in the light intensity (Nultsch 1975). All these mechanisms of orientation are utilized by a population to find a suitable environment and to adapt to the continuously changing conditions (Nelson and Castenholz 1982).

A number of taxonomically different microorganisms have been found to be extremely sensitive towards solar UV-B radiation (Damkaer et al. 1980; Ohnishi et al. 1982; Jagger 1983). These organisms, which range from prokaryotes to eukaryotes, from gliding to flaggelated forms, and from photosynthetic to non-photosynthetic organisms, are under UV-B stress even at current levels and would be seriously endangered by an increase in the UV-B radiation caused by a partial reduction of the ozone layer due to anthropogenic gaseous pollutants such as chlorofluorocarbons (Maugh 1984; Weiss et al. 1985).

In addition to immediate lethal effects on the organisms, several responses to UV-B radiation can be distinguished, all of which eventually destroy a population of microorganisms. The most obvious effect observed is an inhibition of motility, which prevents a population from escaping from hazardous influences in its environment. A more indirect effect is the inhibition of photoorientation, which occurs at even lower UV-B doses. As a consequence, the organisms are still able to move but are unable to respond to changes in their environment and are eventually killed by the unfavorable conditions in their environment. A discussion of these problems with a few examples follows.

Filamentous cyanobacteria live in aquatic (marine and freshwater) habitats as well as in soil and other terrestrial ecosystems and produce a large fraction of the organic material (Fay 1983). The organisms glide using a mechanism not yet fully understood. When exposed to low doses of UV-B irradiation, the populations are killed within a few days after exposure. The analysis of the behavior using an artificial UV-B source showed that both motility and photoorientation are drastically impaired (Häder 1985a).

Investigations at an experimental station near Lisboa, Portugal, demonstrated that the organisms do not survive long in solar radiation. Neither the visible component of the radiation nor the temperature increase caused the organism in the experiment to die but rather the UV-B component in the solar radiation killed them (Häder et al., in press). When exposed to unfiltered solar radiation at noon during the summer, the filamentous Phormidium was found to stop moving within 35 minutes. An action spectrum measured in the Large Spectrograph in Okazaki, Japan, indicated that wavelengths below 300 nm were extremely effective (Hader et al., in press). It could be shown that the UV-B target is not DNA since no photorepair could be induced after UV damage (Eker 1983; Yamamoto et al. 1983). A mechanism involving photodynamic effects could also be excluded by studying the effects of diagnostic reagents (Ito 1978; Spikes and Straight 1981; Nultsch and Häder 1984). The conclusion of these experiments was that an intrinsic component of the motor apparatus of the organisms, probably a membrane-bound protein, is the molecular target of UV-B radiation.

Cyanobacteria use a primitive, but rather effective mechanism to orient themselves with respect to their photoenvironment (Häder 1979). Each time a filament leaves a bright area by gliding into a shadow, it reverses direction and returns to the bright area. This so-called "photophobic response" guarantees that the population stays in the light, which is essential for a photosynthetic organism. Light fields that are too bright are avoided by phobic responses upon entering the bright area. The remarkable precision of orientation can be demonstrated by a simple experiment in which a photographic negative is projected into a homogeneous suspension of organisms, which then accumulate in areas of suitable light intensities and form a sharp and detailed positive image (Häder 1984).

Most cyanobacteria are typical low light organisms: When they are exposed to high light intensities, their photosynthetic pigments are photooxidized and the cells die within a few days (Walsby 1968). The consequence is that populations of cyanobacteria are not only endangered by increased UV-B radiation, which affects motility and directly kills the organisms, but also by lower UV-B doses, which impair photoorientation of the organisms. As a

result they either move into the substratum, where they die due to the lack of light or they can no longer prevent themselves from being exposed to excessively bright light, which bleaches and eventually kills the cells as well.

Only a few organisms have developed mechanisms to escape UV-B radiation while most organisms are insensitive toward the UV-B. The archaebacterium Halobacterium uses different sets of photoreceptors to accumulate in favorable light fields and to escape from hazardous radiation (Wagner 1984). Upon an increase in the short wavelength radiation, the blue/UV receptor mediates the repellent signal and triggers an instantaneous reversal of movement.

The cellular slime mold Dictyostelium is also extremely sensitive toward UV-B radiation because its development and motility are impaired by very low doses (Häder 1983a, b). In its multicellular stage, this organism uses a lens effect to detect the direction of laterally incident light. Because the refractive index of the cytoplasm is higher than that of the surrounding air parallel, light is focused to the rear flank of the organisms (Häder and Burkart, 1983). The resulting light gradient is used for photoorientation towards the light. In the UV-B range, the lens effect is cancelled by a strongly absorbing substance so that the optical properties are reversed and the organisms move away from the UV-B source (Häder 1985c).

Flagellates, such as the green uniflagellar Euglena gracilis, find their suitable environment by two antagonistic orientation mechanisms: At low light intensities, the cells move toward the light source (positive phototaxis) and at high intensities away from the light source (negative phototaxis). In their natural environment, the cells swim upward until they reach the optimal light intensity, where they form a dense population.

Hader(1985b) found that solar UV-B affects swimming in these organisms. No motile cell was found in samples exposed to solar radiation for 2.5 hours around noontime. The cells did not recover from the damage. Even when the population was transferred to darkness or weak light after a short UV-B irradiation, most of the cells died in the subsequent hours indicating a damaging effect even of low UV-B doses.

Photoorientation was measured by an automatic computer-controlled video analysis (Häder and Lebert 1985). The video image of the cells swimming under the microscope was digitized in real time and stored in an electronic memory. A microcomputer had access to the memory and was programmed to detect and follow individual organisms. The deviation of the organisms from the incident light direction was calculated and stored for subsequent statistical treatment and histogram analysis. Under appropriate culture conditions Euglena moves with a high precision of orientation. After artificial or solar UV-B irradiation, the swimming tracks become increasingly erratic and the organisms move in random directions independent of the light direction.

Both effects of solar UV-B radiation on the flagellate Euglena are disastrous for the survival of the population. When motility, photoorientation, or both are impaired, the organisms are no longer able to select a suitable habitat. Even when we allow for a considerable attenuation of the UV-B radiation by the column of water above the cells, solar UV-B has to be regarded as a natural stress factor even at current levels. Any increase in

the UV-B doses would increase the stress, and would eventually threaten the survival of these and other ecologically important microorganisms.

ACKNOWLEDGMENTS

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Is the Impact of UV-B Radiation on Marine Zooplankton of Any Significance?

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ABSTRACT

Over the past decade several studies have examined the effects of UV-B radiation on a number of major marine zooplankton groups. While the adult organisms are commonly found in the upper meters of the ocean, the eggs and larval forms may be concentrated in the surface micro-layer. It has generally been noted that larval forms of an organism are more susceptible to damage by UV-B than later life stages. The species that have been studied are critical components of bioenergetic pathways that lead to larger animals that are of nutritional and commercial value to man. The studies indicate that present-day levels of UV-B radiation significantly affect the developmental life stages of these organisms. For some species a 10% decrease in atmospheric ozone could lead to as much as an 18% increase in the number of abnormal larvae.

ATMOSPHERIC OZONE EVOLUTION

It is generally accepted that the earth's primeval atmosphere resulted from the outgassing of volatiles from the interior of the planet. The atmosphere was devoid of oxygen and consisted primarily of methane, carbon dioxide, ammonia, and hydrogen sulfide. The absence of an effective filter allowed short wavelength UV from the sun to penetrate the surface of the earth. While solar ultraviolet radiation can be viewed as a major constraint on the evolution of life, at the same time it may have been a vital force in the formation of biological macromolecules. It is thought that associations of macromolecules at some depth in the could utilize the diminished solar energy reaching to that depth. These precursors to what we now call phytoplankton were the principal source of oxygen to the early atmosphere. Berkner and Marshall (1965) have implicated the development of atmospheric oxygen, and therefore, ozone as an important contributing factor to explosive evolutionary advances in the fossil record. Two thresholds of oxygen concentration have been postulated. These correspond to two periods in the fossil record, one in the Cambrian and another in the Silurian.

THE IMPACT OF UV RADIATION ON MARINE ZOOPLANKTON

The first investigations into the effect of the ultraviolet radiation component of sunlight on aquatic organisms were conducted as early as 1925 (Huntsman). By 1930 several studies using a variety of marine organisms indicated there was a detrimental impact of the ultraviolet portion of sunlight (Klugh 1929, 1930; Harvey 1930). These early pioneering studies serve as benchmarks and are of historical interest, but have little relevance to the current ozone issue because of the lack of instrumentation to precisely and absolutely measure the quantity and quality of the radiation. It was not until 1970 that instrumentation for precise UV irradiance measurements became available.

Worrest (1982) has reviewed the literature relating to the impact of UV-B radiation upon marine organisms. This report updates that information, adds to its scope, and provides some comparisons between results from a variety of experimental methods. The data presented will clarify that ultraviolet radiation does have an impact on this portion of the biosphere. The extent of this impact is a function of primarily three variables:

- The species being exposed to UV
- The developmental life stage of the species
- The dose of radiation the organism receives.

The data presented in Table 1 is compiled from a number of investigations over the last decade by various authors using different experimental techniques and criteria for assessing the impact of UV-B radiation. Because standardized techniques were not used in collecting the data, some conversions of the data have been made for the sake of comparison. Nomographs (Damkaer and Dey 1982) have been used in an attempt to account for differences in UV exposures among authors using different methods of exposure. In spite of these approximations there are some general statements that can be made and in fact some remarkable parallels among the different sets of data:

- First, the younger the developmental stage of the organism at the time of exposure the more sensitive the organism is to the harmful effects of UV-B. This is demonstrated by looking at the total dose required for an early larval stage to exhibit a significant effect and comparing it with the total dose required by a later larval stage of that species.
- Next, organisms appear to have differing sensitivities to UV-B. This can be seen by comparing the response of different groups of zooplankton at analogous stages of development.
- The third point is that the rate at which the total dose is received is a factor in the extent of impact. In looking at the copepod data a doubling of the dose rate roughly halves the time for the end point to be reached.

- Finally, the data in Table 1 show that the impact of UV-B on marine zooplankton larvae is significant at present levels. For comparison purposes the dose rate and total dose for oyster larvae are about what we would expect to measure on a clear sunny day at 45N latitude in late June or early July.

Table 1. Estimated Biologically Effective UV-B Doses Leading to Significant Effects in Major Marine Zooplankton Groups (Biological weighting referenced to action spectrum normalized to 300 nm.)

	Zooplankton Group	Dose Rate DNA(300) W/m2	Total DNA Dose kJ/M2/d	Time for Effect
I.	Shrimp/Euphausiid larvae	6.00 E-2	2.55 E3	4 days
	Euphausiid adult	9.90 E-2	6.45 E3	6 days
II.	Copepod larvae	3.39 E-1	1.35 E3	1.0 hr
	Copepod postlarvae	1.59 E-1	2.70 E3	4.5 hr
	Copepod adult	3.39 E-1	3.00 E3	2.5 hr
III.	Crab larvae	9.90 E-2	6.45 E3	6 days
	Crab postlarvae	2.79 E-1	6.00 E4	20 days
IV.	Anchovy/Mackerel larvae	6.00 E-2	2.50 E4	12 days
V.	Oyster/Mussel larvae	1.20 E-1	2.16 E3	5.0 hr

Source: Data from: Damkaer et al. 1980, 1981; Hunter, Kaupp, and Taylor 1982; Karanas, Worrest, and Van Dyke 1979, 1981; Thomson et al. unpublished.

The most recent data in Table 1 come from studies conducted in our lab. Individuals from four species of bivalve molluscs (Crassostrea gigas, Saxidomas giganteus, Mytilus edulis, and Clinocardium nuttallii) were spawned separately. Fertilized eggs were exposed to UV-B radiation for periods ranging from 1 to 6 hours. The eggs were then held for 24 to 72 hours at which time they were examined for abnormal development. This examination was done at the end of the trochophore stage; the onset of the straight-hinged larvae stage. The response of the four species is shown in Figure 1. A third degree polynomial curve was fitted through the means of several hundred data points. The shape of the curve is typical of survival curves in that there are two inflection points. Beyond a threshold value there is an increase in the rate of abnormal larvae as the UV dose increases. At the upper end of the curve, beyond a second threshold, the impact levels off.

Using these data as a basis, we calculated the increase in abnormal larval development as a function of decreases in atmospheric ozone (Figure 2). The calculations were referenced to ambient daily doses that would occur during the spawning peak of these species. For species such as the bay mussel and oyster a 15% decrease in ozone thickness can lead to nearly a 30% increase in the number of abnormal larvae. The impact of UV-B may be greater on some species while species such as the cockle and butter clam may be less sensitive. Using the butter clam as an example, a 15% reduction in atmospheric ozone could lead to a 15% decrease in the harvest. In comparison, a 1% change in the harvest from a commercial salmon hatchery can be the difference between negative cash flow and economic viability.

FUTURE AGENDA

Phytoplankton serve as a major conduit through which the sun's energy flows into marine life; small plants floating at or near the ocean's surface or at depths not exceeding the euphotic zone depth. A portion of this energy is transferred to the zooplankton that graze in this "breadbasket of the sea." As predator becomes prey, the sun's energy is transferred to the next strand in a web that can ultimately lead to man. The impact of UV-B radiation on this transfer of energy through a food web needs to be examined. Two levels of impact are possible; direct impact on the predator organisms or its larval stages and indirect effects as a result of the availability of prey. Organisms surviving increased levels of UV-B may do so at a biochemical cost that alters the value of that species as a food item. Preliminary investigation of this question has begun. Initial results indicate that predator organisms grow at different rates when fed different species of phytoplankton. In addition it appears that biological molecules in otherwise "healthy" phytoplankton are altered by exposure to UV-B.

Documentation of the effects of UV-B on marine zooplankton should continue with increased attention to the need for uniformity in methodologies among investigators. This would permit greater accuracy in comparing results and predicting effects. The effort to investigate species that are critical components of food webs needs to be increased. Detection of biochemical alterations in some key species would constitute a refinement in our ability to assess the impact of UV-B. Without this additional information, accurate prediction of the effect of decreased atmospheric ozone on the marine ecosystem is difficult.

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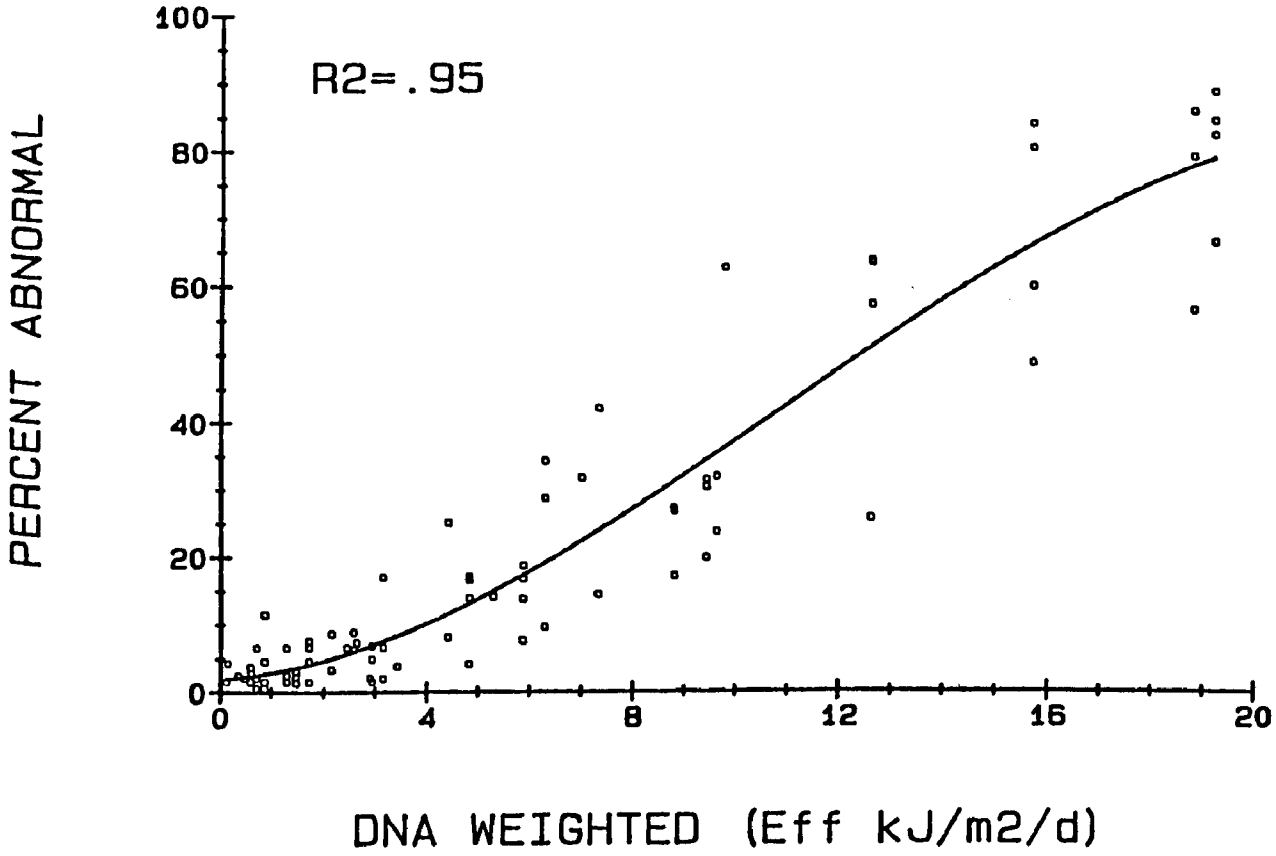

Figure 1. Bivalve Larvae Following UV-B Exposure. Fertilized eggs from four species of bivalves molluscs were exposed to various levels of UV-B for 1 to 6 hours. The eggs were then held through the larval trochophore stage (24-72 hr.) and were examined for abnormal development at the onset of the straight-hinged stage. Data points represent the means of four replicates (n=400). (Unpublished data, Thomson, Worrest, and Robinson)

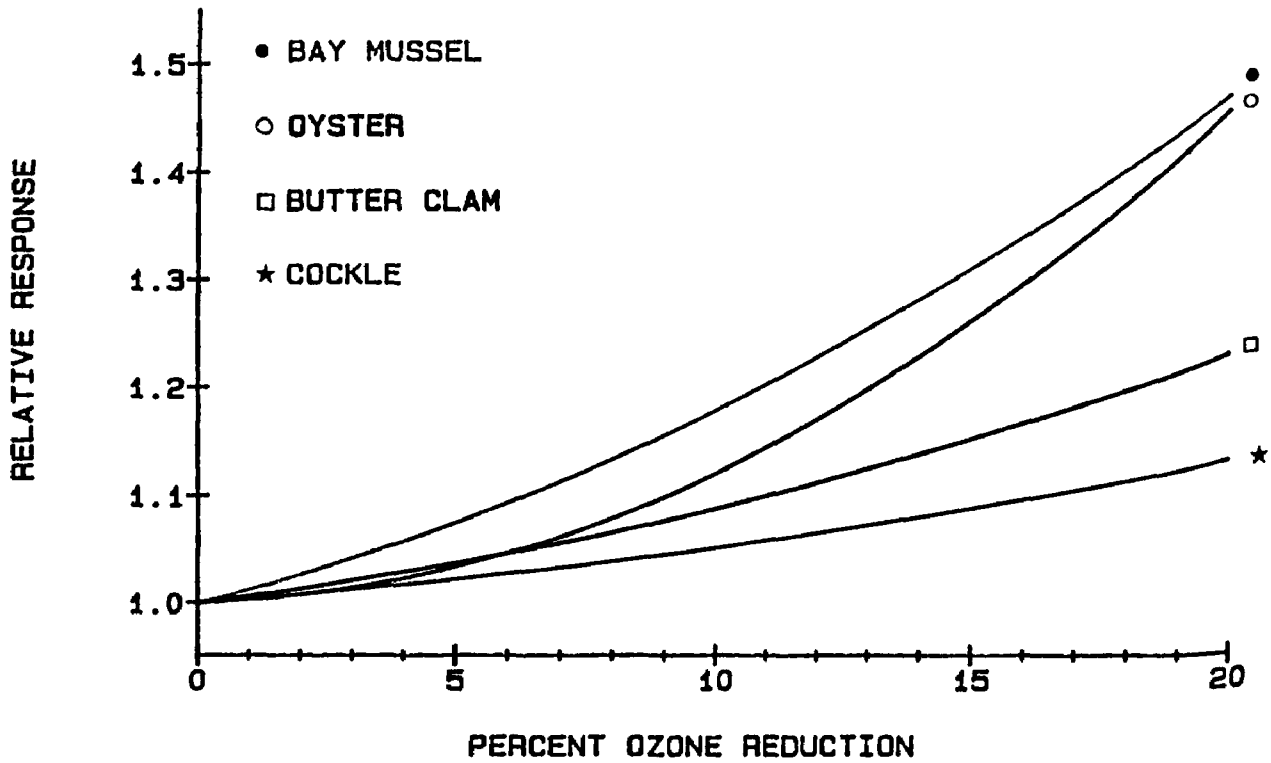

Figure 2. Abnormal Bivalve Larval Development. The response of the four individual species of bivalve larvae to increased levels of UV-B was calculated using the response of that species at standard ozone thickness (0.32 cm-atm) as the reference. The reference levels of UV-B bracketed the spawning season for these species.

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An Estimate of the Role of Current Levels of Solar Ultraviolet Radiation in Aquatic Ecosystems

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ABSTRACT

Organisms that use sunlight are exposed to solar UV-B radiation. Ultraviolet radiation in the UV-B band, 280-320 nm, can produce lethal or mutagenic lesions in DNA and RNA. Successful species must be able to cope with the UV-B exposure; the measures that render solar UV-B tolerable are not often recognized. Solar UV-B exposure may be mitigated by three pathways: avoidance of exposure, shielding, and repair of DNA lesions after injury. It is well known that: (a) many forms of biological activity occur in the early morning or late evening, avoiding the midday when UV-B is most intense; (b) animals heavily exposed to sunlight tend to be shielded by intense pigmentation, while cave or ocean-bottom species are often pale or colorless; and (c) there are multiple DNA repair systems that can repair solar UV-B injury, one of which (photoreactivation) has no known function aside from the ability to repair the type of lesions induced by sunlight. Because the actions that minimize solar UV-B injury (avoidance, shielding, and repair) expend resources that might be used for other purposes, the best adapted species would have no more capacity to cope with solar UV than is actually required. Observations of a number of aquatic organisms suggest their tolerance of solar UV-B is remarkably close to their present exposure. When tolerance and current exposure are essentially equal, then a significant increase in solar UV would be expected to have an adverse effect on many organisms and be especially damaging to species that are threatened by other environmental factors.

INTRODUCTION

Almost all the living organisms familiar to human experience seem to thrive in the sunlight; so it is counter to our intuition that the sun's rays could be a lethal agent which, if increased, might produce ecological disaster for sensitive species. In spite of the general impression, studies show that solar ultraviolet radiation is a potent biological agent that shapes the form

and behavior of most living organisms in such subtle ways that even biological scientists seldom consider.

Humans are among the largest animals, and the limited penetration of UV into tissue might be expected to provide complete and automatic protection from solar UV damage. But this is not the case, and solar UV radiation is as clear a threat to humans as it is to other species. Humans protect themselves from solar UV injury in the same general ways used by other living organisms: avoidance, internal shielding, and repair. People avoid the most intense sunlight (siestas) and reduce it by clothing (sombreros, parasols, sunbonnets, etc). Intense constitutive pigmentation is widespread among humans indigenous to the tropics. Protective tanning of skin is clearly a response to solar UV damage (Giese 1976). It has been recently discovered that individuals lacking DNA repair capacity (suffering from the disease xeroderma pigmentosum) may live if protected from sunlight but suffer early severe cancer if chronically exposed to solar UV (Cleaver 1968). While these forms of protection from solar UV are obvious and well-known, they have often been subject to misinterpretation. It was once thought that people with black skin were protected from the heating effect of sunlight when obviously white, not black, skin would reduce solar heating. If such a large organism as a human requires redundant protection from solar UV, then it is obvious that small organisms may be at extreme risk from solar UV exposure. While the interior of a human is well-protected, all the cells of small animals or plants are exposed. Small animals and especially unicellular organisms, which are more common in aquatic ecosystems, may lack the sensing capacity and the rapid and properly directed mobility required to avoid solar UV; they also may not have sufficient body size to permit an effective internal sunscreen.

While these arguments suggest that solar UV may be a major environmental hazard, more direct evidence is required to determine the particular relationship of solar UV and aquatic ecosystems. The oceans and natural waters cover three quarters of the earth's surface; human life and welfare is intimately bound to the earth's waters and the organisms that live in the seas. A significant part of the human food supply is harvested from the water, a harvest especially rich in protein and generally deficient in plant-derived foods. Clean healthy waters also provide an increasing role in human sports, pleasure, and recreation.

The most likely consequence of stratospheric ozone depletion would be a worldwide increase of solar UV, increasing the present effects of UV on all natural waters. Thus, the possibility that increased UV from the sun could have important consequences on marine life and the multitude of human activities depending on it deserves careful analysis. The aquatic biota, the organisms living in the oceans and fresh waters, seem to be particularly vulnerable to general changes in ozone because all waters would be affected. Furthermore, aquatic organisms are not subject to direct human control. Humans can provide shielding or compensation through strain or specie selection, which might minimize injury in the case of crops, domestic animals, and human exposure; but the options would not be available to aquatic ecosystems. Even if harmful effects were so small as to be individually undetectable, such as a 5% decrease in the harvest of a certain fish, a worldwide accumulation of such low level effects could represent a serious loss of resources.

The objective of the research reported here is to assess currently-available data and develop analytical methods that will permit the prediction of the potential effects of ozone depletion on aquatic ecosystems or will suggest the methods and additional data needed to make such predictions.

PATTERNS OF ORGANIZATION OF AQUATIC ECOSYSTEMS

Photosynthesis, the conversion of radiant energy from the sun into chemical energy (food) is the basis of essentially all life on earth. Photosynthesis by land plants ordinarily proceeds in large multicellular structures (higher plants) at a fixed location and with many specialized tissues and structures that obtain and transport nutrients and water, conduct photosynthesis, ensure reproduction, and protect the plant from various kinds of physical, chemical, and biological stresses.

Aquatic plant life is organized along entirely different patterns (see Russell-Hunter 1970). Photosynthesis is also the source of the energy used by the aquatic biota; but the primary photosynthetic plants are quite different from land plants in body form, reproductive patterns, behaviour, and almost all basic structures. Photosynthesis requires light; but adequate sunlight seldom penetrates to the bottom of natural waters. Thus, aquatic photosynthesis is largely confined to a relatively thin layer at the water surface termed the euphotic zone, the surface layer where plants create more chemical energy by photosynthesis than they use for their own metabolism.

The essential nutrients required by plants in aquatic systems are available only in very low concentration; thus, smaller plants have an advantage as they have a large surface to volume ratio, making them efficient in capturing these essential but diluted nutrients from the water. In fact, aquatic photosynthesis is primarily conducted by single-celled organisms, many species being very small (Russell-Hunter 1970). Microscopic organisms are unable to swim or otherwise move about in the water even against currents of low velocity, and thus are carried with the bulk movements of the waters in which they are suspended. Those suspended organisms carried about with the water are termed plankton ("drifters"). Planktonic organisms capable of photosynthesis are termed phytoplankton, while those organisms that live by eating (grazing on) phytoplankton or other floating organisms are termed zooplankton. Organisms that swim strongly (nekton) are not included with the plankton; however, the embryonic forms of many aquatic higher animals are planktonic. As feeding progresses from the phytoplankton through the zooplankton to fish and other higher organisms (the food web), the chemical energy generated by aquatic photosynthesis becomes more and more concentrated into organisms of larger size, reaching organisms suitable for harvesting for human use.

SOLAR RADIATION

Wavelengths from 200 to 280 nm are termed UV-C and are the most biologically injurious solar UV component when incident on living organisms. Fortunately, UV-C is strongly absorbed by the ozone (O_3) molecules present in the stratosphere, the outer layer of the atmosphere beginning ten to fifteen kilometers (six to nine miles) above the earth's surface (note Figure 1). The strong absorption of UV-C by various atmospheric components (primarily ozone) means that biologically active levels of solar UV-C do not reach the earth's surface.

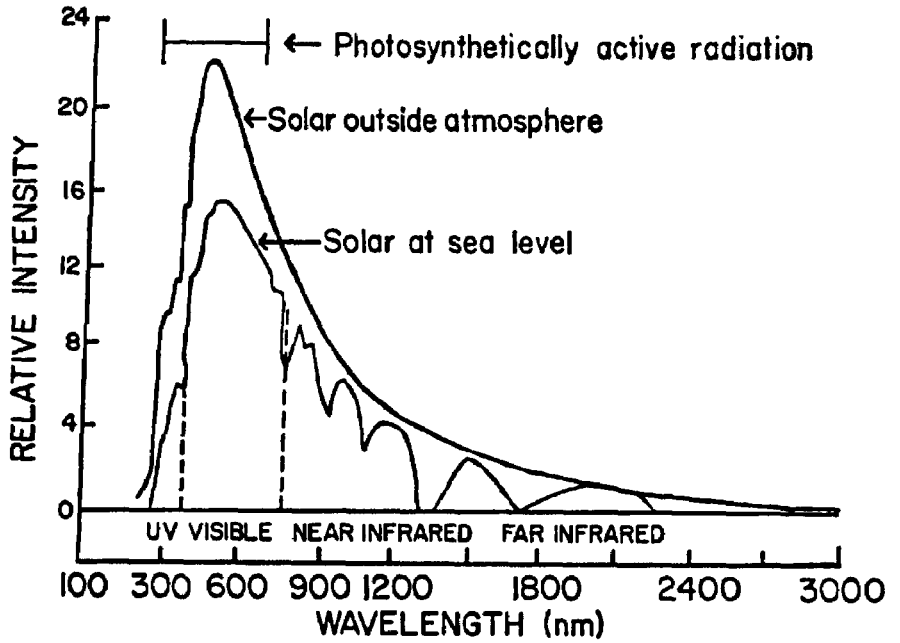

Figure 1a. Solar radiation before atmospheric absorption and after passage through the atmosphere. There is a general absorption and much stronger absorption by atmospheric gases (O_3, CO_2, H_2O, O_2) in particular wavelength bands. Adapted from Giese 1976.

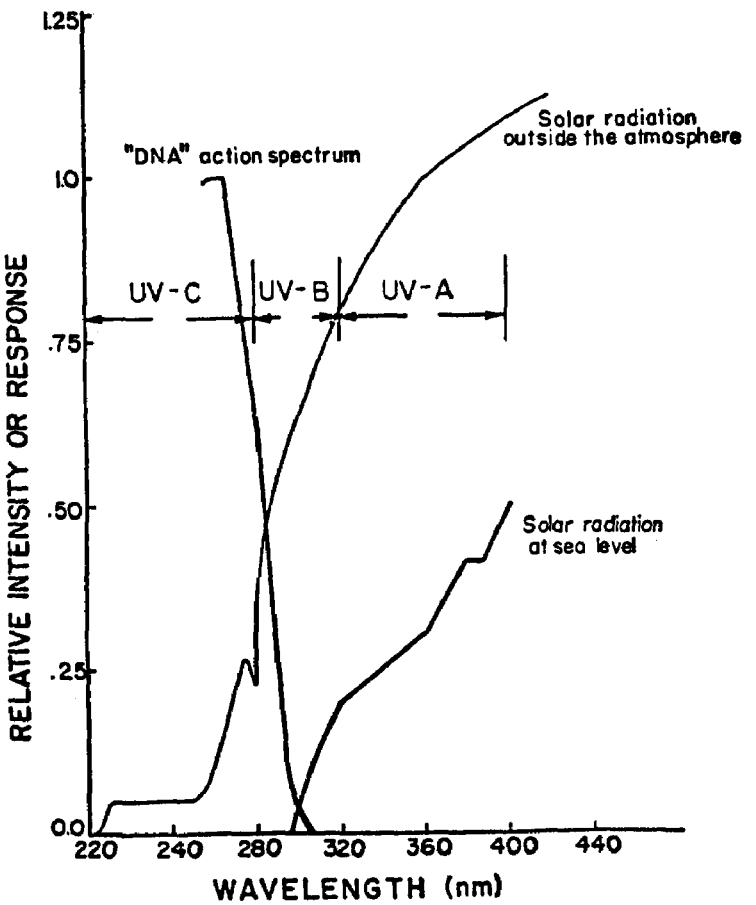

Figure 1b. A comparison of solar UV intensity (adapted from Green and Schippnik 1982) and biological effectiveness (Setlow 1974) as a function of wavelength. Note that the biological action, which is dependent on the product of effectiveness and intensity, results from the crossing tails of two rapidly falling functions and is most significant in the UV-B wavelength band.

Radiation of wavelengths from 320 to 400 nm are termed UV-A. The atmosphere is transparent for UV-A; and this band of radiation from the sun reaches the earth's surface with high intensity; UV-A produces little adverse biological effect. Species easily damaged by UV-A or visible light do not dwell in the sunlight. However, some animals sensitive to UV-A can be found as nocturnal organisms, in caves, or deep in the oceans where sunlight is absent.

Between the UV-C and the UV-A lies the narrow UV-B band of radiation extending from 280 to 320 nm. Small but significant amounts of UV-B are present in sunlight reaching the oceans; and it has a high potential for injury to most living organisms found at the earth's surface. Atmospheric absorption of short wavelength solar UV does not end sharply at 280 nm nor does UV damage begin abruptly at 320 nm. Figure 1 illustrates that the UV-B encompasses a transition zone where solar radiation intensity rapidly falls and the capacity to injure or kill living organisms rises as the wavelength shortens below 320 nm.

The strength of the absorption of various spectral components of electromagnetic radiation in both the atmosphere and in natural waters is often expressed by an absorption coefficient (K). The intensity of radiation incident on the absorber (I_o) is reduced to a value I by passage through a thickness of absorber (Z). It is usually found that the relationship between I, I_o, and Z can be expressed by

$$I = I_o e^{-KZ} \qquad \text{Eq. 1}$$

the absorption coefficient (K) will depend on the nature of the material traversed (air, ozone, clear ocean water, or turbid fresh water) and the wavelength of the particular radiation.

Sunlight is generally absorbed in natural waters, heating the sunlit zone while the lower water remains cold. Because water becomes less dense as it warms, sunlight generates a stable, low density warm layer overlying cold, dense water. The boundary between the warm and cold layers is termed the thermocline and is of great importance in aquatic biology. Wind-generated currents frequently mix the waters above the thermocline producing a mixed layer, but the mixing does not ordinarily penetrate into the dense layer below the thermocline. Because solar energy both generates the thermocline and provides the photosynthetic light for the aquatic plants, it is often found that the euphotic zone essentially coincides with the mixed layer. In small water bodies, cooling the water surface at night produces dense water over the warmer, less dense mixed layer and the entire water body can be remixed. In larger water bodies, the thermocline can remain from spring until cold winter weather cools the mixed layer to the temperature of the water below; then deep mixing will occur and replenish the nutrients, which probably would have been depleted by the photosynthesis in the euphotic zone. A common pattern in fresh water bodies is that deep mixing occurs in the fall or winter as noted above and also in the spring when the cold (0°-4°C), low density surface water is warmed to the temperature of underlying water and the density barrier to mixing disappears.

CONCEPTUAL MODELS

In the absence of definitive knowledge of how a particular human activity will affect the global environment, it seems reasonable to strive to maintain the present condition. However, technologies or activities that become so widespread as to perceptively modify the environment must provide substantial benefits. Some method must be developed to weigh the benefits from the environmentally perturbing activity against the benefits of the status quo. The focus of this paper will be on methods to evaluate the harm that might occur in aquatic exosystems if UV-B were increased. The benefits that may be derived from the technologies that reduce stratospheric ozone and thus increase UV-B are not considered.

There are several ways to quantify the ecological damage from a chemical or agent. In some cases, historical data can be analyzed to evaluate the injury or loss of resources due to the agent. Another widely-used technique is to construct a microcosm, a small scale model of the ecosystem, that can be subjected to the expected levels of the agent. Although there is a strong latitudinal dependence of UV-B intensity, it is not clear how this or any other historical data can be applied to aquatic systems to estimate the impact of increased UV-B. Microcosm research holds some promise, but it is difficult to establish how well the microcosm simulates the natural ecosystem especially for results requiring long (weeks or months) exposures or organisms needing a large range to thrive. A third approach is to measure selected critical parameters, and, by appropriate modeling, deduce some of the probable consequences of various UV-B exposures.

The essence of the analysis is the capacity to quantify the risks from defined changes in solar UV-B. Considering that aquatic ecosystems are outside human control and the relevant data presently available are so limited as to be almost negligible, quantitation would seem to be an impossible task. In the subsequent sections of this paper, approaches are defined that may provide quantitation for limited but important areas of the problem. Quantitation of effect is achieved by developing models that produce responses from relevant and measurable variables; in this case the primary variable is the UV-B intensity and its changes. In general, the models presented are extreme simplifications of actual conditions but are necessary to clarify the nature of the problems.

Lethality Models: Organism Level

The most clearly-defined response to UV-B exposure in aquatic organisms is lethality, and it is particularly easy to quantify the predominant components of the plankton. Certain acute lethality is the most adverse effect that UV-B could exert on a living organism. Methods for assaying radiation-induced lethality are well developed. Although dose-response relationships for lethality can assume many forms, the combinations illustrated in Figure 2 cover the various responses observed in irradiated organisms. Figures 2A and 2B show a threshold type response. Up to a critical (threshold) dose, little or no lethality would be expected; above the threshold a small increment of dose produces a large response. The response illustrated in Figures 2C, 2D, and 2E show the "classical single hit" response often observed in irradiated microorganisms. Single hit response can be expressed mathematically as

$$S = S_o e^{-D/D_o} \qquad \text{Eq. 2}$$

where S is the number of survivors measured after exposure to dose D, S_o is the number of survivors measured with o dose and D_o is a constant expressing the sensitivity of the particular species. Single hit response begins at the lowest doses and implies that some types of single event ("hit") produces lethality; the larger the dose, the larger the probability the organisms will receive a lethal "hit." If a species shows the single hit type response, then even small exposures (and current levels of UV-B) produce some lethality; and it is easy to compute the increased lethality result from increased incident UV-B.

$$\frac{dS}{dD} = \frac{1}{D_0} e^{-D/D_0} \qquad \text{Eq. 3}$$

Substituting $S/S_o = e^{-D/D_o}$ it is evident that the incremental survival per unit dose dS/dD is proportional to the product of $1/D_o$ and the survival ratio S/S_o.

The threshold model suggests that a species may suffer no lethality from given levels of UV-B because exposures fall below the threshold level. For threshold responses, it is important to know the relationship between the threshold dose and current UV-B radiation levels. If the threshold dose is above the exposure, then the organism survives current exposure, and the difference represents a reserve of tolerance that permits a limited increase of UV-B exposure without any increased lethality (the good news). However, the bad news is that if the threshold is exceeded, then a small increment in the dose may produce a large probability of lethality (note Figures 2a and 2b).

It is possible to simulate solar UV-B or use real sunlight to determine UV-B dose-response curves. It is also possible to estimate the typical solar UV-B exposures that individuals will receive in natural waters; from such estimates the incremental killing of organisms showing "single hit" response and the reserve tolerance of threshold type responding species can be computed.

Lethality Models: Population Level

If data on individuals can be obtained, then the response of individuals should be applied to populations to define the expected ecosystem response. A traditional modeling scheme (illustrated in Figure 3) might use the Lotka-Voltera approach. We may assume a three-species ecosystem having: a primary producer, which might be a bacteria (or photosynthetic algae, etc.); an omnivore or primary herbivore, represented by a protozoan (but could be rotifer, arthropod, etc.); and a secondary carnivore flatworm (arthropods or fish, etc.). This model ecosystem can achieve a stable population distribution. If we assume, for illustration, that only the middle member of the food web would be subject to an abrupt increase of solar UV-B damage, then its population would be depressed but there would also be a population-restoring tendency because the food supply would increase and the predation would (after a time lag) decrease. Considering the complexities of even this simplified model and the vast difference between it and an actual aquatic food web, it is very unlikely that this form of modeling will provide useful or believable conclusions for evaluation of solar UV-B effects.

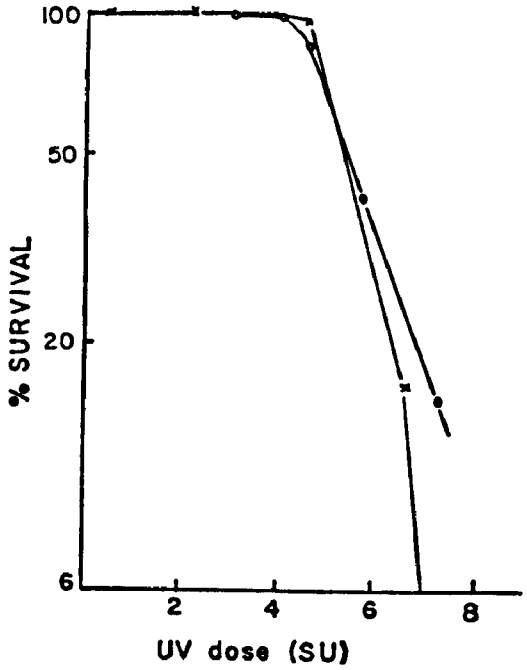

Figure 2a. Survival of the algae Chlamydomonas exposed to simulated solar UV-B and real sunlight, (modified from McKnight and Nachtwey 1975). Doses were measured using the Robertson-Berger meter; the good response agreement for the two very different wavelength distributions indicate the Robertson-Berger meter weighting distribution is very satisfactory for this organism. Note the sharp threshold; the algae survive without measurable injury up to 5 SU but when this level is exceeded mortality is very large.

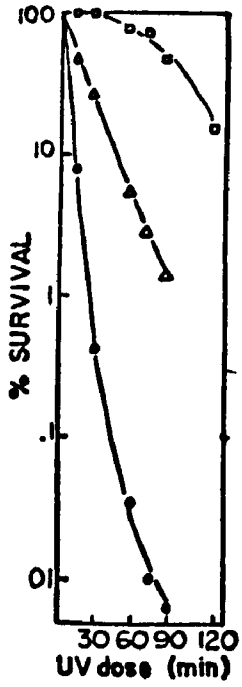

Figure 2b. The killing or repair competent yeast (N) and two repair defective strains (A, B) by sunlight (from Resnick 1970). Note that substan tial killing occurs in a matter of minutes and that repair defective strains show "single hit" killing, while the wild type yeast shows the threshold type response.

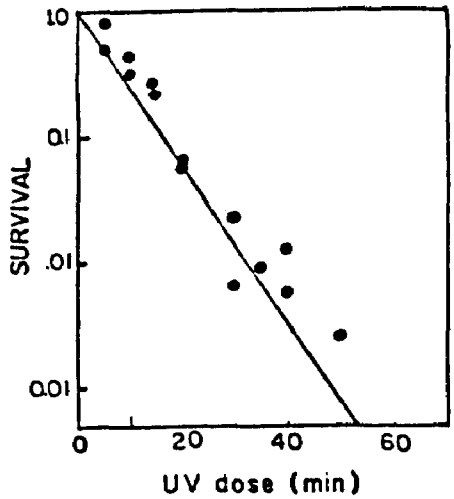

Figure 2c. The killing of E. coli by natural sunlight (Lukeish 1946).

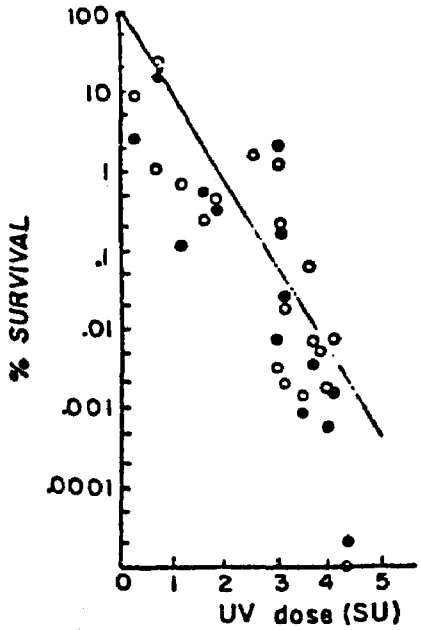

Figure 2d. The killing of coliform bacteria by sunlight in a waste-water lagoon system (Calkins et al. 1976).

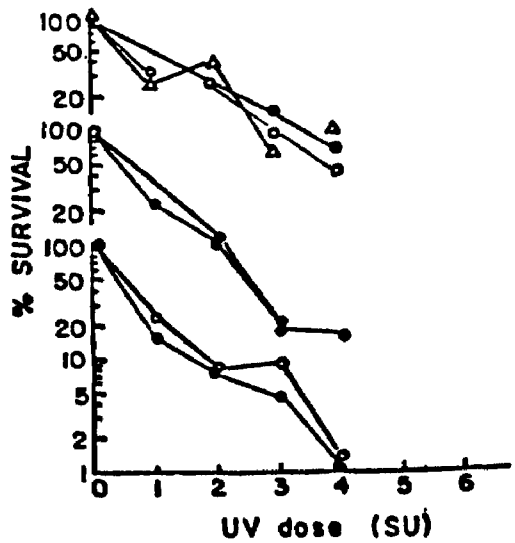

Figure 2e. The killing of three marine diatoms collected from the waters off Iceland by simulated solar UV-B (modified from Calkins and Thordardottir 1980).

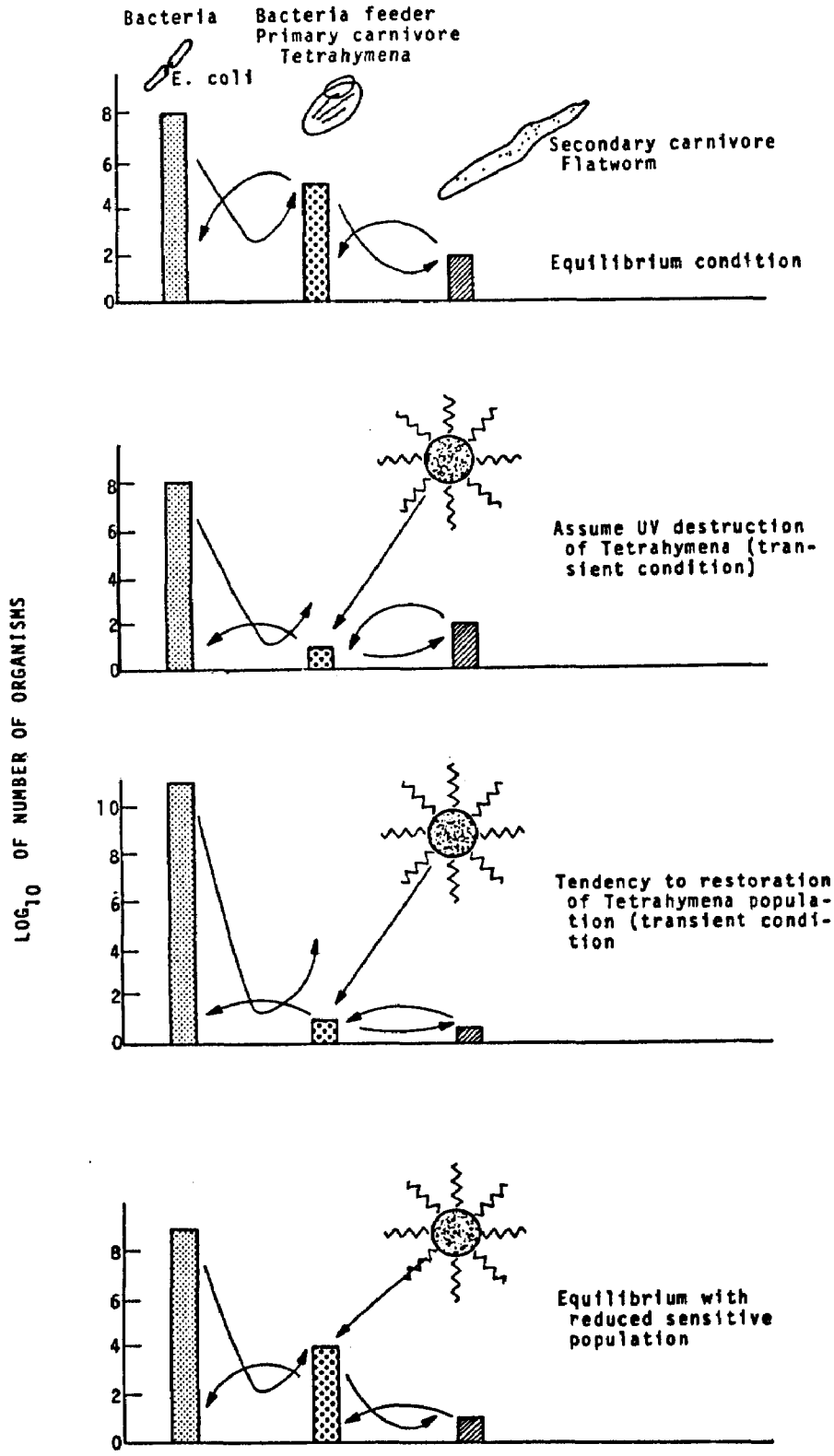

Figure 3. A Schematic Representation of Equilibrium-Type Modeling of a Three-Component Ecosystem With and Without UVB.

An even simpler model provides some definitive and surprising conclusions. While the level of depression of a particular population by a newly introduced agent such as UV-B seems beyond computation, it is possible to determine the maximum level of an injurious agent (UV-B) that a particular species can tolerate. If the daily UV-B incident (given optimum growth conditions and with all other sources of lethality such as predation eliminated) produces lethality that exceeds the capacity of the subject population to replace the killed component, then population will, under prolonged treatment, be depleted to extinction (Figure 4; Calkins 1974). In actual ecosystems, optimum growth conditions are seldom found and most aquatic species are food for some predators; however, if a population cannot survive with the most favorable conditions, then it clearly could not survive in the real ecosystem. In the laboratory, it is possible to determine the parameters for the maximum tolerable dose (the replacement limiting dose, RLD): the optimum growth rate, the growth delaying effect of radiation, and the dose response for lethality. When the RLD is compared to the actual exposure in nature, one obtains an estimate of the reserve of tolerance a species may have for increased solar UV-B.

EXPOSURE MODELS

There are a number of ways of establishing the UV-B levels incident at the water, which we consider below. Because natural waters are semitransparent for UV-B wavelengths, Equation 1 can be used to determine UV-B intensity at any particular depth and thus, knowing the UV-B intensity at the surface and the depth of the organism, the instantaneous exposure can be computed (See Figure 5). In some cases, aquatic organisms hold fixed positions such as at the bottom of a shallow pond or stream. However, a fixed vertical position is rare and some definitive knowledge of position is required.

Planktonic organisms in many cases are moved with the water mass in which they live. When there is effective wind-driven mixing of the layer above the thermocline, a common condition, then the organism receives the average exposure (I_{av}) in the mixed layer, i.e.,

$$I_{av} = I_0 \frac{(1-e^{-KZ})}{KZ}$$

Source: Morowitz 1950 Equation Eq. 4

where z is the depth of the mixed layer and the other symbols have the same significance as in Equation 1 (See Figure 5b).

PRIMARY PRODUCTIVITY MODEL

Solar UV directly reduces the rate of photosynthesis in plants. The spectral distribution of this inhibitory effect is quite different from the lethal effect. Because long wavelengths of UV and even visible light inhibit photosynthesis, ozone reduction producing a small increase in UV-B would appear to have a negligible effect on the primary photosynthesis in aquatic systems (Lorenzen 1979; Smith et al. 1980).

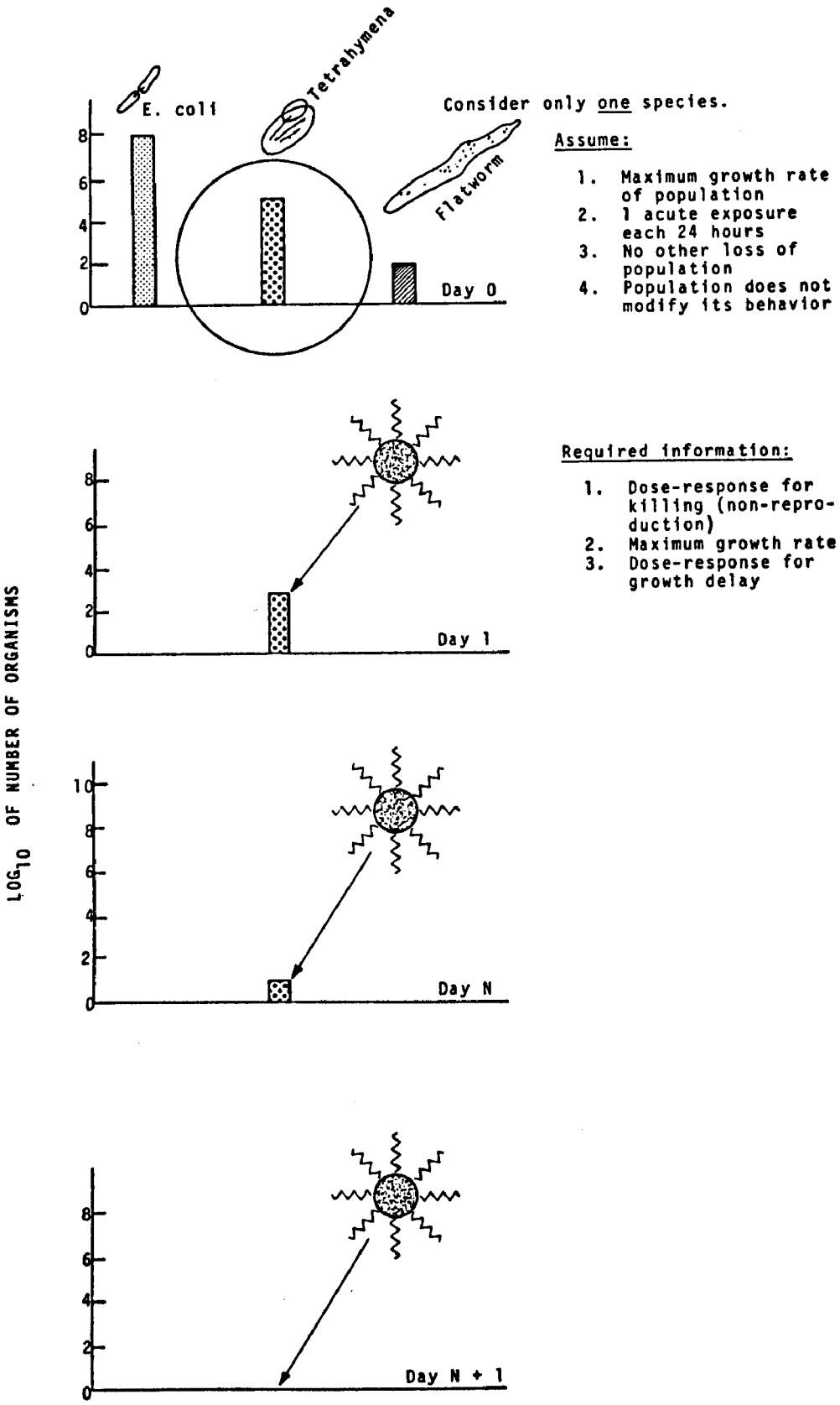

Figure 4. The Replacement-Limiting Model

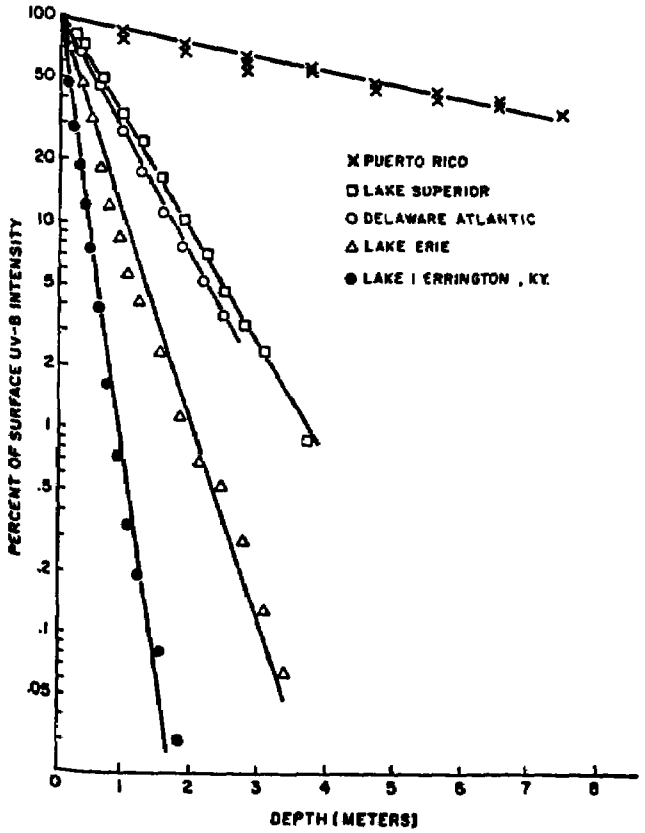

Figure 5a. The attenuation of UV-B as measured by the Robertson-Berger meter in some representative natural waters.

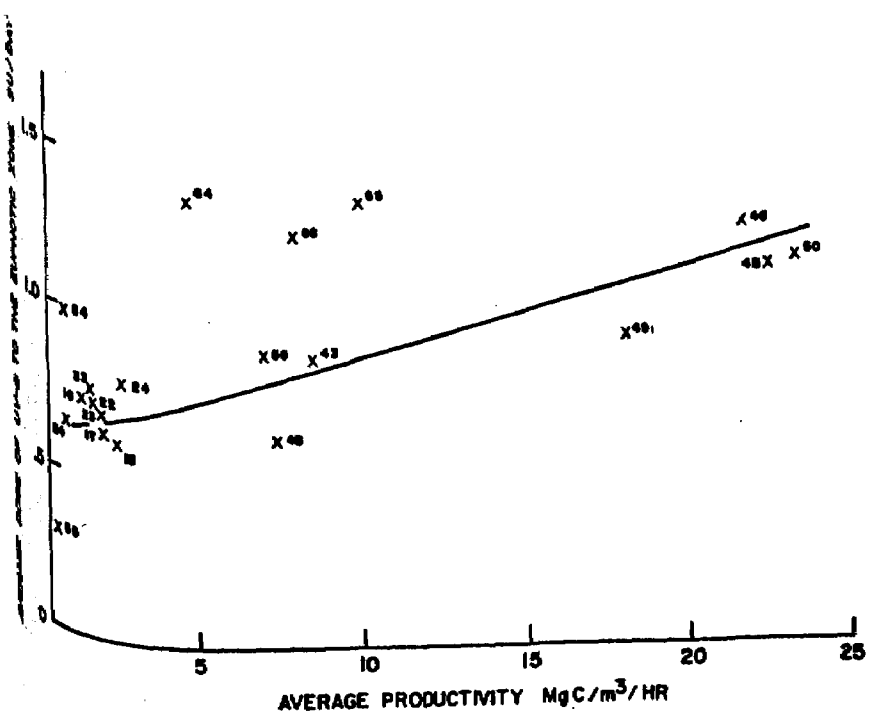

Figure 5b. The average UV-B dose to the euphotic zone at various locations off Iceland as a function of the productivity of the waters. It is assumed that 10 SU per day are incident at the water surface.

There are indirect ways by which the increase of UV-B might produce a larger effect than predicted from the direct photoinhibition of photosynpthesis. Killing phytoplankton should show the DNA type action spectrum and thus reduce photosynthesis in a way similar to other lethality. However, the effect of lethality on photosynthesis would require much longer times to develop than is required for measurement of direct photoinhibition. If lethality contributes to the loss of photosynthesis capacity, it must be modeled by more complex models. There are very few data on UV-B killing algae and also little information as to the vertical location at various times of day. Considering the relatively large attenuation of UV-B in the more productive waters (coastal and upwelling zones), it would appear that the phytoplankton are well protected from UV-B.

Another approach has been proposed. Although photosynthetic productivity measurements are usually made with samples held at fixed depths for a half or a whole day, mixing is common in natural waters. In the well-mixed situation, all the photoplankton receive the same amount of light per day and the same UV-B exposure. A "ferris wheel" model has been proposed (Calkins et al. 1982) where algae enter the circulation (by rising) and are transported (by Langmuir cells, etc.) along the surface where they accumulate a UV-B exposure that produces a sinking (increased density) stimulus that removes the algae from the circulation into the stable water at or below the thermocline (Figure 6). The postulated cycle would ensure that the phytoplankton species received the maximum possible photosynthetic light without exceeding its UV-B limits. If UV-B exposure is not random, but is controlled by the organism, then an increased incident UV-B will reduce the time the organism remains in the mixed layer. The loss of photosynthetic light due to UV-B increase will be directly related to the ratio of absorption coefficient of UV-B and photosynthetic light in the mixed layer (Calkins 1982), quantities that are easily measurable.

BIOLOGICAL DOSIMETRY

The solar UV-B is the principal parameter of all the analytical models. This might seem to be an easily determined quantity but, referring back to Figure 1, it should be noted that the biological effectiveness of UV-B energy drops by a factor of 1000 or more as the wavelength changes from 290 to 320 nm. Clearly, it is impossible to simply measure UV-B intensity without distinguishing between components that differ 1000-fold in biological action. Ideally, UV-B dose should be measured in very small wavelength bands and then each multiplied by an appropriate <u>biological weighting factor</u> to obtain the instantaneous intensity (Figure 7). The product of the intensity times the time of its application for the entire exposure should be added to give the <u>biologically weighted dose</u>. This is a difficult procedure requiring a high resolution spectroradiometer and definitive knowledge of the proper weighting factor which may not be known for the organism under study.

The physical devices used for measuring solar UV intensity usually measure the quantity termed <u>irradiance</u> (power per unit area in Watts/m^2) and the summation or time integral of this quantity is termed the <u>irradiation</u> (energy/unit area, commonly expressed in Joules/m^2). The biological weighting unit is simply an efficiency ratio so the biologically weighted dose has the units of irradiation, J/m^2.

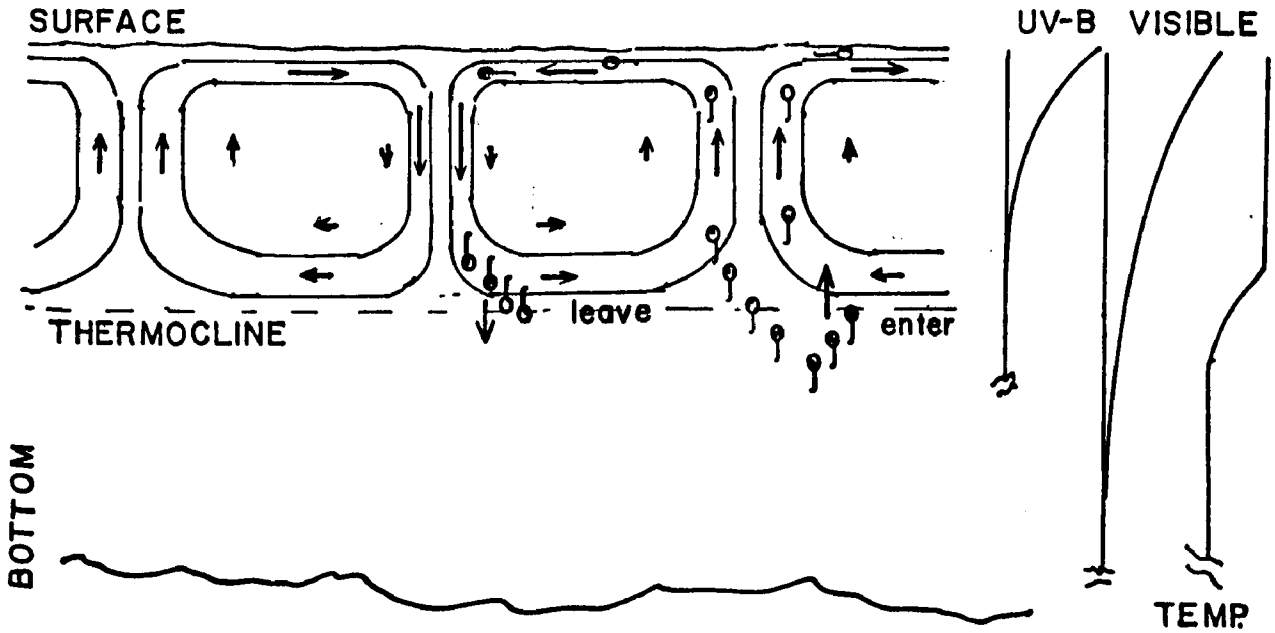

Figure 6. The "Ferris Wheel" model of control of UV exposure. It is suggested that aquatic organisms may use the motions of the mixed layer to obtain the average light exposure. At dawn, the phytoplankton rise into the circulation and ride the currents. When the UV-B exposure becomes limiting, the algae move downward out of the circulation into the still waters under the thermocline. A small capacity for movement plus the circulation in the mixed layer permit the weak swimming phytoplankton to control their position for maximum photosynthetic light while receiving a tolerated UV-B exposure. Increased UV-B would reduce the time spent in the mixed layer each day.

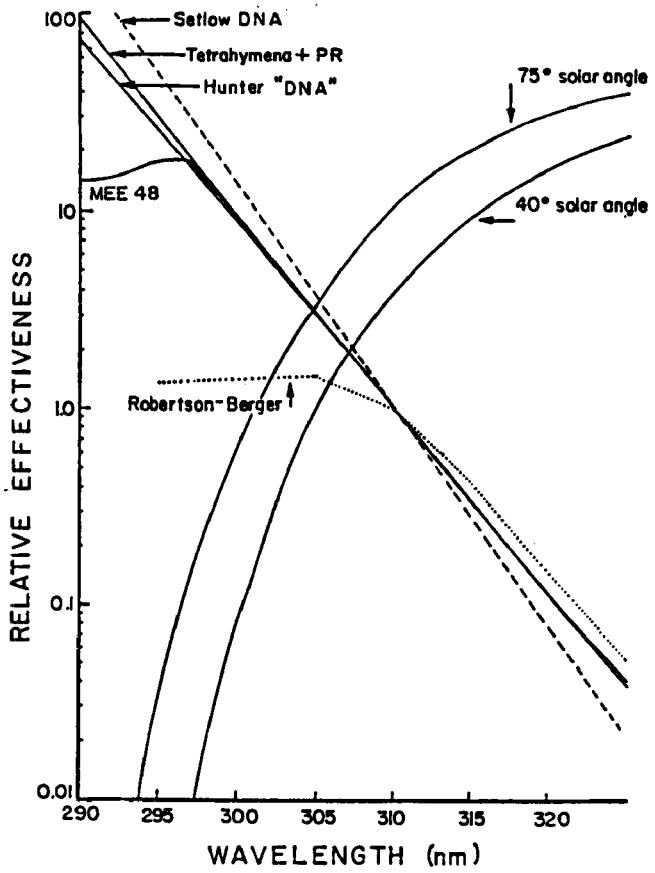

Figure 7a. A logarithmic plot of biological effectiveness and solar UV intensity for two sun angles (.32 cm O_3). The 75° solar angle approximates the highest solar angle in the temperate zone, the 40° solar angle would be more typical of the temperate zone incident UV-B wavelength distribution since much of the UV energy arises when the sun is at lower solar angles (adapted from Shettle and Green 1974). Examples of the trend of biological responses used to generate weighting functions for UV-B dosimetry. The Set Low DNA function (from Peak et al. 1984) fits many observed responses; the Hunter DNA (from Hunter et al. 1981) is a modification of the Setlow DNA fitting fish larvae response; the MEE 48 (Cole et. al. 1986) is a response fitting skin cancer production in mice; the Tetrahymena+ PR is the response of the protozoan after maximal photoreactivation (unpublished observations). The Robertson-Berger (from Berger 1976) shows the response of the sunburning meter widely used to measure biologically weighted UV-B intensity.

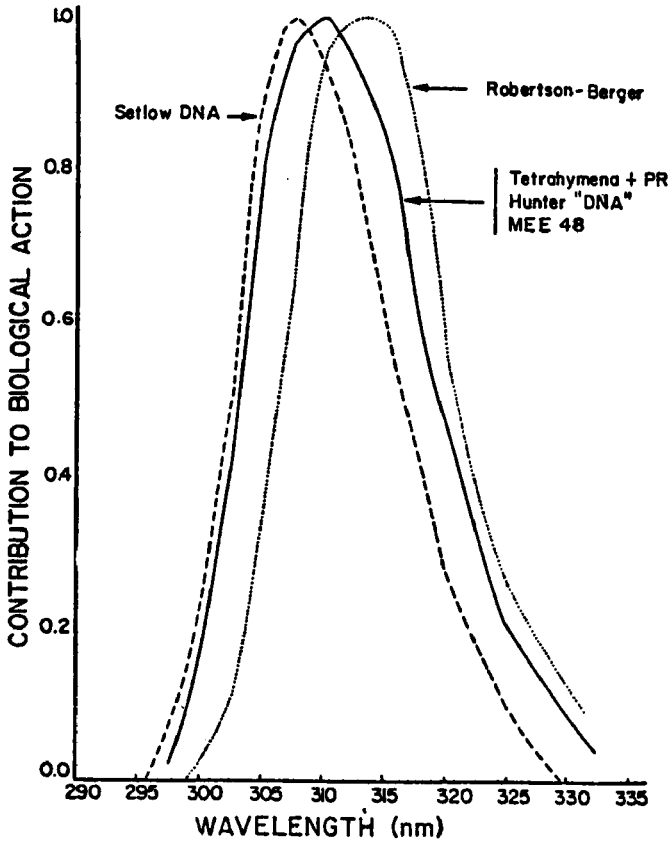

Figure 7b. A normalized wavelength contribution to biological action for a solar angle of 75%, computed from data in Figure 1. The area under the curves would measure a "biologically weighted" iradiance. Note that the Robertson-Berger meter tends to underweight the short wave components while the Setlow DNA tends to slightly overweight the short wavelength components compared to the three actior alternative spectra that produce essentially identical weighting.

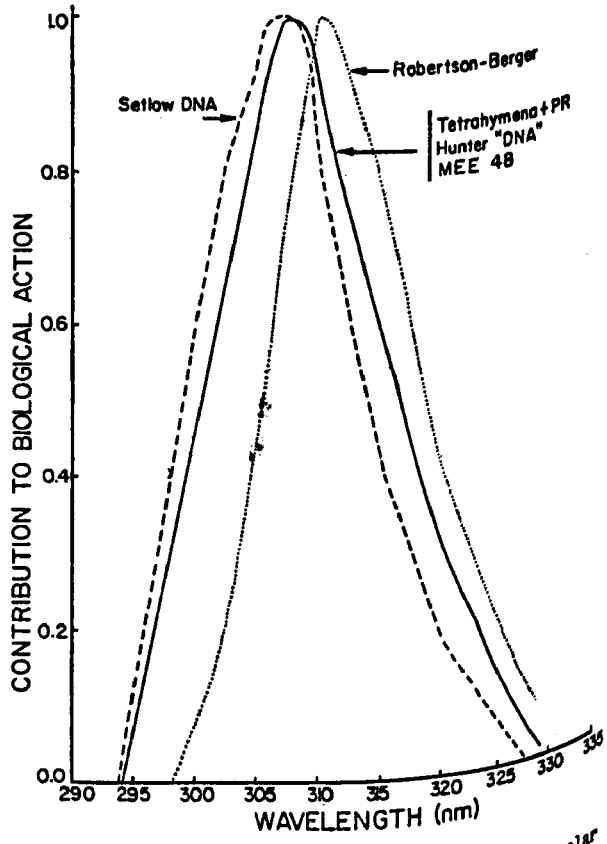

Figure 7c. Biological action for a solar angle of 40° plotted as in Figure 3b.

Biologically weighted dose is often calculated from a knowledge of the spectral distribution of the irradiation and a weighting factor derived from action spectrum (the measured relative effectiveness of different wavelengths to produce some particular biological effect) known or assumed to be appropriate for the organism under study. The "DNA" action spectrum has been most widely used to derive weighting factors but this action spectrum does not seem appropriate in some cases. Another widely used device for obtaining biologically weighted doses is the Robertson Berger meter. This instrument measures irradiation (expressed in a special unit termed the SU) including a weighting factor. Approximately 20 SU are incident on a sunny midsummer day in Lexington, Kentucky; about 10 SU on a similar day in Reykjavik, Iceland.

Organisms that show shouldered survival responses can be analyzed for reserve tolerance to UV-B. Among the Icelandic diatoms, the two strains of Thalassiosira have reserve tolerance in excess of the typical UV-B exposure they would receive in the mixed layer. The other organisms are at present close to the threshold dose. Tetrahymena exhibit a threshold dose about twice the average daily exposure in a typical 1-m deep pond. Most other organisms we have tested, showing threshold type survival responses, have the threshold dose below the average daily exposure in a 1-m deep pond (Figure 8). It is possible that they do not live in such shallow ponds or that they have special sensing and remove themselves when UV-B irradiance is excessive. The important point is that making crude but quantitative estimates of exposure and factors critical to tolerance of UV-B shows that most of the aquatic organisms tested are very near their tolerance limits.

REPLACEMENT LIMITING DOSE

Figure 8 shows the replacement limiting dose (RLD) computed for a wide variety of aquatic organisms compared to the exposure they might receive on a sunny midsummer day using the models and data already noted. Because the RLD is the upper limit of a species-tolerance, it is evident that some additional protection against solar UV must be included in species currently exceeding their RLD. Some of these factors have been noted. Organisms reduce solar UV exposure by sinking to the bottom during high UV periods or collecting at sunlight shielding objects in the water. The filamentous algae Rhizoclonium in a habitat where exposure exceeds the computed tolerance remains at the surface of the ponds where it was collected, but all new growth is downward under the protection of old moribund filaments. Coliform bacteria die out in natural waters in a way suggesting that solar UV is a major cause of lethality. However, their UV-B tolerance is great enough to suggest that they can survive in natural waters long enough to transfer from host to host.

The models used in this presentation are great simplifications of actual ecosystems. It might be concluded that tolerance and exposure just naturally come out approximately equal. Figure 9 shows that this is not the case. The replacement limiting dose of ionizing radiation (cosmic rays and natural radioactivity) can be computed in exactly the same manner as was done for solar UV. It is evident that tolerance to ionizing radiation vastly exceeds exposure while these two factors are almost identical for solar UV. One can conclude that current levels of solar UV represent a positive evolutionary force. Each species must have the observed UV-B resistance to live in its particular ecological niche, a value very close to the exposure level. A mutant that might gain resources by losing a protection factor (such as not

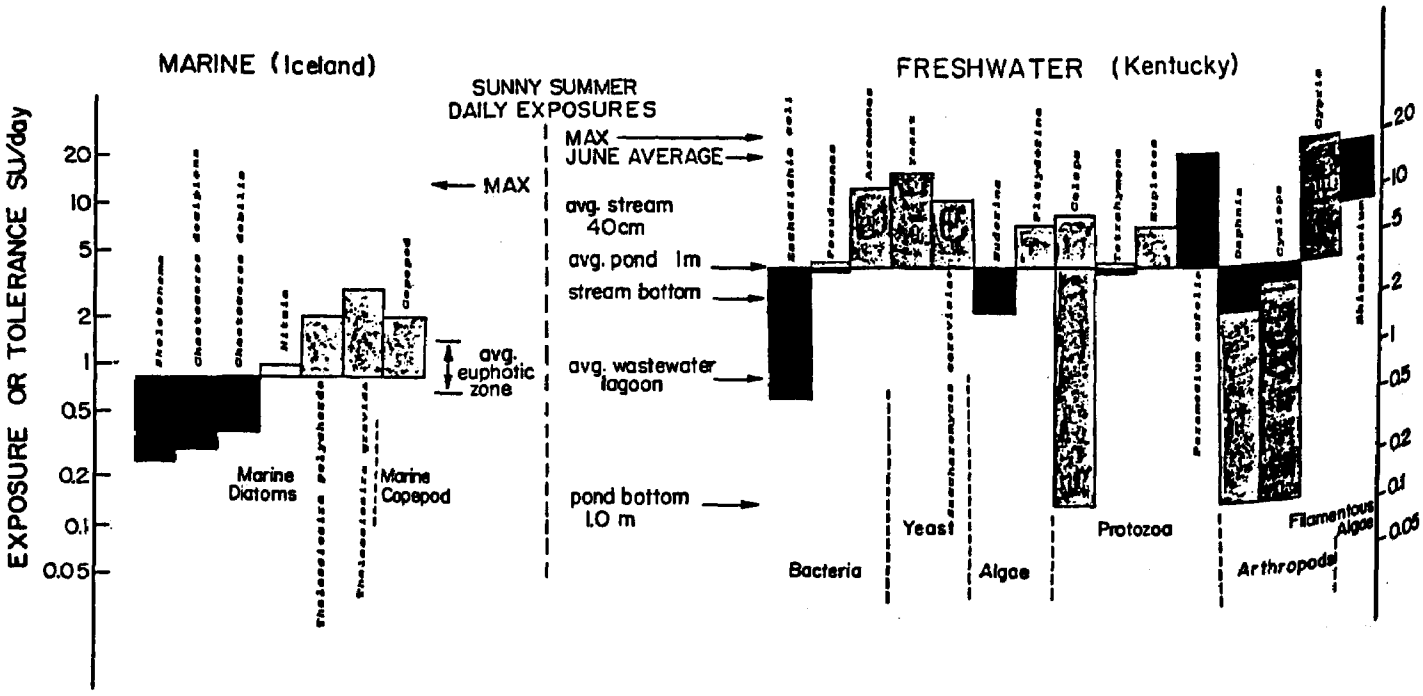

Figure 8. A comparison of solar UV-B exposure and tolerance of various organisms. Dark shading indicates exposure exceeds tolerance; light shading indicates tolerance greater than exposure (see text for methodology and discussion.)

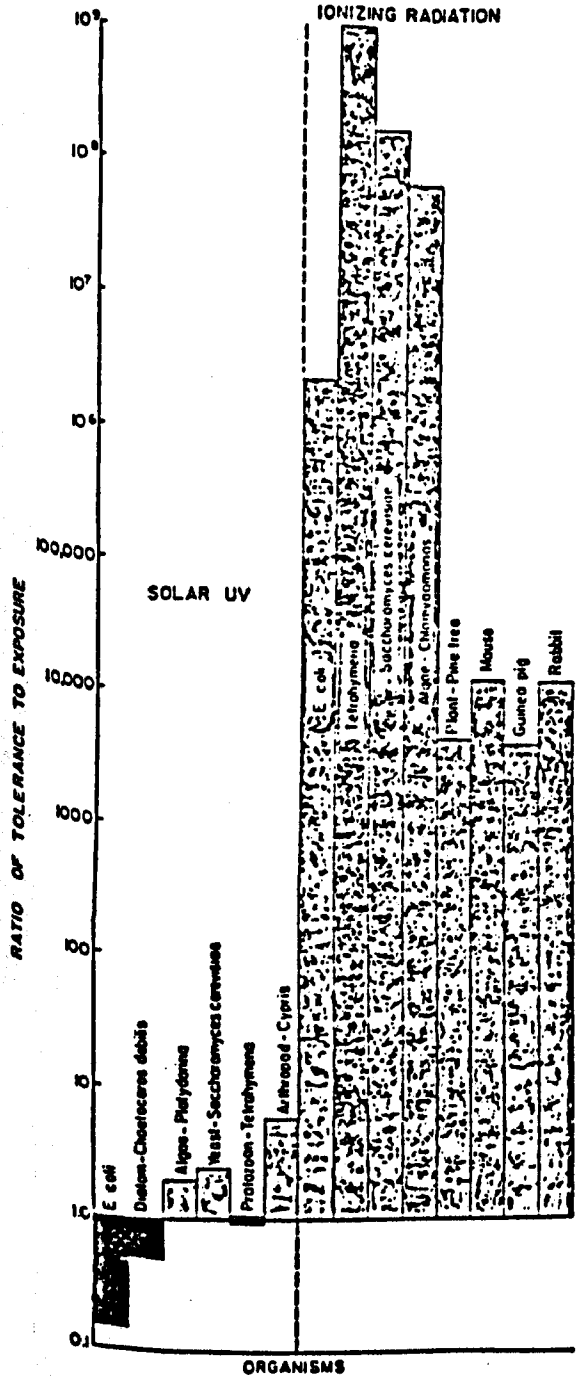

Figure 9. The ratio tolerance to exposure for solar UV (left panel) and natural ionizing radiation (right panel). The appropriate equality of tolerance and exposure of solar UV suggests resistance to solar UV to be an active characteristic which would be maintained in the population. The large excess of tolerance over exposure to natural ionizing radiation suggests that resistance to ionizing radiation may arise indirectly and characteristics that increase resistance to ionizing radiation but produces no other effect would not be strongly favored.

making a repair enzyme or a protective pigment or not avoiding high UV exposure) will be killed by the UV. The best adapted strain has exactly the UV-B tolerance required but does not waste resources protecting itself from UV levels not actually encountered. If this status (tolerance equals exposure) is the current condition as indicated by the available data, then it must be concluded that an increase in solar UV-B levels will perturb aquatic ecosystems; they then will lose some of the available photosynthetic light upon increased UV-B. Figure 10 shows the simplest mode, algae that occupy fixed positions. From Figure 11 one can see that the loss of photosynthetic light (PSL) will equal the percent increase in UV-B times the ratio of the attentuation coefficient for photosensitive light (K_{ps1}) divided by the attenuation of UV-B (photosynthetic) (K_{vis}) in the water.

$$PSL\ (\%) = UV\text{-}B\ (\%)\ \frac{K_{PSL}}{K_{UV\text{-}B}} \qquad \text{Eq. 5}$$

For organisms that circulate in the mixed layer but remove themselves below the thermocline upon excessive UV-B exposure (see Figure 11 for a potential model of this effect), the loss of light approximates the same ratio but is somewhat more complex to compute and depends on the particular assumption regarding depth of the mixed layer, incident UV-B, and tolerance limits. In any event, if the phytoplankton have mechanisms to limit UV-B exposure by avoidance and if this mechanism is utilized with current levels of UV-B, then enhanced UV-B would replace the visible light available for photosynthesis presumably without increasing lethality.

DISCUSSION AND CONCLUSIONS

It is very difficult to evaluate the potential effects of solar UV on aquatic ecosystems. The systems are so dynamic and human consequences, such as fish catch, are so variable that any attempt to directly measure small effects would be "lost in the noise." The laboratory simulations and microcosm data suggest that enhanced UV-B would modify aquatic ecosystems, but it is difficult to judge how much reliance can be placed on conclusions derived from laboratory experiments or microcosms where the subject species is exposed to far fewer variables than in natural systems.

The "replacement limiting dose" model and corresponding data suggest that many species are at present close to their tolerance limit for solar ultraviolet radiation. Various species show higher or lower UV-B resistance in close correlation to their exposure and lifestyle. If solar UV is presently a significant limitation on aquatic organisms, then changes in incident UV-B will probably produce ecological changes. The changes could in fact be favorable, for example, elimination of coliform pollution of water in a reduced time. However, in the absence of convincing evidence as to the nature of changes that might occur, it seems prudent to try to maintain the status quo.

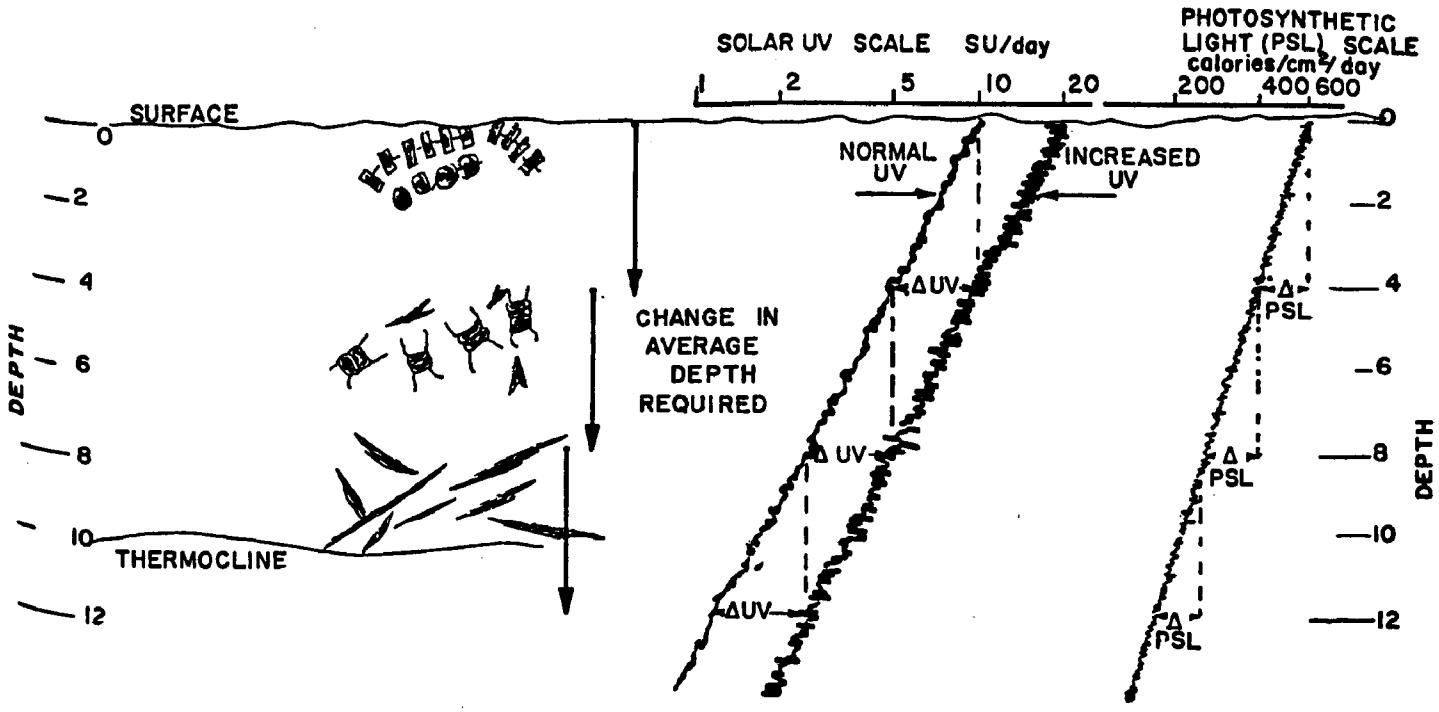

Figure 10. A model illustrating the loss of photosynthetic light whenever organisms are forced to move deeper in the water column to avoid increased UV-B.

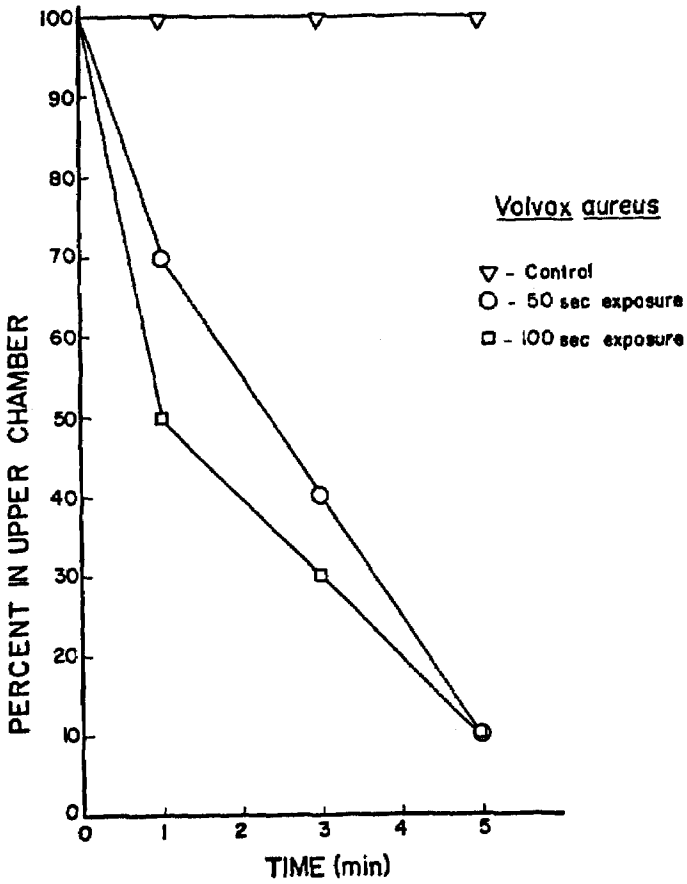

Figure 11a. The time course of movement of Volvox Aureus after exposure to real sunlight and a control. After exposure, the algae were layered on the top of a cylinder. The data points indicated the percentage remaining in the upper half of the cylinder.

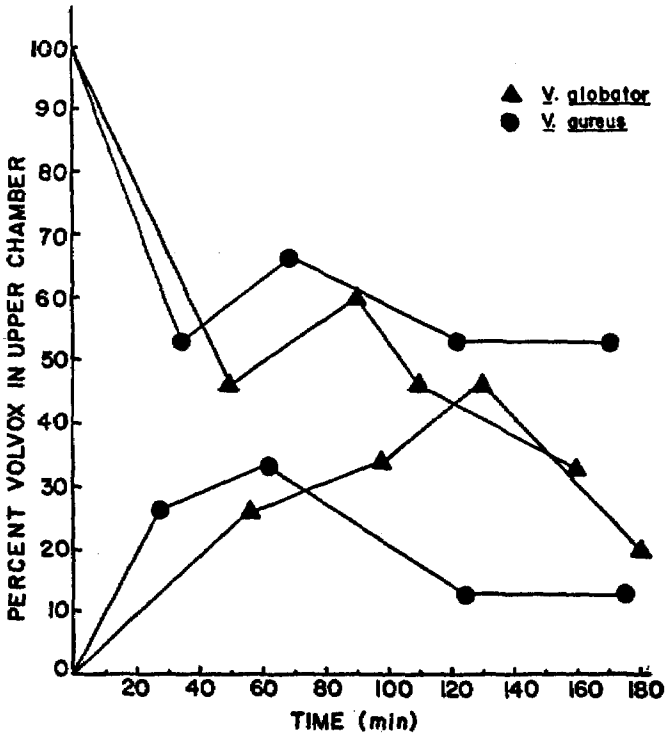

Figure 11b. The time course of movement of two specieis of Volvox in the dark without prior UV exposure. Samples were placed at the surface and the bottom of the test chambers.

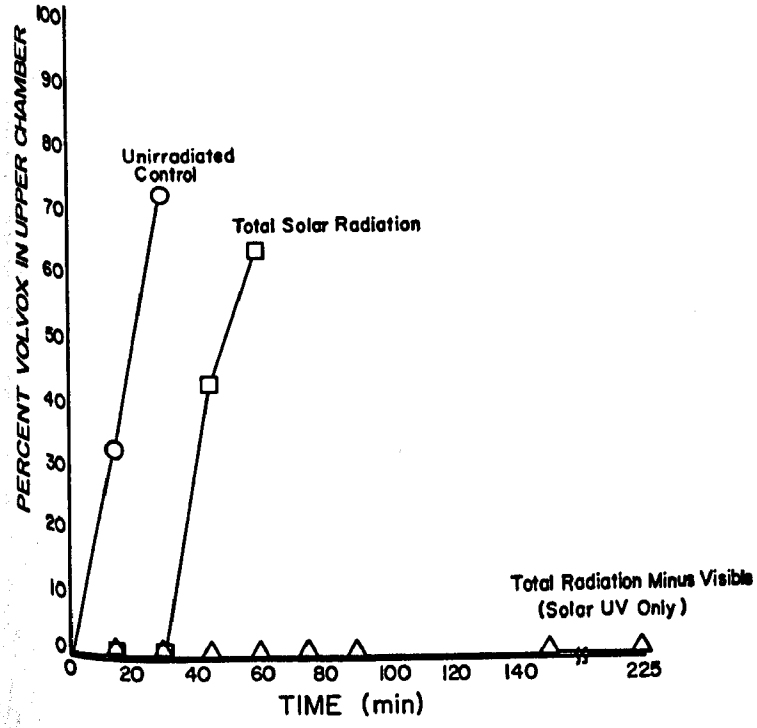

Figure 11c. The movement of Volvox aureus under exposure of an irradiance of 5.2 W/m^2 light from Westinghouse Agrolamps. The "unirradiated" control had been held in room light, approximately 1.6 W/m^2 prior to testing. The two test populations (N and A) were exposed to June sunlight for 5 minutes. The squares received the total solar irradiance. The triangles were exposed under UV transmissive Schott glass filters. They received essentially the same total UV irradiance as the "total solar irradiation" group but with the visible component removed. Note that although the irradiance was much less in UV-only group, the depression of upward movement was extreme. The UV-only group appeared to be killed upon subsequent observation, while the total irradiation group appeared viable.

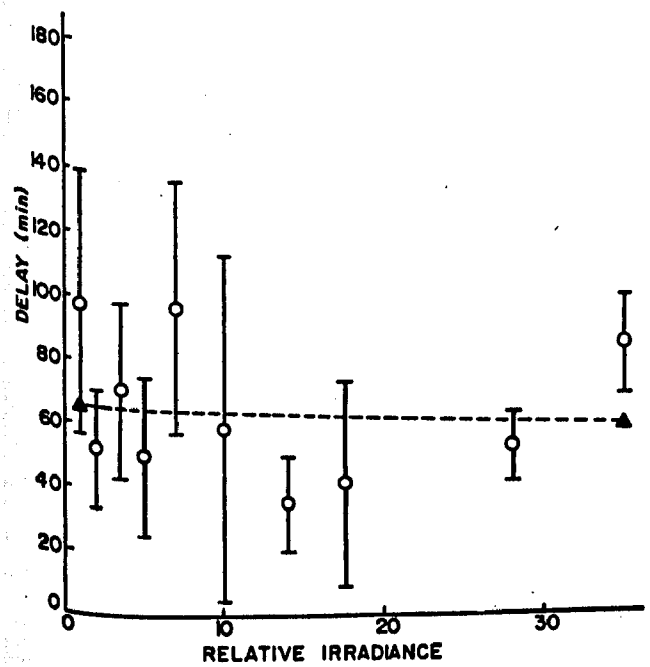

Figure 11d. A test for reciprocity. The points represent the mean of at least 4 tests using irradiances ranging from 1.0×10^{-2} to .37 W/m^2. The same total irradiation of 22 J/m^2 was delivered. The delay in minutes for 50% of the irradiated population to move into the upper chamber when started at the bottom over the time for controls to make this movement was considered to be the delay induced by the UV radiation. The source of the UV radiation was 2 to 4 FS-20 sunlamps filtered by a .3cm Pyrex glass filter and a UG-5 Schott glass filter, 1mm thick.

The more direct models of aquatic photosynthesis suggest that increased solar UV-B would have little effect on primary productivity. On the other hand, some observations suggest that solar UV-B acts as a limit on light exposure for some of the phytoplankton. If there is a mechanism whereby algae sense and avoid excessive UV-B exposure by restricting their time or location, then such organisms, although they would survive as well as they do at present, would reduce their photosynthesis (if light limited) by an amount computed from Equation 5. Using absorption coefficients from natural waters, the primary productivity loss for a 10% increase in UV-B leads to a loss of visible light from 3% to 5%.

There is not an adequate data base to reach firm conclusions or predictions as to how aquatic systems whould respond to changed solar ultraviolet radiation. Aquatic species survive large short-term changes in solar UV exposure. But the near equality of exposure and the tolerance suggests present average levels of solar UV to be a limiting factor for many species, and thus, if the average solar UV-B level were to increase, there would be shifts in the composition of aquatic ecosystems. Only much more extensive research could suggest the magnitude of the shifts and which species might benefit and which species would lose.

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How Might Enhanced Levels of Solar UV-B Radiation Affect Marine Ecosystems?

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ABSTRACT

One of the most challenging tasks facing environmental scientists is to determine the likely consequences of an external change for complex natural ecosystems on the basis of observations of the impact on individual organisms or processes. Assessing the potential impacts of enhanced solar UV-B radiation on the marine environment presents such a challenge. The potential UV-B stress is broad-scale; the response of marine ecosystems involves many spatial and temporal scales, and also involves subtle changes that may be difficult to detect. A particularly acute concern exists, however, for lasting changes--changes made possible because of long residence times for many oceanic processes, as well as the lack of mitigating measures given the spatial extent of both stress and effects. Thus, acknowledging the speculative nature of extrapolations, the potential problems are of a quality requiring significant a priori examination. In this paper, I suggest several types of ecological changes of concern. The examples--including possible effects on food webs, fisheries, and biogeochemical cycles--are chosen to illustrate the scope and urgency of the problem.

UV-B INCREASE AND MARINE ECOSYSTEMS: WHY SHOULD WE BE CONCERNED?

Every type of environmental alteration seems to carry special features that make assessment and resolution of potential ecological problems difficult. In the case of projected stratospheric ozone depletion and increased UV-B striking the earth's surface, the spatial scale of the environmental modification is one such confounding feature. The global nature of the problems brings associated challenges: the task of observing ecological changes, perhaps subtle, across the vast expanse of the oceans; and the problem of considering effects upon the variety of ecological systems from the nearshore to the deep sea, from polar to tropical regions. For all of these ecosystems, but particularly for much of the deep sea surface waters away from land and least studied in situ, documenting changes distinct from

natural variation may prove especially frustrating because little background historical information exists. Yet, ecological changes keyed to currents and movements of water masses have the potential to set in motion biological and/or biogeochemical effects of uncertain magnitude that endure for the time scales of oceanic circulation--processes with residence times of centuries and longer. Unfortunately, also associated with the spatial aspect is the probable lack of potential for mitigating observed effects. Furthermore, mitigation of the causes of those effects, even if it were achieved quickly, would do little to alter the lengthy recovery times of any circulation-related ecological changes that have been already induced.

A valid concern is that significant changes might occur in many marine ecological systems well before we know it; and we might be stuck with these changes for a very long time. This concern adds urgency to the assessment of the potential ecological problems.

A crucial part of this assessment concerns the effect of enhanced UV-B upon marine ecosystems. Clear and convincing evidence from research of the past decade indicates that UV-B radiation can injure the health of some marine organisms. In this paper I focus on the effects upon whole ecological systems. The level at which I address the problem is not limited to mechanisms of physiological damage to tissues of individual organisms, or, for example, on possible reductions in certain sensitive surface-dwelling populations. Those are major aspects of the assessment problem, but it is difficult to extrapolate from such directed effects to the overall consequences for natural ecosystems which, in and of themselves, are of concern to humans. In the absence of information on ecological effects at the ecosystem level, I speculate about those ecological processes, components, and systems that could be agents for, or targets of UV-B induced ecological change. The major contribution of this effort is to suggest facets of the problem that do and do not lend themselves to direct ecosystem-level assessments.

WHAT COMPONENTS AND PROCESSES ARE DIRECTLY AT RISK?

The level of UV-B striking the sea varies seasonally and with latitude. The penetration of UV-B into seawater varies with turbidity, concentration of dissolved organic matter, absorption by plants, and other factors. In spite of temporal and spatial variance in UV-B attenuation, the global concern relates to ecological impacts acting directly upon biological and chemical activity only in a relatively shallow (perhaps several meters deep) surface layer of water. Though this represents a very limited portion of the marine environment, it includes an important portion of the zone where the primary food base for marine ecosystems originates and where many processes occur to influence the distribution of organisms, energy, and matter for the whole ocean to its depths.

There is convincing evidence that direct effects on certain near-surface organisms and processes in marine waters are caused by exposures to enhanced levels of UV-B radiation (Calkins 1982; NRC 1984). Biological effects range from cellular-level damage and physiological impairment to reduced reproductive potential and direct mortality. Organisms that can be affected include the two major biological components of the sea--photoautotrophic and heterotrophic organisms, or, roughly speaking, both plants and animals.

Several particular effects raise significant concerns for marine ecosystems. I focus on these to suggest potential consequences.

Photoautotrophic species of free-floating phyto- and bacterio-plankton, along with attached algae and submerged vascular plants in the very shallow coastal areas of the sea, are the "primary producers" of the aquatic realm--those using the energy of incident solar photosynthetically active radiation (PAR) to produce their own organic tissues. These autotrophic organisms, active only to water depths to which sufficient PAR penetrates (known as the "euphotic" surface layers of the ocean), are key to the entire marine food web. Two kinds of noted effects on autotrophic organisms are of special interest. Experimental evidence suggests that rates of primary production of carbon into organic matter (both by some species of floating and attached bottom-dwelling plants) are significantly reduced when natural autotrophs are exposed to slightly increased UV-B doses (see Worrest 1982); such a mechanism is responsible for "photoinhibition" of primary production seen at high light intensities at the very top of a water column. Furthermore, evidence from laboratory aquarium "microcosm" studies also suggests that certain species of phytoplankton are more susceptible than others, so that when a mix of species is exposed to increased UV-B doses, a different plankton community can develop in comparison to normal control levels of UV-B (Worrest 1982).

Heterotrophic species, ranging from microscopic forms to the great whales, depend for their energy and nutrition upon organic tissues synthesized by autotrophic forms. Evidence shows that some of the smaller heterotrophs may be at risk, especially those not afforded UV-B protection by pigmentation. Although the animals normally living in the upper layers of the sea seem generally more resistant to UV-B damage than those living at lower depths, effects on surface-dwelling forms of zooplankton (including copepods, euphausids, and planktonic larvae of shrimp and crabs) have been shown to occur with increased UV-B. These effects include increased mortality of adults, decreased survival of early larval stages, and decreased fecundity of survivors (reviewed by NRC 1984). Additionally, fish eggs that float at the sea surface and larval life stages of some fish (e.g., anchovy) show cytogenic abnormalities and reduced survival caused by UV-B radiation.

The Value of Direct Effects

Direct biological effects from UV-B exposures, observed for individuals or even calculated by extension to conditions encountered by natural populations, are considered in at least partial isolation from the other ecosystem components and processes. These controlled experiments, conducted without the many complicating variables inherent to the natural situation, are designed to be simple tests to determine if UV-B radiation enhancement of the magnitude presently envisioned through past and future decades is sufficient to cause biological damage to individuals of certain species. These findings have created legitimate and significant concern regarding possible changes to marine ecosystems. We know for certain that some individual marine organisms and processes which we care about are at risk from increasing UV-B levels. Thus, the value of the scientific demonstration of directly caused UV-B damage is unquestioned. However, a next step in the assessment--extension of results to the complex natural environment--is difficult and tenuous.

ECOLOGICAL EFFECTS IN NATURAL ECOSYSTEMS

Ecosystem modification of direct effects can arise from biological, physical, and chemical mechanisms that are features of natural ecosystems, but are not features contained in direct effects tests. In nature, "indirect" effects can be transmitted via ecological linkages to components or processes themselves not directly sensitive to UV-B. UV-B increases itself could also cause environmental modifications and indirect effects. For example, increased photolysis of some dissolved organic or inorganic chemical compounds in surface seawaters might occur (e.g., Zepp 1982); some modifications of the aqueous chemical environment can indirectly cause biological changes. Although we are conscious of these features of ecosystems, we so far have gathered data relating to effects only on select parts of marine ecosystems; it is uncertain that any of the effects carry, unqualified or unmodified, to nature (Figure 1).

The possibilities for indirect effects, embedded within a perspective on the potential ecosystem responses to UV-B stress, are indicated in a simple diagram relating biological effects and ecological changes (Figure 1). Stress agents can act initially upon individual organisms directly or by modifying the environment. Such individuals can then affect other individuals (populations and communities) by many biotic mechanisms including predation, competition for resources, and altered reproductive success. Additionally, however, an affected individual (or more usually a group of individuals) can create a change in ecosystem processes (e.g., the rate of primary production, rates of oxidation of organic matter, patterns or timing of the biogeochemical cycling of elements). These processes are depicted (Figure 1) as at the interface between organisms and their environment. Almost invariably, changes in processes modify the chemical broth in which organisms bathe; sometimes the physical environment is also altered. For example, a change in the balance of organic production, consumption, and decomposition processes changes the concentration of plant biomass, which in turn changes solar radiation extinction coefficients and the light energies available throughout the underlying water column.

In general, affected individuals in ecosystems can spawn indirect effects by these paths--biological interactions and alteration of process rates. Each effect upon individual organisms can beget others, creating change in the biological structure (e.g., changes in populations, species interactions, and food webs) and processes (e.g., biological rates, biogeochemical interactions) in the ecosystem. Sometimes changes will be very unexpected or far removed from the original impact in time or space (e.g., Levin et al. 1984). Yet extrapolations need to deal with even more than the issue of enhancement of direct effects. At the other horn of our extrapolation dilemmas sits the possibility that the same two general paths for indirect effects will produce compensating mechanisms, ameliorating direct effects so that little discernible change happens even where some individuals are clearly injured. For compensation, biotic and chemical mechanisms exist, but physical features of aquatic ecosystems in particular can also modify the extent and severity of change.

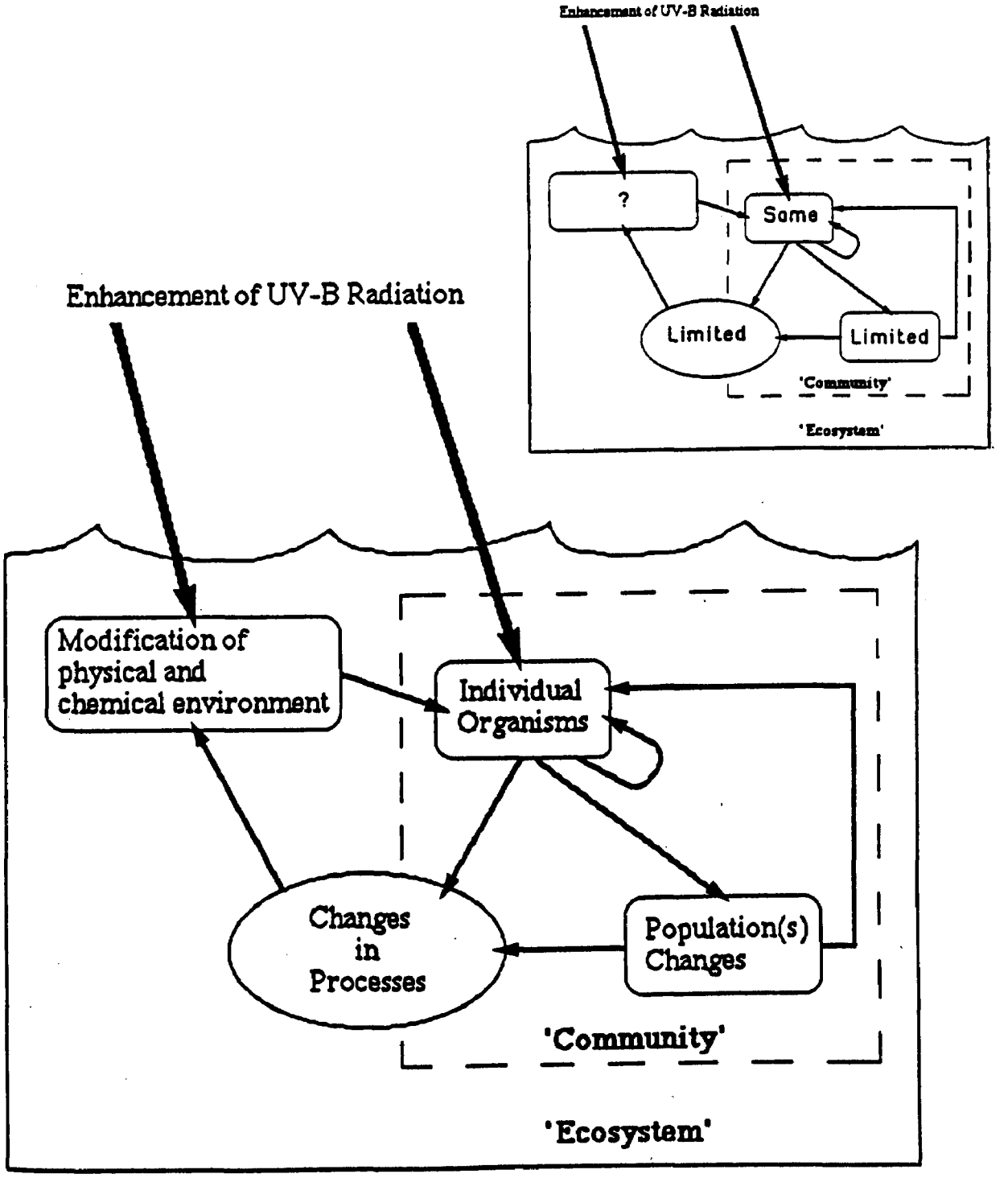

Figure 1. Potential ecological effects of enhanced UV-B in marine ecosystems are shown above. The diagram shows direct and indirect pathways for producing changes in individuals, populations, communities, and ecosystems. The shadow insert suggests the current state of information existing for UV-B effects on different pathways and at different levels in the marine environment.

I next illustrate compensatory changes and indirect ecosystem effects for two cases where extrapolation of effects is important. These are effects that, if verified, should arouse human concerns for regional or global repercussions. The examples involve:

- Reduction in rates of photosynthetic production of the organic tissues forming the primary resource for marine food chains

- Reduction of surface-dwelling stages of fish larvae and their adult population stocks.

I do not comprehensively consider all aspects of the extrapolation problem; only certain classes are given. I simply wish to illustrate a number of existing uncertainties before I suggest the potential consequences for marine ecosystems should such reductions occur.

ECOSYSTEM COMPENSATION AND A KEY PROCESS

The first scenario considers the possible reduction in primary production. The level of primary production influences many aspects of marine ecology and biogeochemistry. These extended effects form the major focus of my speculated consequences for the oceans. First, I examine the extrapolation problem. Assume (following Smith and Baker 1982) that with enhanced UV-B, productivity reduces up to 40% at the surface, decreasing to a 0% reduction within 2 attentuation lengths (a value roughly on the order of 1 to 10 m, but dependent upon the optical characteristics of the water (Smith and Baker 1979, 1982; Smith et al. 1980). The question of concern is not, "Does UV-B inhibition of primary production occur in the very surface waters?" but rather, "Does it change the productivity of the whole water column?"

One way to suggest the net quantitative change in an entire water column is to make calculations via mathematical models. Smith and Baker (1982) examined the potential magnitude of photosynthesis reduction for the whole euphotic zone using a simple, depth-integrated model without the "nonlinear" (including indirect effects) or compensating mechanisms of Figure 1. Their calculations predicted that with a 25% reduction in ozone thickness, primary production would decline 9%, regardless of the absolute productivity level. (Note: the relation between ozone and total productivity in the model is not quite linear, as a 5% primary production decrease would occur with a 16% ozone reduction.) Smith and Baker (1982) strongly emphasize limitations to their model, focusing on some possible nonlinear relationships relating to spectral quality of the solar radiation and concomitant changes in effective biological doses to marine organisms. They express concern that several indirect effects not considered by the model might easily add order of magnitude uncertainties to predictions. Complicating features of the natural ecosystem include the potential for change in the autotrophic community to phytoplankton species less sensitive to enhanced UV-B (e.g., in Figure 1--reduction in numbers of individuals leading to increases in individuals of another species). Additionally, they suggest complications due to the influence of water movement upon the depth of the surface mixed layer. Vertical mixing of organisms makes it difficult to estimate biologically effective doses and an organism's exposure regime for PAR or UV-B with precision. Yet their considerations, although perhaps quite comprehensive in relating productivity

and solar energies, do not include other facts that may be limiting to primary production.

One can envision yet another wrinkle, which I believe stands as a good example of potential ecosystem compensation. Plants need nutrients for growth as well as light energy; the availability of nutrients in the photic zone limits production, although in some marine areas, particularly the high latitudes, light may be more limiting through portions of the year. Except for heavily enriched coastal areas of the oceans and intense upwelling areas, the level of marine autotrophic production is partially, if not primarily, sustained by heterotrophic regeneration of dissolved nutrients (such as nitrogen and phosphorus) within the ecosystem (e.g., Kelly and Levin 1986). Thus, there exists an internal ecosystem factor influencing production to complicate the solar radiation/photosynthesis relationship. But if production is reduced within an upper portion of the water column, we cannot suggest with assurance that internal supplies of nutrients used to support a production level would not now be available below the new "photoinhibited" zone. To accomplish this compensation, mixing or simple diffusion of the unused nutrients only several downward vertical meters needs to occur; there are phytoplankton species that live much deeper than this in the water column, are adapted to lower light intensities, and whose growth would be enhanced by greater nutrients. Thus, within the range of the results for enhanced UV-B and productivity lies the possibility that no net change in the overall water column rate of primary production will occur.

Note that this scenario easily has room for some ecological change outside of an impact on the process of immediate concern. The ecological change from deeper UV-B photoinhibition could involve a slight deepening, or expansion, of the "neuston" community of organisms that dwells at the sea-air interface and is more adapted to high light intensities. Underneath this "expanded neuston layer" there could also be some changes in the mix of species responsible for the process of primary production. Species changes don't necessarily alter the overall level of primary production, which is more likely set by the supply of available nutrients. The main point of this example, however, is that the magnitude of euphotic zone primary production rates, even with surface inhibition and changes in autotrophic community composition, need not be altered as a whole.

PHYSICAL FEATURES, INDIRECT EFFECTS, AND COMPENSATION: A POPULATION EXAMPLE

The first simple extrapolation problem added a compensation mechanism to uncertainties associated with modeling photosynthetic reductions. My second example adds another level of complexity and examines an individual fish species. Direct effects on young fish could be a serious problem with enhanced UV-B because much of the world's commercially important fish species (e.g., tunas, mackerels, flatfishes, pilchards, anchovies, cods) produce "pelagic" (living in the water column as opposed to dwelling on the ocean floor) eggs and larvae; both of these life stages are translucent and allow UV penetration deep into tissues. Consequently, "most pelagic eggs and larval stages of fishes studied to date show high sensitivity to UV radiation and often die if exposed in shallow containers to solar UV" (Hunter et al. 1982).

Aspects of the extrapolation problem for larval populations of northern anchovy have been discussed rather thoroughly by Hunter et al. (1982). In the

anchovy case, known direct effects include: lesions in eyes, brain, or other body tissues; increased mortality; and reduced growth in surviving larvae. To illustrate the potential concern for the anchovy larval population as a whole, by making simplifying assumptions of a stable vertical distribution of larvae and no vertical mixing of eggs and larvae and using short-term exposures (12 days) for mortality effects, Hunter et al. (1982) calculated that annual production of larvae lost to UV-B mortality might rise by a factor of 1.38 with a 25% reduction in ozone thickness.

Relative to the previous primary production example, there are similar uncertainties with vertical mixing for small animal forms; however, more can be made of the issue of whether there is actual damage to individuals in the field because the larval organisms have more ability to move away from damaging exposures than do most phytoplankton. Yet in direct analogy with the key process case, a similar central question remains--does individual damage mean that the numbers or biomass of the adult population will change?

Three relevant aspects of the ecosystem that contribute to extrapolation uncertainties illustrate different classes of concerns: physical oceanographic features to moderate effects upon individuals, a biological mechanism to compensate for loss of individual eggs and larvae to maintain adult population levels, and indirect food chain effects that might add synergistically to enhance direct effects.

Ocean surface waters are not static vertical strata and mixing occurs that complicates projections of the overall exposure regime for organisms. Implications of this feature, in terms of effects, have been noted by Kullenberg (1982), and by Smith and Baker (1982) for photoinhibition, and were considered by Hunter et al. (1982) in their analysis of enhanced UV-B consequences for the northern anchovy. Because mixing intensities occur across a broad range in the ocean and are locally variable, calculations using exposure regimes and dose-effect relationships from direct effects tests have considerable uncertainty. Hunter et al. (1982) also add the uncertainties of the vertical distribution of the larvae, seasonality of larval production, seasonality of incident UV-B, and variations in UV-B penetration caused by the concentration of plants with which larvae associate for food. They conclude that vertical mixing alone could reduce estimated UV (direct) effects to zero, and that the variables of mixing and vertical distribution could account for the full range of possible UV effects, independently of other variables.

Even if physical and distributional (habitat) variables were well known in some specific case, there are other problems. Biological compensation for early life stage effects could occur so that effects do not alter year class success of the adult population. Density-dependent mechanisms, including changes in survivorship at different life stages or changes in reproductive capacity, might ameliorate effects seen in an individual larva. For example, there could be increased survivorship in unexposed individuals. Additionally, UV-B mortality might be, although higher, still relatively small compared to other sources of natural mortality. Over some time, greater fecundity (more viable eggs per adult female) could also develop, compensating for a certain percentage of young lost to UV-B. A further compensation could be that a lesser number of surviving individuals might grow bigger faster because there is less competition for a food resource. These simple examples of known

density-dependent mechanisms could therefore act to maintain adult numbers or biomass within normal bounds of natural variation.

Compensatory changes might well negate egg or larval effects, yet interactions with primary production might act, synergistically, to enhance the population decline. Besides direct UV-B related mortality of young developing stages of fish, there could be indirect mortality from starvation. This could occur if primary production decreased or the autotrophic species producing the preferred food of a given fish were replaced by indirect ecological changes (Figure 1). Perhaps there would even be an amplified mortality in that some larvae die from direct exposure, others from lack of food, and still others from the combination of reduction in food and weakening from exposure (by being less able to compete with other organisms for limited food resources). One can thus foresee the possibility of a catastrophic drop in a fish population, even if the direct mortality from UV-B alone would not convey that sense of concern.

These simple examples illustrate that the problem of projecting biological effects to marine ecosystems is itself at a young stage of development; this task represents a significant scientific challenge. What is not yet known about ecosystem changes adds up to such a potpourri of uncertainty that there are cases where we can't confidently predict whether a particular component or a process will increase, decrease, or remain the same.

POTENTIAL CONSEQUENCES FOLLOWING EFFECTS

Having given due emphasis to the tenuous nature of extrapolations, I am not the least bit hesitant to suggest that significant ecosystem changes could ensue. Ecological repercussions could arise especially from the following effects, if they are expressed:

- Reduction of certain individual species critical to the maintenance of structure or function in the ecological system
- Change in the mixture of species of primary producers
- Reduced rates of primary production of organic matter.

Some aspects of these have been mentioned. I'll touch briefly, with examples, upon potential consequences in these three areas.

Mortality or Impairment of Certain Populations

In general, mortality or impairment of certain populations can lead to significant changes in biological structure. For such "key" or "critical" species, which can include phytoplankton, zooplankton, and fish, some chains of effects are well documented. Removal of a key predator changes the distribution and abundance of more than just prey organisms, leading to species diversity changes and changes in community dynamics (e.g., Paine 1966). The structure of an entire pelagic food web can be altered, for example, by changes in fish feeding pressure. Feeding pressure can reduce some zooplankton species, allowing greater growth in other zooplankton; these changes in the zooplankton community then contribute, indirectly, to alter the type of algal assemblage present.

Cascading from effects on key species, there can even be alteration of the physical environment. Levin et al. (1984) relate an example in which removal of sea otters along the U.S. Pacific coast increased animal grazing on large attached brown algae (kelp) and eventually reduced the kelp beds, whose absence exposed the shoreline to greater physical disruption from waves. Other nearshore areas, such as the Gulf and Atlantic coasts, are exposed to less wave action than the west coast. Yet even in these less severe physical environments, reductions in growth of some attached plants, either directly by UV-repressed photosynthesis or indirectly by a sequence of interactions within the heterotrophic community, would alter physical aspects of the ecosystem. Removal of the physical habitat structure lent to shallow waters by seagrasses, for example, would likely modify wave and current energies along shorelines, which would change spatial patterns in sediment deposition and water clarity. Such physical consequences would be significant; they would involve alteration of two primary environmental features that influence the distribution of marine organisms and food webs.

Whether or not those heterotrophic species of larval fish, crab or shrimp larvae, or zooplankton that are known to be directly sensitive and at risk from UV-B (e.g., Damkaer et al. 1980; Damkaer and Dey 1982; Worrest 1982; Hunter et al. 1982) are critical species remains to be demonstrated. But the primary producers throughout marine ecosystems certainly are key components, for they set the table for the rest of the ecosystem.

Changes in the Species Composition of Primary Producers

Changes in the species composition of primary producers can influence both food webs and ecosystem processes. Worrest et al. (1981a, 1981b) show that shifts in community composition are possible with UV-B. Worrest (in Harwell and Hutchinson 1985) suggests that compositional change would alter size distribution of primary autochthonous particles and directly change nutritional quality, an effect already noted with enhanced UV-B (i.e., depressed protein content, dry weight, and pigment concentration of autotrophs). Changing sizes of food particles might alter the energy allotment required for consumption, perhaps reducing feeding efficiencies in consumer species, even if wholesale heterotrophic community changes were not instituted. Energy efficiency change in either individual species or in food webs (by simplification or diversification of the species interactions) could change ecosystems; changes in particle size distributions or in bulk nutritional quality could translate to a different level of food energy being available to top consumers of a food web. Such changes might be of special concern when considering, for example, what nutrition or size alterations might do to the pelagic food chain in the Antarctic that seems to center around the transfer of phytoplankton biomass to only one next major component, the krill (e.g., Everson 1984). Effects upon such a critical linkage, at a latitude where light levels are indeed important to production and where ozone depletions have been noted, would have major implications for the direct consumers of krill--whales, seals, birds, fish, squid, and even bottom dwelling organisms.

Particle size alteration and nutritional change with species composition could affect other ecological features, particularly biogeochemical processes. For example, settling rates of primary particles vary with size and shape. Because primary particles have many chemical elements incorporated

within their hard parts and soft tissues, as well as adsorbed to their surfaces, removal rates of elements and compounds from the surface ocean-mixed layer by settling, an important marine geochemical process, could be altered. But biogeochemical linkages are perhaps more likely to be affected in a significant manner if the magnitude, rather than quality, of primary production changed with UV-B enhancement.

Reduced Rates of Primary Production

I see reduced rates of primary production as potentially driving a number of important ecosystem changes, notable individually and collectively for their possible broadscale nature. One of these involves biogeochemical processes, which, as noted in Figure 1, are a major feature at the ecosystem level. The other relates to potential fish yields from the sea.

Of interest are those changes associated with element cycles and organic matter distribution throughout the ocean which key to the production, transport, and decomposition of primary produced organic matter. That the cycling of many elements in the oceans is very tightly coupled to biological activity, especially primary production, has been recognized for much of this century. For example, oxidation of the primary production of the euphotic zone, although a process most active closer to the surface, occurs throughout depth. This decomposition of organic matter is, in part, responsible for the vertical and horizontal distribution of some dissolved gases, including oxygen and carbon dioxide, and dissolved inorganic plant nutrients of nitrogen and phosphorus, as well as other biologically active elements throughout the ocean (e.g., Riley 1951; Wyrtki 1962; Redfield 1934, 1958; Redfield et al. 1963). Additionally, from a summary by Suess (1980), we can also see that the transport of the organic carbon to any depth in the ocean can be represented in a simple general relationship, normalized to the level of production. With lowering of primary production, Seuss and others suggest that the organic carbon reaching any depth in the ocean will decrease, leaving less food for the secondary production of the heterotrophic organisms dependent upon that carbon supply. We don't know if the magnitude of a change in primary production with UV-B would cause changes we could easily observe in sedimentation processes, or whether it would cause geochemical changes we would point to as "of immediate environmental concern." However, because the major oceanic biogeochemical cycles are tied to surface biological activity, but also relate to the depths and circulation of the oceans, important changes could be felt across many temporal and spatial scales.

One should not consider marine consequences without discussing fisheries, which is perhaps the most common "endpoint" of human concern. I have already mentioned some problems with fisheries, but how would direct reduction in primary production translate to reductions in fisheries yield? In the absence of information, we can make the simple linear extrapolation, suggesting that the percentage reduction in fish biomass yield would be the same as that for primary production; however, at least one summary suggests that this relation is nonlinear.

Nixon and Pilson (1984) presented a compilation of data for fisheries yield and primary production of some marine systems. Although such data are themselves subject to great errors and uncertainty (e.g., how do we account for heterogeneities in production? How does one choose the size of the

system? How good or comparable are yield data?) and the scatter is such that predictions for yield at an individual level of production would be inadvisable, a rough general correlative relationship is evident from the summary. Examination of the broad pattern in the figure by Nixon and Pilson reveals a nonlinear relationship, with less fish yield per unit of primary production (as grams of carbon) seen at lower primary production levels. From those data, for example, it would appear that about 1000 kg fish per ha per yr may accompany primary production of about 1000 gC per m^2 per yr whereas there may be only 5-7 kg fish per ha per yr associated with 100 gC per m^2 per yr. The explanation for such a pattern is unknown. Perhaps with lower primary production, other fish, not commercially sought, are produced. Maybe the efficiency of energy yield to higher trophic levels changes across different ocean areas and with different production levels. In either event, due to the scatter observed in the range of average ocean and coastal production levels (i.e., 100 to 500 gC per m^2 per yr) and fisheries yields, I would be uncomfortable in suggesting a mechanism or in suggesting how much greater the percent of fishery production would be than the percent of reduction in primary production.

If we use the relationship the ramifications are that with reductions in production from UV-B we should worry about (a) the fisheries yield decreasing everywhere, and (b) a reduction in primary production resulting in a greater than proportional reduction in fisheries yield.

ASSESSING ECOSYSTEM CHANGES: PROSPECTS AND LIMITATIONS

I hope my examples spark some argument, discussion, and research on these and other issues. We are uncertain how the convincing evidence for direct effects on important species and processes (effects having potential for producing large-scale ecological consequences) might actually be expressed in the oceans. Not only the magnitude, but even the very nature of ecosystem effects is unclear. Given this situation, I think we are left with two basic questions: What tools do we have and what approaches must we use to reduce the uncertainties as quickly as possible? Are there aspects of the uncertainty that we are not likely to resolve before the changes occur?

To address ecosystem changes, the best tool we have is experimental manipulation of ecosystems themselves. Although this is not possible for marine ecosystems (other than the global experiment itself), we do have physical models of some ecosystems, called microcosms or mesocosms, in which we can look directly at some ecosystem-level effects (e.g., Grice and Reeve 1982; Kuipers 1984). For example, perturbation studies on simulated shallow coastal ecosystems have been conducted at one such facility, the MERL mesocosms, at the University of Rhode Island along Narragansett Bay in the U.S. (e.g., Nixon et al. 1984; Kelly et al. 1985). Such research, using complex ecosystems, is urgently needed for assessing UV-B effects. It will give us a better sense for the possibility and extent of indirect effects and compensating mechanisms in a most efficient manner.

We could use such ecosystem experiments to identify sensitive components and processes of natural ecosystems. This emphasis could give us indicators for monitoring and assist in mapping effects in nature related to the penetration of UV-B in different marine waters. Correlation of known sensitive features with UV-B penetration changes is valuable information for

scientific assessment of environmental risk, although unequivocal data of this nature are difficult to gather.

Of equal importance to identifying sensitive components or processes, however, is identifying critical or sensitive ecosystems. I believe we need to direct some attention to this problem; so far we have only looked at the generic level assessed. The last decades, especially with increased use of remote sensing for oceans, have given us an increased awareness of heterogeneity in oceans. With this comes an appreciation for the variety of physical habitats and their interactions across a variety of time and space scales inherent to the sea. The UV-B concerns apply across these scales, and it would be efficient and wise to identify and focus intensively upon at least three classes of marine ecosystems: the sensitive ones, those having the least capacity for quick recovery, and those of greatest direct value to humans.

I must end on a note of pessimism by returning to issues in the introduction. Although, for example, mesocosm experiments are essential features to the environmental assessment of UV-B, the principal limitation of such research centers on the issue of scale. Some components of ecosystems cannot be included in experiments because the spatial scale of the enclosure is not large enough; this is particularly the case with the larger consumers that often cause us the most concern. Many processes, especially microbial aspects and primary production processes, can be examined directly in mesocosm experiments in the proper ecosystem context. However, large-scale processes associated with physical features or global biogeochemical cycles obviously cannot be accommodated. I remain concerned that we will be unable to scientifically assess in the near term, to suitable levels of confidence, some major aspects of potential oceanic effects--especially large-scale, sporadic features, or those happening in the clear waters of the deep sea where we have the poorest background information available for comparison to know if effects are occurring.

ACKNOWLEDGMENTS

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TERRESTRIAL PLANTS

The Potential Consequences of Ozone Depletion Upon Global Agriculture

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Our global agricultural system depends on only a handful of key crop species. Of the 350,000 species of plants that have been described in the world, over 80,000 are edible (Table 1). Of these, only about 3,000 are actually harvested for use as food (Vietmeyer 1986). Of these 3,000, only about 80 species have been domesticated and only 15 species supply most of the food calories and three quarters of the protein to the world's people. Three members of the grass family--rice, wheat, and corn--supply two-thirds of these calories and one-half of the protein (Table 2).

Over the course of the past decade several hundred scientific reports have been published documenting the UV sensitivity of more than 200 plant species and varieties under a wide variety of growth chamber, greenhouse, and field experiments (Teramura 1983; National Academy of Sciences 1984). Of the crops examined, approximately two-thirds are sensitive to UV radiation, with one out of every five identified as extremely sensitive. This finding has promoted considerable concern over the potential consequences of ozone depletion. However, several critical caveats must be included with such a "doomsday scenario." First, experiments conducted in growth chambers or greenhouses tend to overestimate and exaggerate plant susceptibility to UV radiation (Teramura, Biggs, and Kossuth 1980; Teramura 1982; Teramura and Murali 1986), partly because natural protective mechanisms are not fully developed under artificial growing conditions. Because over 90% of our knowledge of UV effectiveness comes from growth chamber studies, we have a worst case view of these effects. The second complication concerns the tremendous amount of variability in UV sensitivity within each species. Most of our crops have numerous varieties or cultivars particularly suited to specific growing regions. For instance, corn grown in Maryland is a different variety from that grown in the Midwest. Over 100 crop varieties or cultivars have been screened for UV responsiveness and approximately two-thirds of these were identified as being sensitive (Biggs, Kossuth, and Teramura 1981; Dumpert and Boscher 1982; Krizek 1978). Overall, nearly the same range of sensitivity can be found among

Table 1. Plants as a Food Source

Category	% of Total
350,000 known species of plants	100
80,000 edible plants	23
3,000 harvested plants	0.9
80 domesticated plants	0.02
15 key crop plants	0.004

Table 2. Major World Food Crop Production

Crop	Production (million metric tons)	Crop	Production
Cereals	1984	Sugar Crops	
wheat	522	sugar cane	936
rice	470	sugar beet	293
corn	449		
barley	172	Root Crops	
sorghum	72		
		potato	312
		sweet potato	117
		cassava	129
Legumes		Tree Crops	
soybean	90	banana	41
bean	32	coconut	31
peanut	21		

Source: Food and Agriculture Organization, 1984

cultivars as among different species. Therefore, a crop such as soybean may be labelled as sensitive to UV based on the response of the majority of cultivars tested despite having some very tolerant cultivars. In other words, most crops are not 100% sensitive or 100% resistant to UV.

The third complicating factor arises as a result of the endpoint or criterion used by various investigators to gauge UV sensitivity. Many studies have used whole plant features such as plant height, number of leaves, or total plant dry weight (Biggs and Kossuth 1978; Van, Garrard, and West 1976; Tevini and Iwanzik 1982). Others have used physiological or biochemical criteria such as photosynthesis, respiration, or protein content (Brandle et al. 1977; Sisson and Caldwell 1976; Vu, Allen, and Garrard 1982). Because of the inherent differences in these diverse gauges of sensitivity, it is not terribly surprising that a plant may be sensitive to UV based on photosynthetic determinations while it is resistant based on changes in dry weight. Although crop yield may ultimately be the crucial feature to study, only a handful of investigators have addressed this question because of the enormous technological difficulties encountered in the field. Despite these and other difficulties, our current uncertainty is not whether plants are affected by UV, but rather the magnitude of this sensitivity. Because only a handful of key species supply most of our agricultural needs, this potential susceptibility warrants great concern.

To illustrate some of the potential consequences of ozone depletion on global agriculture, I use soybean as a model. Table 3 shows that soybean has become a major plant source of oil and protein for the world, ranking fifth in the world in terms of tonnage, behind wheat, rice, corn, and barley. It is the third largest crop in the United States, which produces nearly 60% of the world's total. Due to its importance in the United States and global agriculture, any decrease in yield would unquestionably have a profound impact on the world economy.

Because of the drawbacks inherent in growth chamber experiments, the most meaningful method to assess risk is by examining the effects of UV radiation on field-grown crops. In such a setting one is faced with a more difficult task in performing the experiment because more UV radiation must be added to that normally received by the plant. The data shown in Table 4 spans a five-year period between 1981 and 1985 simulating a 25% ozone depletion for one cultivar of soybean, Essex, which is one of the most widely grown cultivars in the United States. Despite a large degree of annual variation, a 25% ozone depletion results in up to a 20%-25% reduction in overall yield. Years with little apparent UV effectiveness (1983 and 1984) were generally hot and dry with plants experiencing prolonged drought. By knowing the pattern of precipitation, temperature, and degree of cloud cover during the growing season, one can predict with a high degree of confidence what the effects of supplemental UV will be on soybean yields (Figure 1). The diagonal line represents a perfect match between predicted and actual yield. The bolder, jagged line represents our model prediction. In addition to reducing the actual weight of the harvest, UV radiation also generally reduces the overall quality of the soybean harvest by reducing its protein and oil content (Table 4); however, we currently know very little about the mechanism of this phenomenon.

Table 3. United States and World Crop Production

Crop	World (million metric tons)	USA	% of World Production
wheat	522	71	14
rice	470	6	1
corn	449	195	43
barley	172	13	8
soybeans	90	51	57

Source: Agricultural Statistics - USA, 1984; FAO Production Yearbook, 1984.

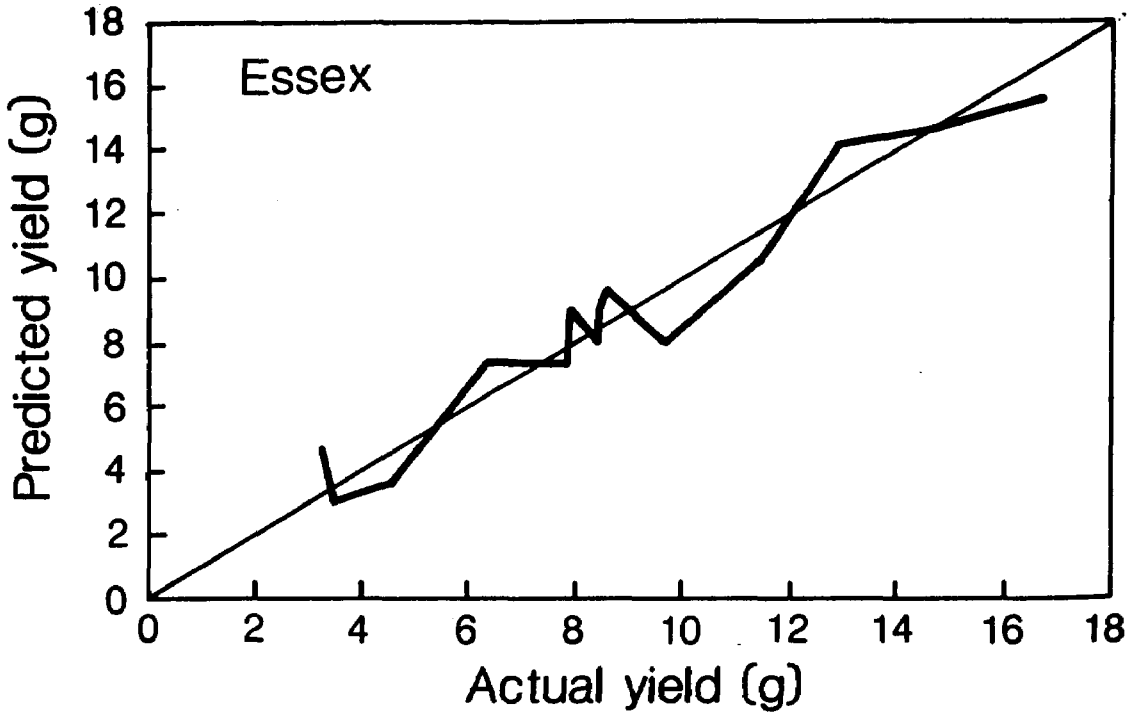

Figure 1. Bold, jagged line represents how closely a model regression equation predicts actual yield. The model inputs include presentation, temperature, and cloud cover data. The diagonal line represents the ideal situation where actual and predicted yields are equivalent.

Table 4. Summary of UV Effects on Soybean Yield and Quality

Year	% change in yield	% change in seed quality (protein)	(oils)
1981	-25	-5	-2
1982	-23	-4	+1
1983	+6	0	-2
1984	-7	0	0

Source: Teramura (unpublished)

What does a 20%-25% reduction in yield mean to global agriculture? For perspective, this UV-induced loss in yield is compared with other current sources of crop loss in soybean in Figure 2. Losses due to weeds reduce soybean yields by 17%, diseases by 12-14%, mechanical harvesting by 10% and insects by 3% (Metcalfe and Elkins 1980). Clearly, if the data presented on Table 4 are universally applicable to all soybean grown in the United States or the world in general, the anticipated loss due to ozone depletion would be greater than our current losses to any one of these factors. Should these experimentally based predictions be correct, we could anticipate a one-third reduction in our useable soybean harvest from 56% down to 36%. Additionally, there is some evidence in the literature (Esser 1980; Carns, Grahm, and Ravity 1978) that under a greater UV environment, crop losses from weeds, diseases, and insects would be even larger due to the weakened state of the crop host. If losses in each category increased by 25%, then our useable soybean harvest might effectively be reduced by one-half (Figure 3).

Up until now, we have quite a depressing picture of what might happen to our soybean harvests; however, there is a brighter side. We know that a tremendous range of UV sensitivity apparently exists among different crop cultivars. The U.S. Department of Agriculture currently has a collection of over 12,000 soybean lines and only a fraction of these have been screened for UV tolerance. Of the 45 cultivars that have been screened (Teramura and Murali 1986; Biggs, Kossuth, and Teramura 1981), nearly one-third were quite tolerant to even large UV doses. This implies that some degree of tolerance is already present in our modern soybean germplasm, and that the potential exists for crop breeding as a means of helping to lessen or ameliorate the anticipated loss in yield due to ozone depletion. Unfortunately, we do not know enough about the bases for these cultivar differences in sensitivity to be able to fully evaluate the likelihood of this possibility. For instance, if UV tolerance were closely associated with inferior genes which might increase drought susceptibility or lower yields, then any breeding program would be of little help.

In conclusion, the 20% to 25% yield reductions reported for soybean in this paper are only preliminary estimates and may portray a worse-case scenario. The data represent the response of only one out of the dozens of

cultivars currently grown throughout the United States. However, in the absence of similar detailed information for other key crops, these data serve to warn us of the potential consequences of ozone depletion upon global agriculture. We can only hope that these data represent an extreme case and not the general status of our global agriculture.

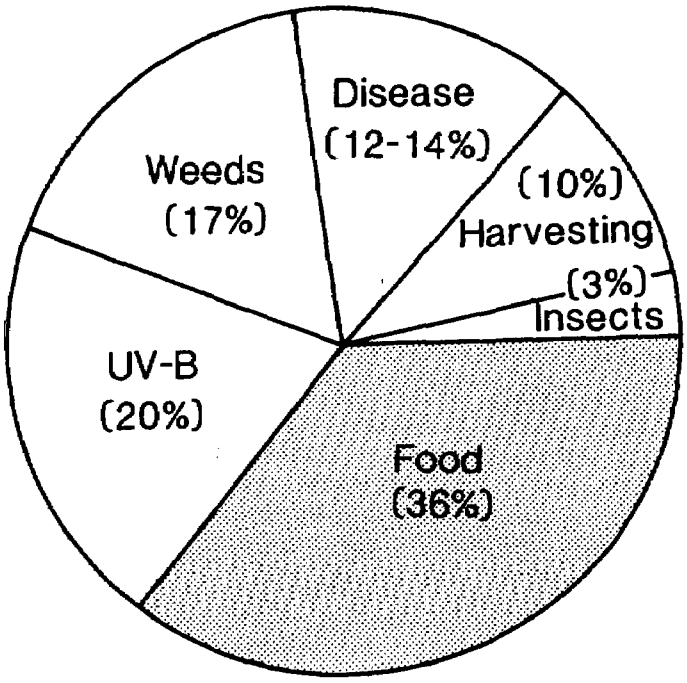

Figure 2. Current sources of soybean crop losses in the United States in relation to anticipated losses due to a 25% ozone depletion.

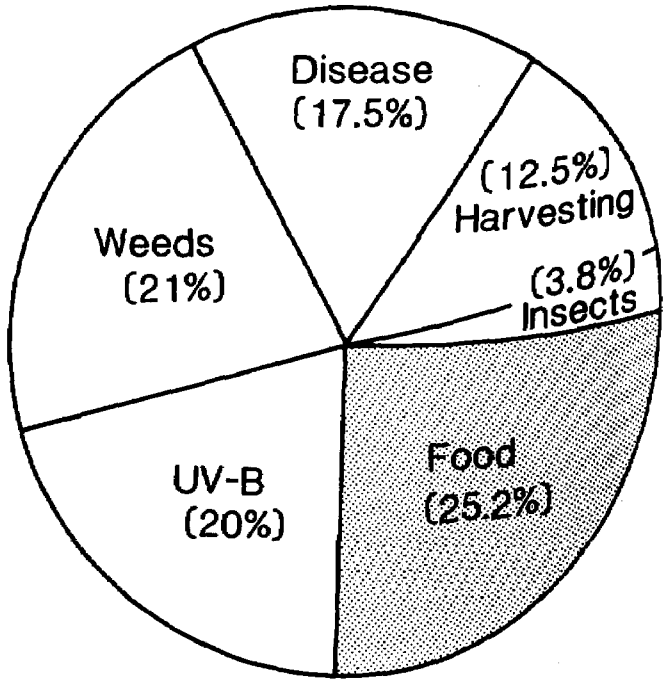

Figure 3. Anticipated yield losses due to a 25% ozone depletion assuming that UV produces a significant (25%) interaction with other sources of yield reductions.

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Inhibition of Photosynthetic Production in Plants by Ultraviolet Radiation[1]

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In this lecture we will try to explain some research findings concerning the inhibition by ultraviolet radiation of photosynthetic production in plants. Because of the abundant scientific literature on this topic we will not attempt to cover all of it, but will concentrate on findings which (a) are relevant to the ozone depletion problem and (b) are of general significance, i.e., not relating to particular plants only. Furthermore, we will try to be nontechnical and not assume a background in plant physiology or photobiology. These are our aims, but the result of our attempt will necessarily be a compromise, due to the short time available.

It is necessary first to say a few words about photosynthesis. The process can be schematically written:

Water + carbon dioxide + light = organic matter + oxygen. Photosynthesis takes place in the green parts of plants, mostly leaves, where the light for the process is absorbed, mainly by the green pigment, chlorophyll. In land plants (but not in aqueous plants) the carbon dioxide enters through microscopic adjustable pores or valves called stomata (see Figure 1). The stomata are adjusted by the plant for the best compromise between carbon dioxide influx and water vapor loss.

The process: water + carbon dioxide + light = organic matter + oxygen, is not completed in one step. There are, in fact, many chemical reactions leading to this net result. The process can be roughly divided into two parts:

- Photosynthetic electron transport and phosphorylation:
  Water + light = oxygen + reducing power + phosphate energy

- Assimilation of carbon dioxide:
  Carbon dioxide + reducing power + phosphate energy = organic matter.

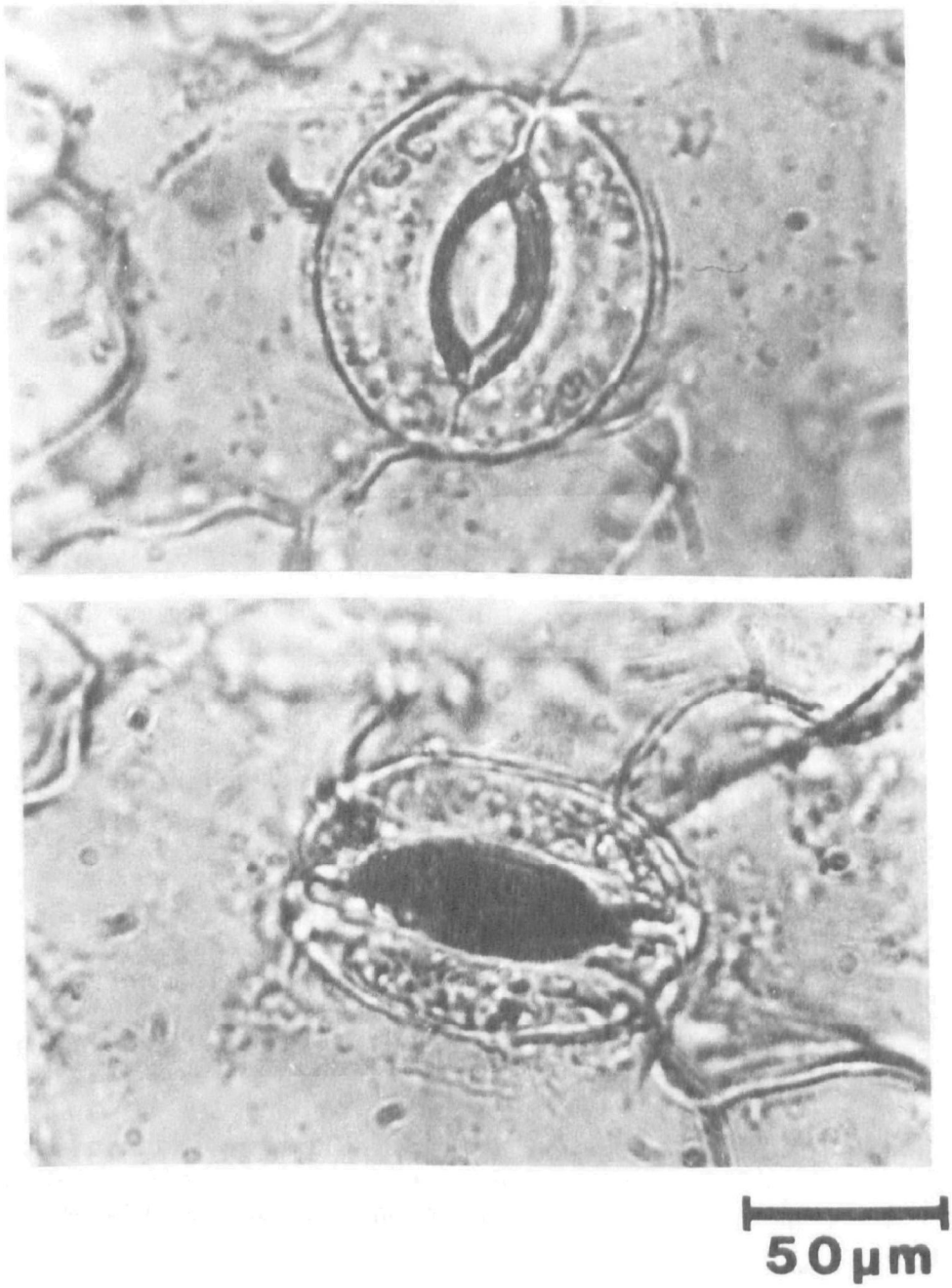

Figure 1. Light micrograph of stoma from broad bean (Vicia faba) leaf in open condition (above, white light only), and stoma closed under the action of UV radiation. The photograph is from an experiment with excised epidermis.

These main parts of the photosynthetic process take place in microscopic green particles in the cells called chloroplasts (Figure 2). Photosynthetic electron transport and phosphorylation take place in the chloroplasts in membranes called thylakoids, where carbon dioxide is assimilated in the space between these membranes, which is filled with a solution (stroma) of the enzymes necessary to catalyze the reactions. The chloroplasts also contain part of the cell's genetic material, DNA, and protein synthesizing system (ribosomes).

Inhibition of photosynthesis by ultraviolet radiation has been established and measured by several types of experiments. The first type are short-term laboratory experiments in which isolated chloroplasts or thylakoids are first exposed to ultraviolet radiation without simultaneous irradiation by visible light. At the end of the ultraviolet radiation the sample is exposed to visible light, so that the effect on the photosynthetic system can be estimated. In such experiments it has been found that photosynthetic electron transport is inhibited by ultraviolet radiation. The electron transport chain is damaged at more than one point, and the points of damage have been approximately determined (see Bornman et al. 1984). In contrast to moderate photoinhibition caused by intense visible light, photoinhibition by UV-B radiation is not rapidly reversed during subsequent dark incubation (Bornman 1986).

Before we proceed, we should be familiar with the concept of action spectrum. An action spectrum is a function describing, or comparing, the effectiveness (average effect per incident photon) of radiation of different wavelength to effect a certain process, such as inactivation of the photosynthetic system. One can think of this as a graph with a wavelength along the horizontal axis and effectiveness, i.e., effect in relation to the intensity and duration of the irradiation, along the vertical axis. Determining action spectra is one of the most important means of characterizing photochemical processes as it may provide information about the molecule absorbing the effective radiation. In connection with the ozone depletion problem, action spectra are important for computing the so-called radiation amplification factor (see Figure 3), and for relating effects of artificial radiation sources to those caused by solar radiation.

Practically all action spectra relating to deleterious effects of UV radiation show a decline of effectiveness from low to high wavelengths, but the slope varies, and there may be "bumps." Measurements in several laboratories using variations on the first type of experiments (Jones and Kok 1966; Hirosawa and Miyachi 1983; Bornman, Bjorn, and Akerlund 1984) have resulted in action spectra with modest slopes (Figure 4). This means a low radiation amplification factor, in marked contrast to spectra measured for inactivation of the genetic system (DNA inactivation, Figure 5), which are very steep.

On the other hand, action spectra for inhibition have also been measured with a second type of experiment, which has yielded different results. In these experiments (Bogenrieder 1982; Caldwell et al. 1986) complete photosynthesis was measured for intact leaves or intact plants with white light impinging on the leaves simultaneously with the UV radiation. These spectra (Figure 6) are steeper than the spectra obtained by the first method. These latter studies are closer to a field situation and may be regarded as more relevant to the ozone depletion problem. They indicate a higher radiation amplification factor and therefore a greater risk. However, a scientist can

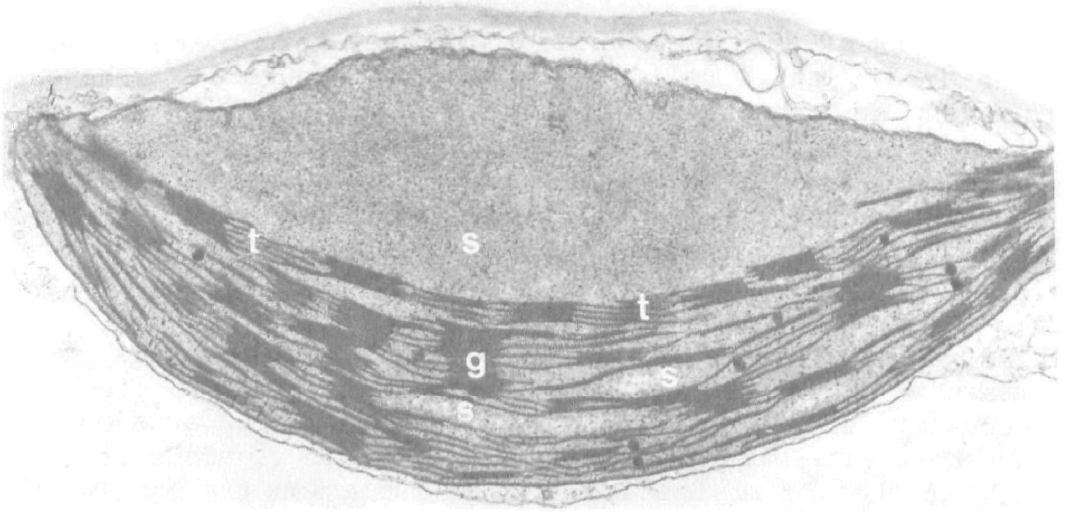

Figure 2. Electron micrograph of part of a cell from a sugarbeet leaf, showing chloroplast with stroma (s) and thylakoids (t). In some places the thylakoids form grana (g). From Bornman et al. (1986).

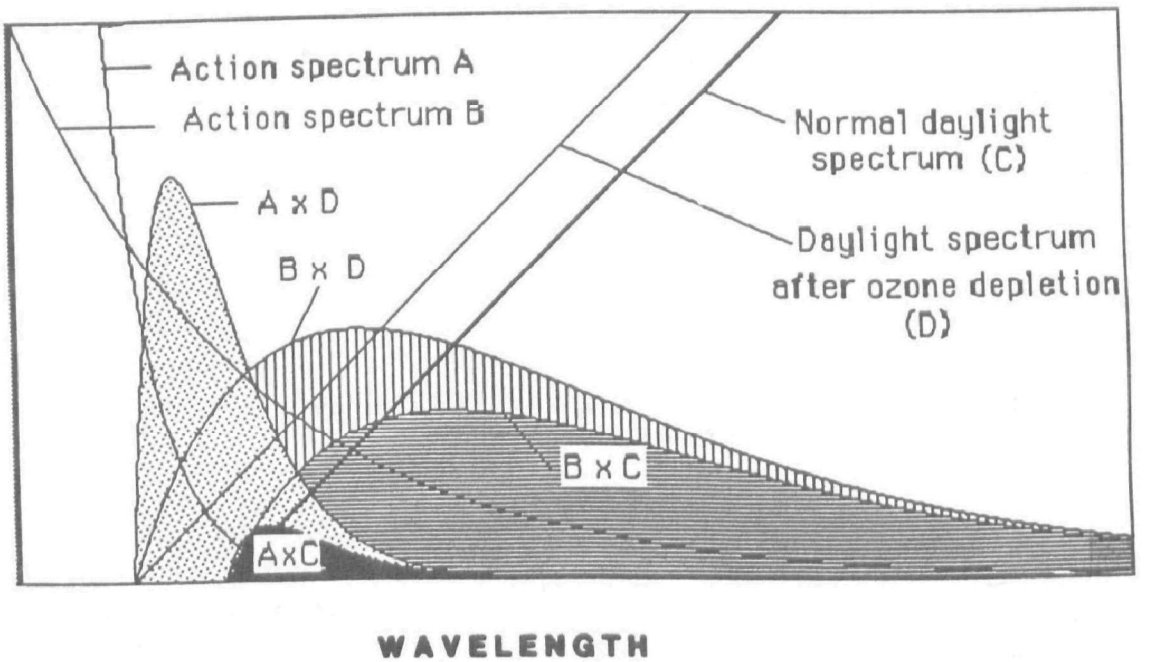

Figure 3. Diagram to illustrate the dependence of radiation amplification factors on action spectra. The whole diagram is generated by a computer program with a set of equations, and does not depict real experimental data. One steep action spectrum (A) and one that is less steep (B) are shown (wavelength on horizontal axis, effectiveness on vertical axis), as well as the short wavelength tails of the normal daylight spectrum (C) and of daylight after ozone depletion (D) (wavelength on horizontal axis, spectral irradiance on vertical axis). The radiation amplification factor for action spectrum A is the ratio between the area below the product curves AxD (stippled) and AxC (black), in relation to the particular ozone depletion that has caused the spectral change from C to D. In the hypothetical example shown, the radiation amplification factor is five times as great for action spectrum B.

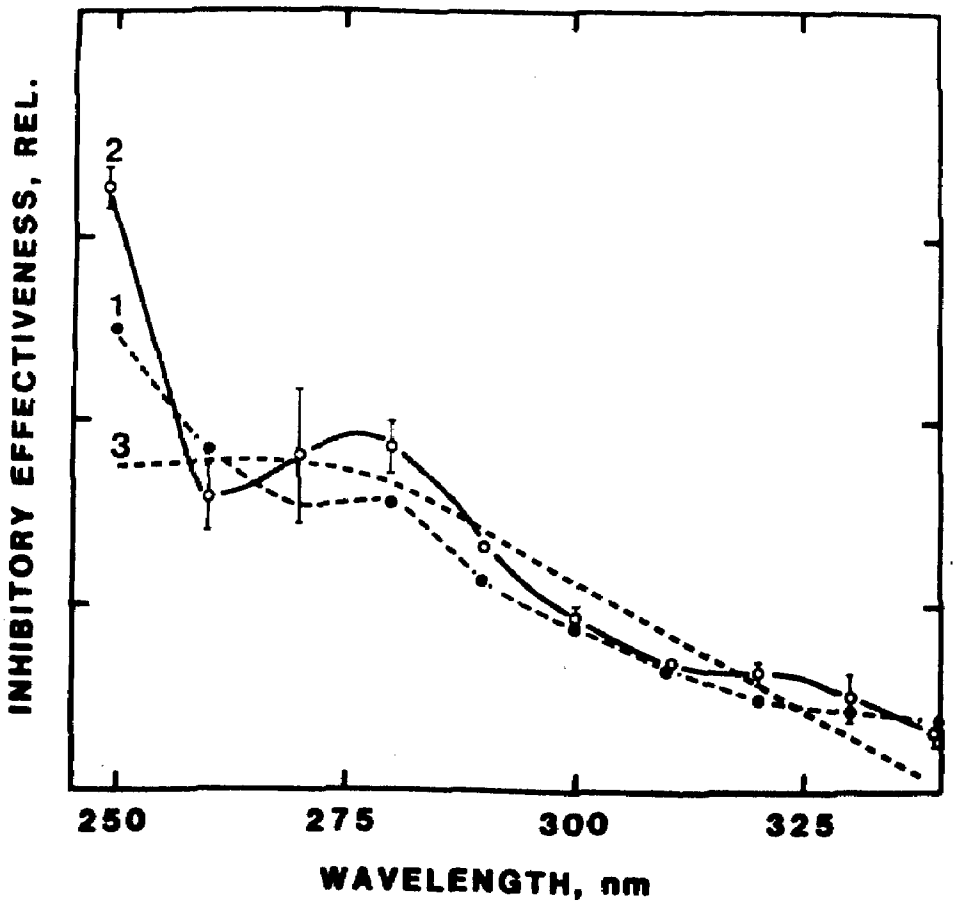

Figure 4. Action spectrum for inhibition of photosynthetic electron transport determined in isolated spinach chloroplasts (curve 1, Jones and Kok 1966), isolated spinach thylakoids (curve 2, Bornman et al. 1984) and isolated cyanobacterial thylakoids (curve 3, Hirosawa and Miyachi 1983). Within the UV-B region (280 to 320 nm) the effectiveness changes about twofold.

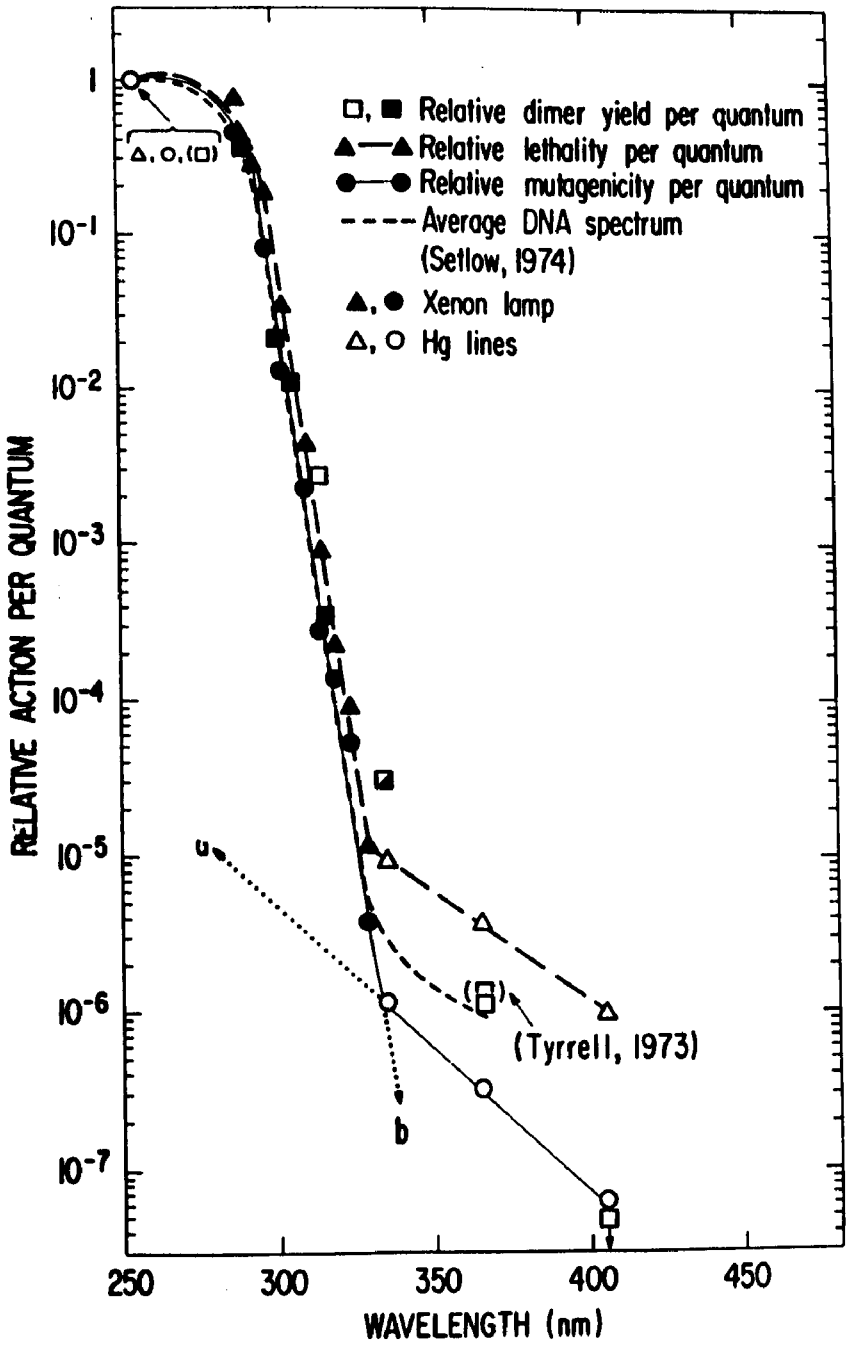

Figure 5. Absorption spectrum for DNA, as well as action spectra for various processes that depend on radiation damage to DNA. Note that the vertical scale is logarithmic. Within the UV-B region the effectiveness (action per quantum) changes about 10,000 fold. Cited from various sources by Peak et al. (1984).

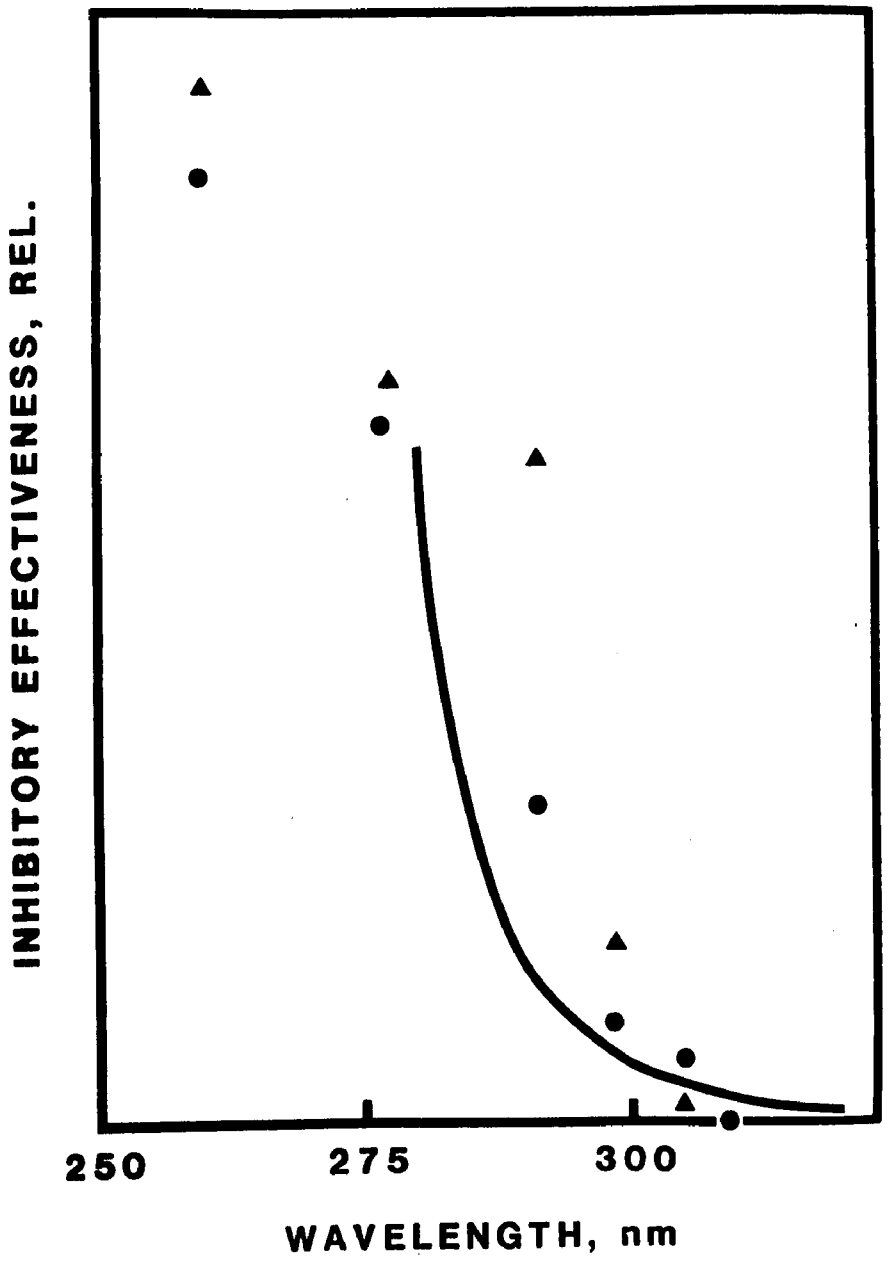

Figure 6. Action spectrum for inhibition of photosynthetic carbon dioxide fixation by intact plants: Lactuca sativa (triangles, Bogenrieder 1982), Rumex alpinus (circles, Bogenrieder 1982) and Rumex patientia (solid line, Caldwell et al. 1986). The slopes are intermediate between the slopes in Figures 3 and 4.

hardly be content with such a statement. We wish neither to underestimate nor overestimate the consequences of ozone depletion, and, to be on solid ground, we must understand the reason for the discrepancy between the two sets of results.

A first guess would be that it is not damage to photosynthetic electron transport (measured in the first method), but damage to the enzymes necessary for carbon dioxide assimilation that is important for inhibiting overall photosynthesis (measured by the second method). Indeed, inactivation of some proteins shows steeper action spectra, and UV-B has been shown to decrease the activity of the carbon dioxide binding enzyme, ribulose bisphosphate carboxylase/oxygenase (Vu, Allen, and Garrard 1982, 1984).

Nevertheless, there is another possibility that also explains the different action spectra obtained, depending on whether white light is given simultaneously with the UV radiation or not. Although there are facts speaking not only for, but also against the following interpretation, we think it is worth presenting as an hypothesis; some background follows to explain it.

One of the components necessary for photosynthetic electron transport is the so-called Q_B protein. This protein binds a quinone that transfers electrons. It is a remarkable protein in many respects (see Arntzen and Pakrasi 1986 for a review). One feature that is important in our context is that it has a very high turnover rate in light, i.e., it is rapidly broken down and resynthesized. The rate of breakdown increases with light intensity; we may regard 15 minutes as a typical lifetime for a molecule of Q_B protein. It is as if the protein molecules were worn out by working hard. If resynthesis is prevented during irradiation with strong light, the photosynthetic system is inactivated. When such a situation occurs, trees are exposed to strong light, but temperature is still too low for efficient protein synthesis, e.g., in the spring in Swedish conifers.

It could be that one important effect of UV radiation is to destroy the chloroplast DNA, in which the blueprint for resynthesis of the Q_B is encoded, thereby slowing down resynthesis and photosynthetic electron transport. If this mechanism dominates inhibition of photosynthesis by UV, one would expect action spectra measured with simultaneous irradiation by UV and visible light to be steeper than those determined with sequential irradiations. This seems to be supported in the results, although we do not have strictly comparable data yet. There is also more direct evidence (Dohler, Biermann, and Zink 1986) that protein synthesis may be inhibited by UV-B radiation.

If the above interpretation is correct, we also have, in principle, a recipe for producing more UV-resistant plants: We should introduce additional copies of the gene encoding for the Q_B protein (the correct designation is the psbA gene) into the chloroplasts. This may be possible, but the recipe is, of course, a bit naive. Other genes are inactivated as well. But all genes are not equally critical. Genes that do their important job in, for example, the growth zones (the meristems) in root and shoot tips can be discounted, for they are shielded and protected from UV.

Plants vary widely in their tolerance, and many factors other than the possibility just mentioned certainly contribute to this. Considerable differences may exist between closely related species (Robberecht and Caldwell 1986), or even between cultivars of the same species (Biggs, Kossuth, and Teramura 1981). Differences already occur at the membrane level: we have found for example that spinach thylakoids are more resistant than even intact leaves of Elodea (Bjorn, Bornman, and Olsson 1986; Figure 7). At the organ level different degrees of protection are offered by the outermost cell layer of the leaf, the epidermis, which often contains substances that strongly absorb ultraviolet radiation. The content of these shielding substances is often adjusted to match the need. As an example, we (Bjorn and Bornman 1986) have observed that the same Oxalis leaf is more sensitive to UV radiation coming from below (where the plant "has no reason to expect" any UV) than to UV impinging upon the upper side of the leaf (Figure 7). Caldwell et al. (1982) have shown a correlation between UV tolerance and UV level in the natural habitat, as well as between UV sensitivity and epidermal UV transmittance. Wellman (1974) and Beggs, Schmeider-Ziebert, and Wellman (1986) have reported the accumulation of UV absorbing flavonoids in response to UV exposure, and have shown how widely various plants differ with regard to this response.

It has also been repeatedly observed (e.g., Klein 1963; Teramura 1980) that plant cell cultures and whole plants tolerate more UV radiation the more they are simultaneously exposed to visible light. The nature of this protective action of visible light is not understood. Even if inhibition is due to DNA damage, the so-called photoreactivating enzyme (which is known to carry out a light dependent DNA repair in other cases) is probably not involved. The photoreactivating enzyme repairs a type of damage (pyrimidine dimerization) that is probably not the most important type of damage in the spectral range of solar UV radiation (Peak et al. 1984). Hirosawa and Miyachi (1983) studied inhibition by UV-A of photosynthetic electron transport in a cyanobacterium and concluded that the reactivation achieved with subsequent visible light is due to light absorption in the photosynthetic system. However, this may be a phenomenon different from the protection from UV-B damage; it seems that no one has studied the spectral dependence of this protective effect. It is possible that plants become more resistant when they are grown in strong white light because of long-term changes in their properties, especially in the amounts of protecting pigments. Another possibility is that they can repair damaged systems faster in strong light because they have more energy available in this case as compared to weak light conditions.

The protective effect of visible light seems to be an argument against the hypothesis that an important UV effect is the inhibition of resynthesis of the Q_B protein, because the need for resynthesis would be greater the stronger the visible light. However, only strong visible light given prior to the UV irradiation has a protective effect (which thus can be described as an acclimatization to strong sunlight), while strong visible light concomitant with UV irradiation enhances UV inhibition (Warner and Caldwell 1983), as would be expected from the hypothesis.

When considering UV effects on whole plant photosynthesis, one should take into account not only direct effects upon the photosynthetic system, but also indirect effects on photosynthesis. Inhibition of leaf area growth, for

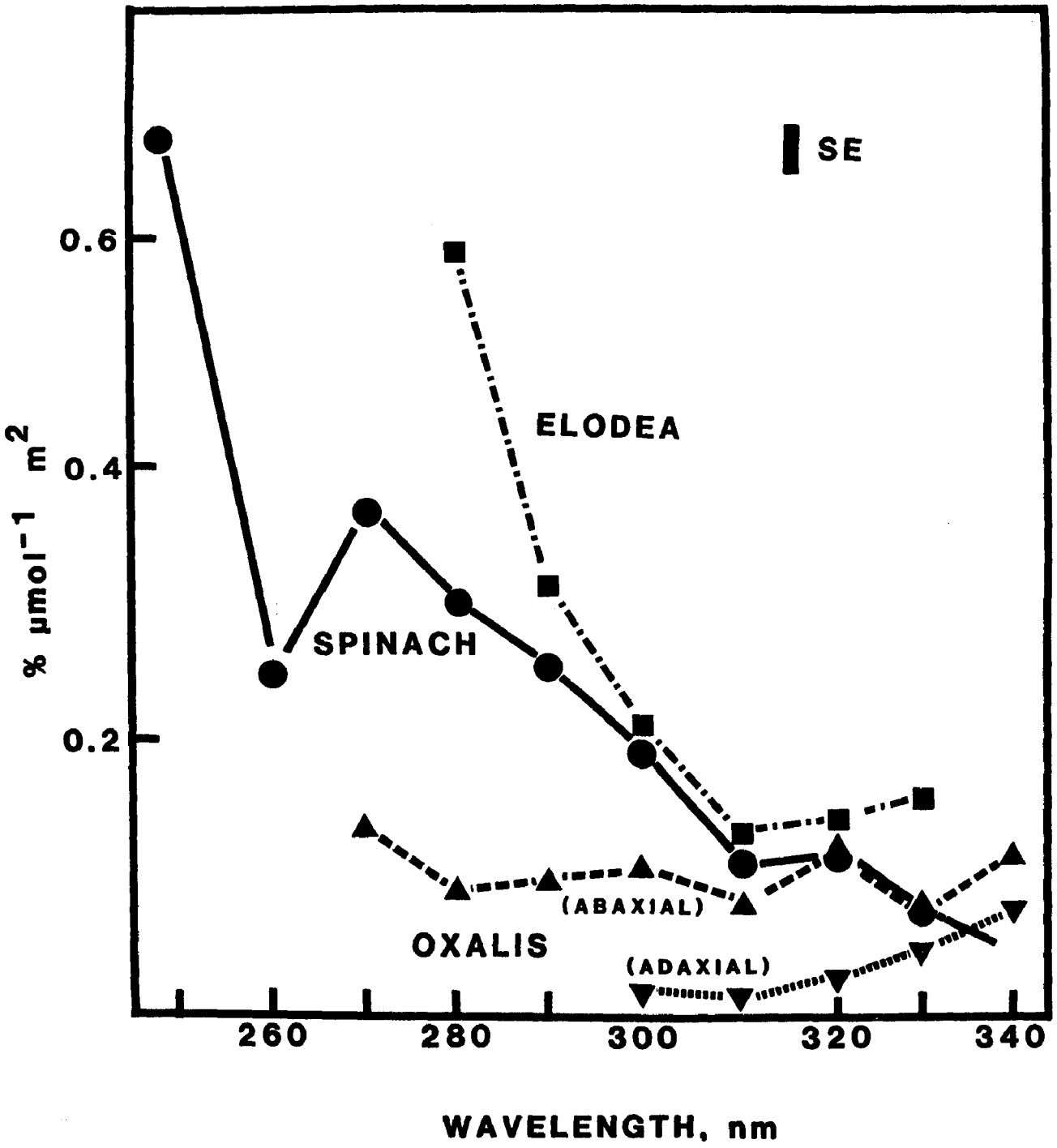

Figure 7. Action spectra for inhibition of electron transport (as measured by the effect on fluorescence induction) in intact leaves of Elodea densa and Oxalis deppei, and isolated spinach thylakoids, all to the same scale. Elodea leaves lack an epidermis, and the sensitivity is the same for both sides. For Oxalis the sensitivity is much greater for UV radiation impinging on the lower (abaxial) side than the upper (adaxial) side. From Bjorn, Bornman, and Olsson (1986).

instance, will lead to a lowered input of light energy, and thus to lowered photosynthetic production. Dr. Häder (this volume) has described how UV inhibition of the ability of free-swimming photosynthetic organisms to swim in the proper direction can affect photosynthetic production. Another UV effect, which has been studied in our laboratory (Negash and Bjorn 1986), is the closing effect on stomata (Figure 1). Closing of stomata prevents carbon dioxide from entering the plant, and thus inhibits photosynthesis. Also in this case visible light counteracts the effect of UV (Figure 8). Our conclusion as regards tef (Eragrostis tef, an important crop in Ethiopia, where UV exposure is high) is that UV closure of stomata is not important under field conditions as long as the water supply is adequate. This conclusion is based on our laboratory experiments and on a computer program (Bjorn and Murphy 1985) for predicting daylight spectral UV irradiance under various conditions. Teramura and Perry (1982) and Teramura et al. (1982, 1984) have measured UV effects on stomata in soybeans, radishes, and cucumbers under field conditions and found that these UV effects on stomata may have some importance in certain plants under water stress conditions.

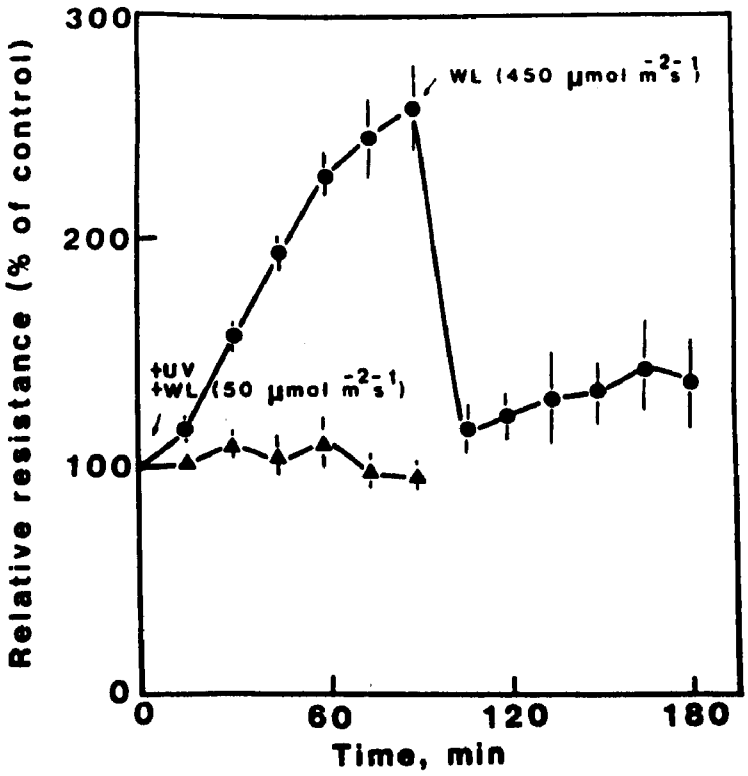

Figure 8. Stomatal resistance in control plants (tef, Eragrostis tef) irradiated with white light (50 µmol $m^{-2}s^{-1}$) only (triangles) and plants simultaneously exposed to UV (285 nm, 2.4 µ mol $m^{-2}s^{-1}$, circles). The UV, exposure commenced at time 0. After 90 minutes white light fluence rate was increased 9 fold. Stomata start to close when exposed to UV radiation, but the closure is reversed by an increase in white light. Note that high resistance values correspond to small stomatal aperture. The roots of the plants were immersed in water. From Negash and Bjorn (1986).

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MISCELLANEOUS

An Assessment of UV-B Radiation Effects on Polymer Materials: A Technical and Economic Study

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INTRODUCTION

In March 1986, the U.S. Environmental Protection Agency sponsored a workshop to review the results of several analyses that predicted increases in future emissions of chlorofluorocarbons (CFCs) and possible depletion of stratospheric ozone.[1] One direct result of ozone depletion would be an increase in the level of UV-B radiation reaching the earth's surface. In turn, the increase in UV-B radiation would lead to accelerated damage in several effects categories, including polymer materials. This paper addresses both technical and economic issues related to damage to plastics brought about by hypothesized increases in UV-B radiation.

Plastics are increasingly used outdoors where they are routinely exposed to sunlight. Exposure to light of certain wavelengths results in a variety of chemical reactions in most commonly used plastics, leading to increased deterioration of useful physical and mechanical properties and therefore shortening the useful lifetime of the plastic. The most potent region of the solar spectrum in this regard is the UV-B region. The longer wavelength ultraviolet light (UV-A) may also contribute to the degradation of plastics exposed to light. The shorter wavelength (<300 nm) ultraviolet radiation, if it were present in sunlight to any significant extent, would result in rapid, extensive degradation of polymeric material.

On a volume basis only a few classes of plastics are used outdoors or in situations where they are exposed to diffused and reflected sunlight (Anonymous 1986). These are shown in Table 1 with some examples of typical products. Polyvinylchloride (PVC) is the most widely used plastic in the residential building industry. It is used in siding, window frames, rainwater

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[1] Protecting the Ozone Layer: Workshop on Demand and Control Technologies, Workshop Sponsored by the U.S. Environmental Protection Agency.

goods, pipes, and flexible sheeting. Polyolefins, reinforced polyesters, acrylics, and polycarbonates are also used outdoors in significant quantities.

The same polymer is often compounded differently to yield materials best suited for specific applications. The various additives (fillers, plasticizers, heat stabilizers, lubricants) used in the compound constitute a significant fraction of the final plastic product and heavily influence its photosusceptibility. Thus the light-sensitivity and the nature of damage suffered by plastics exposed to light are determined not only by the type of polymer but also by the compound composition (Weiler 1984; Ho 1984).

Table 1. Plastics Used Outdoors

Plastic Class	Typical Use
1. Polyvinylchloride rigid [PVC]	Siding, window frame, pipes, and conduits
2. Polyvinylchloride plasticized [PVC]	Roofing membrane, wire/cable coating, hose
3. Polyolefins [PE/PP] (polyethylene, polypropylene)	Packaging, agricultural film, stadium and outdoor furniture
4. Polyester (thermoset) [UPE]	Panels/siding, tanks and pipes, glazing
5. Polycarbonates [PC]	Glazing

A given polymer product used outdoors may suffer damages or undesirable changes in its properties. The rate at which such damage occurs is determined by the kinetics of the reactions responsible for the change. Consequently, it is possible to identify a sequence of progressive damage events where several desirable characteristics are in turn deteriorated below acceptable levels. The most rapidly occurring damage determines the lifetime of the product, and is termed the "critical mode of damage" (CMD). Table 2 gives the CMD of several important classes of polymers used outdoors under current spectral and climatological conditions.

Polyvinylchloride compounded specifically for siding or window frames illustrates the effects of UV-B radiation on polymers used outdoors. The choice of PVC for the purpose of illustration is appropriate because it is used in building applications more than any other plastic and because of the

Table 2. Modes of Damage Experienced by Polymers Used Outdoors

Polymer	Application	Damage
PVC	(a) siding, window frames	[yellowing] + chalking - impact properties - tensile properties + surface distortion
	(b) roofing materials	[+ brittleness] [+ discoloration]
UPE	outdoor surfaces paneling	[+ surface erosion] + discoloration - strength
PE/PP	irrigation pipe, outdoor furniture synthetic turf, stadium seats, packaging	[+ brittleness] - tensile properties - electrical properties
PC	glazing material	[+ yellowing] - transparency

+ = increase
- = decrease
[] = Brackets indicate critical mode of damage (CMD)

availability of at least a limited amount of relevant research data on the polymer. A typical rigid PVC composition for outdoor use is stabilized solely by 10% to 13% parts by weight of titanium dioxide pigment (Titow, 1984). The pigment particles shield the plastic matrix below it from light-induced degradation. On exposure to light, the rigid PVC, originally white in color, suffers both yellowing and partial photobleaching. The subsequent uneven discoloration is the critical mode damage for rigid PVC in applications such as siding and window frames.

In the next section we review technical issues that arise in an analysis of the effects of increased UV light on plastics. The principal output of the technical analyses is a damage equation which relates a measure of the CMD to the amount of titanium dioxide stabilizer used in the PVC resin compound. We then integrate this damage equation into a supply and demand analysis of the PVC market to obtain estimates of the potential economic damage that would result from the hypothesized depletion in ozone. We summarize our conclusions in the final section.

EFFECT OF INCREASED UV LIGHT ON PLASTICS USED OUTDOORS[2]

CIAP Approach

The Climatic Impact Assessment Program (CIAP) (Shultz, Gordon, and Hawkins 1975) study employed the Beer-Lambert equation to calculate approximate increases in UV-absorbing stabilizers required to offset the effects of increased UV light. While the approach is sound and simple to use, its applicability is limited to the transparent, homogeneous polymers. With the exception of window glazing, polymers used outdoors are generally opaque and are often stabilized using light screeners. Therefore, a parallel model based on light-screening stabilization, applicable to opaque materials, was developed in the course of the study.

Both equations require the use of an empirical constant that depends on polymer and stabilizer for its use. The equations developed for each model are shown in Table 3.

Table 3. Models for Prediction of Stabilizer Requirements

Transparent Polymeric Materials CIAP equation based on Beer Lambert Law	Opaque Polymeric Materials Equation derived in present study
UV-absorption is the main mode of stabilization	UV-screening is the main mode of stabilization
$\text{Log}\ (I_\ell / I_o) = \epsilon C \cdot \ell$	$\text{Log}\ (I_\ell / I_o) = \ell / 2r_3 \log(1-3V/2)$
C = concentration of absorber	V = Volume fraction of screener
ϵ = extinction coefficient	r_3 = area-average radius of screen particle
x = factor increase in light intensity y = factor increase in stabilizer concentration to offset the effects of increased light.	
$\text{Log}\ x = (y-1) \cdot \epsilon C \ell$	$\text{Log}\ x = \ell / 2r_3((1-3V/2)/(1-3Vy/2))$
Requires $(\epsilon C \ell)$ for predictive use	Requires $(\ell / 2r_3)$ for predictive use

[2] A more detailed description of the technical analysis is available in Andrady, A.L. 1986. An analysis of technical issues related to the effect of UV-B on polymers. Draft report submitted to the U.S. Environmental Protection Agency.

Both approaches suffer from the same drawbacks. First, they do not take into account the spectral sensitivity of the degradation process (i.e., the action spectra). Second, they do not take into account the dose-response relationship for photo-induced deterioration. Finally, they do not take into account the possible nonlinearity of effectiveness of stabilizers at different concentrations. In spite of these serious shortcomings, the method yields approximate estimates of the increased stabilizer requirements at a given increased light level.

Comprehensive Approach

The extent (and even the nature) of damage suffered by a polymer exposed to sunlight depends on wavelength. Since any possible deterioration of the ozone layer will cause significant changes in the spectral distribution of sunlight, both the altered sunlight spectrum and the action spectrum for a given mode of damage is required to estimate the effect of ozone depletion on material deterioration. The terrestrial solar spectrum has an irradiance $H(\lambda)$ and is altered by a function $\Delta(\lambda)$ caused by ozone depletion. The altered spectrum $H(\lambda)\Delta(\lambda)$ weighted by the appropriate action spectrum $F(\lambda)$ will yield an estimate of the damage at the wavelength λ. Provided that the damage is additive and is a linear function of available light energy, the following integral gives the total damage (Cutchis 1984).

$$\text{Total damage } D = \int_{\lambda} H(\lambda)\Delta(\lambda)F(\lambda)\, d\lambda$$

The integral might be evaluated numerically with $\Delta(\lambda) = 1$ to represent current baseline conditions. Using the absorption spectrum of ozone, $\Delta(\lambda)$ at various levels of ozone depletion might be obtained. However, the lack of a suitable action spectrum (i.e., one relating to properly compounded PVC containing appropriate quantities of titanium dioxide) limits the usefulness of the approach. The only action spectra available in the literature relate to transparent PVC films and to types of damage other than yellowing. However, a very approximate estimate might be made using the action spectra for polyene formation based on Reinische, Gloria, and Wilson (1966). Yellowing in PVC is a direct result of the generation of long polyene sequences. The ratio of integral D' calculated for a given level of ozone depletion to that at zero ozone depletion, D, is a measure of the expected increase in damage. Typical values are shown in Table 4.

The question of how much stabilizer should be used to counter the increased yellowing damage is more difficult to estimate. The main difficulty is that the data on stabilizer efficiency with respect to unaltered sunlight conditions cannot be reliably extrapolated to stabilization under high UV light conditions. Stabilizer effectiveness of titanium dioxide in rigid PVC was reported by Summers (1983) for weathering under Arizona conditions. Assuming that the dependence of stabilization effectiveness on the pigment concentration as reported by Summers is applicable under high UV conditions, approximate ranges for increased pigment levels required to maintain the light-resistance of PVC might be calculated. The data on yellowing damage at the various pigment levels reported are consistent with the following relationship.

Table 4. Estimated Ranges of Factor Increase in Damage and the Factor Increase in Stabilizer Needed to Counter the Change for Yellowing of Rigid PVC Compositions

Percent Loss of Ozone	Zenith Angle 30°		Zenith Angle 60°	
	D'/D	S'/S	D'/D	S'/S
0-5	~1.01	1.01-1.02	1.01-1.02	1.01-1.05
5-10	1.01-1.02	1.01-1.05	1.03-1.04	1.03-1.09
10-20	1.02-1.05	1.03-1.11	1.04-1.09	1.05-1.20
20-30	1.03-1.08	1.03-1.18	1.07-1.18	1.08-1.38

Note: D'/D = factor increase in damage
S'/S = factor increase in titanium dioxide stabilizer

Zenith angles selected to reflect North American locations.

$$\text{Log [Damage]} = -[\text{pigment}]\ k + a$$

Since acceptable levels of damage (D*) obtained at the current use levels of pigment (S*) are known, the factor increase in pigment (S'/S*) corresponding to a given factor increase in damage (D'/D*) can be calculated using known values of k. The results are shown in Table 4.

The estimates could be improved considerably by using action spectra, dose-response information, and stabilizer effectiveness information for the particular polymer/pigment system of interest. Efforts to obtain some of the relevant data are currently underway.

The use of increased amounts of titanium dioxide pigment poses several difficulties. These include increased melt viscosity and therefore higher power consumption, increased wear and tear on machinery, increased compounding of ingredients to maintain processibility and the other mechanical properties of material, and possible increased susceptibility to oxidation. Thus, increasing the levels of titanium dioxide is a limited solution which will not necessarily be cost-effective beyond a certain level of increased UV light.

ECONOMIC DAMAGES TO PVC PRODUCTS[3]

Methodology

Economic benefits are generated whenever a transaction such as the sale of a good or service takes place. Economists generally agree that any attempt to measure these benefits should be based on an individual's own valuation of the transaction, demonstrated by their "willingness-to-pay" to engage in the transaction (Just, Hueth, and Schmitz 1982). Willingness-to-pay can be inferred from the market demand curve. In particular, since the marginal purchaser (i.e., the purchaser who would not buy if the price were any higher) is by definition willing to pay exactly the price he pays and no more, the area beneath the demand curve up to a given quantity can be shown to be the appropriate measure of total willingness-to-pay. This situation is illustrated in Figure 1, where total willingness-to-pay is given by area $abcq_0o$.

In benefit-cost analysis willingness-to-pay is frequently the measured net of any charges paid by consumers for the good or service. When this is done, the result is called net willingness-to-pay, or more often, consumers' surplus. The consumers' surplus measure represents what consumers would be willing to pay over and above what they do pay. In Figure 1, if price is p_o, consumer surplus is given by the triangular area acp_0.

Any action or event that leads to a change in market demand or supply will change the surplus measure. In Figure 1, if market price increases to p_1 as a result of a depletion in stratospheric ozone, consumers' surplus will be reduced by the area p_1bcp_0. This is the measure of welfare change reported in this case study. To be precise, we measure the willingness-to-pay by individuals to remain in the initial situation (with price p_0) and to avoid the increased costs associated with ozone depletion. These costs, if not avoided, represent a real economic loss to society since the costs limit other productive opportunities in the economy.

In the next two subsections we discuss the data and methods used to estimate demand and supply curves for PVC products used in building and construction. We then compute economic damages under the assumption that the producers of the PVC products will alter the resin formulation as UV-B levels increase to maintain product lifetime and quality. This change in the resin compound increases production costs and is the source of the measured surplus change. We note that the measured damages do not include the increased costs associated with early maintenance or replacement of in-place stock.

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3 Description of the economic analysis is available in Horst, R.L., et al. 1986. The Economic Impact of Increased UV-B Radiation on Polymer Materials: A Case Study of rigid PVC. Report in preparation for the U.S. Environmental Protection Agency.

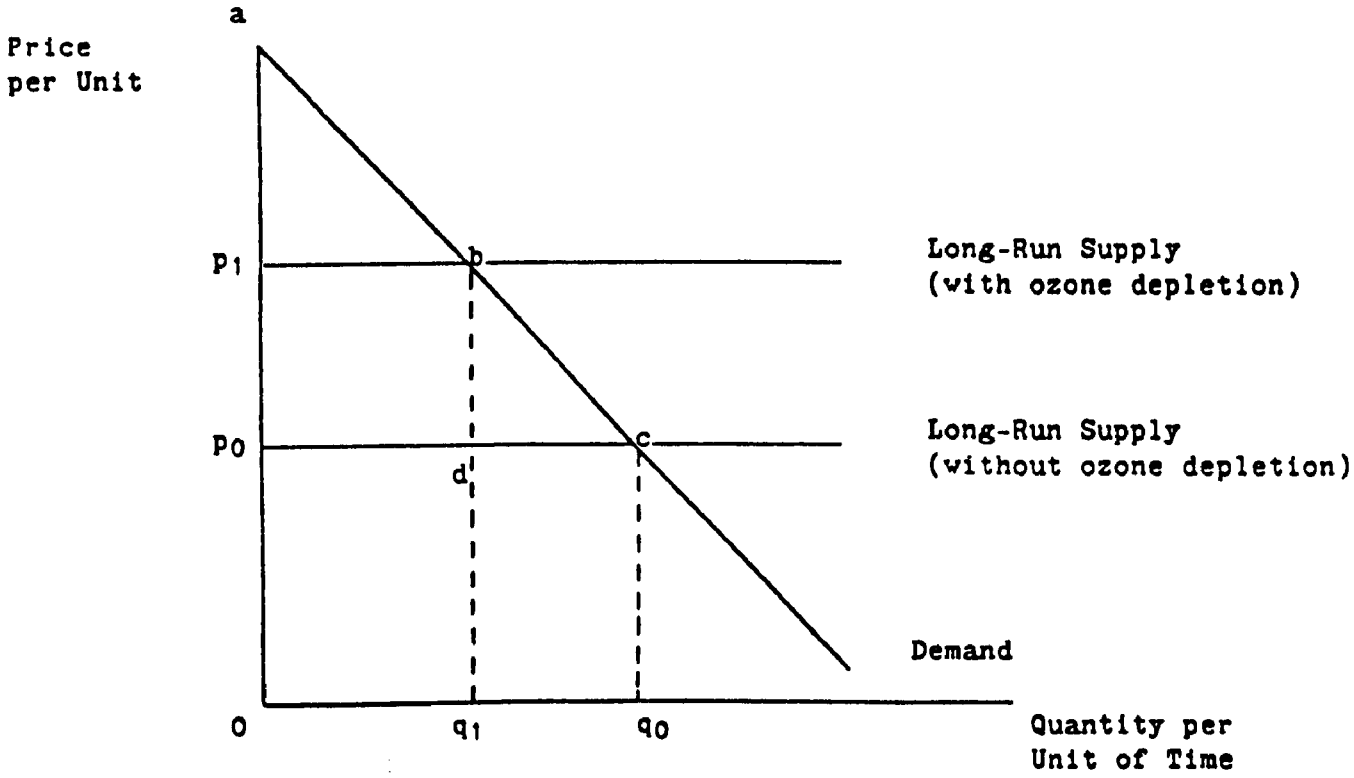

Figure 1. Aggregate Demand and Supply in the Market for PVC Products.

Aggregate Demand

A demand equation for PVC used in construction is estimated using annual data for 1970 to 1983. The demand for PVC is a derived demand; that is, it is derived from a more general demand for construction activity. The explanatory variables included in the equation are the price of PVC, wood, and fabricated metal products, and the value of construction put in place.

The results of a two-stage least squares regression analysis are shown in Table 5. The explanatory variables are statistically different from zero at standard levels of significance. The signs of the coefficients agree with a priori expectations. Since the specification is double-logarithmic, the coefficients are interpreted as elasticities. Thus, the own-price elasticity of demand for PVC is -1.95. This is elastic, which would be expected for a product with good substitutes.

The equation shown in Table 5 represents current preferences. However, the case study requires that welfare impacts be evaluated to the year 2075. Consequently, PVC consumption must be forecast over time. The approach used to obtain future values of PVC consumption is based on the assumption that the

Table 5. Derived Demand for PVC Used in Building and Construction*

Variable**	Parameter Estimate	Standard Error	t-Statistic
Intercept	-5.396	4.160	-1.297
LPVCHAT	-1.956	0.316	-6.190
LPMET	2.288	0.423	5.406
LPWOOD	0.740	0.367	2.018
LCON	1.343	0.635	2.115

Observations: 14

Dependent Variable: LQPVC

Adjusted R^2: 0.9573

Durbin-Watson D: 1.872

F-Statistic: 73.817

* The equation is estimated using the REG procedure in the SAS software package.

** Variable names are: LPVCHAT is the fitted value of the natural log of PVC price; LPMET is the natural log of the price of fabricated metal products; LPWOOD is the natural log of the price of soft lumber products; LCON is the natural log of the index of construction activity; and LQPVC is the natural log of the quantity of PVC used in building and construction.

demand curve estimated with historical data also represents future preferences. Therefore, given forecasts for each of the independent variables in the demand equation, a forecast of the dependent variable (PVC) can be made. Forecasts of each independent variable in a future year are estimated using a combination of time trend models and autoregressive models (Pindyck and Rubinfeld 1976). Table 6 reports the 50% prediction interval for PVC demand in selected future years. For the mid-range forecast, the projections are consistent with about a 2% annual rate of growth in PVC demand.

Aggregate Supply

The supply side of the market for PVC products used in building and construction is determined through a model plant analysis. Representative plants are constructed for each of the three PVC products considered in the case study: pipe and conduit, siding, and window profiles. Data for the model plant analysis were obtained from the literature and through conversations with PVC fabricators, PVC compounders, PVC resin producers, and extruder manufacturers (Perry 1977).

The data collected for the model plant analysis reflect operating characteristics for a single level of output. If a supply curve for the PVC market is to be estimated econometrically, a significant amount of other data is required. For example, resource costs, administrative costs, and production methods may be expected to change as output levels vary. Unfortunately, there is insufficient data available in published statistics to develop the detailed information that would permit a formal econometric analysis of production costs. Furthermore, it is unlikely that an econometric analysis would be able to pick up any variations in production costs caused by variations in the amount of titanium dioxide used in the resin compound.

As an alternative to the econometric approach, we used a less formal method. For each of the model plants we assumed that the market for PVC products used in building and construction is perfectly competitive and that the output level associated with model plant operation is consistent with the economic conditions for profit maximization. We also assumed that each firm (model plant) is in long-run equilibrium, which implies that equilibrium price and quantity are associated with the minimum of the firms' long-run average cost curves. Finally, we assumed that the industry for each of the three PVC products is characterized by constant costs. This assumption implies that the long-run supply curve for each industry will be horizontal. In turn, under these conditions, price equals long-run average cost and also equals long-run marginal cost. Consequently, the supply curve for the PVC products market can be determined from knowledge of total production costs and output, which is the information provided by the model plant analysis.

The average costs ($/lb) of production (inclusive of normal profit) are estimated to be $0.52, $0.75, and $1.02 for pipe, siding, and profiles, respectively. An aggregate price index for these three products is calculated as a weighted average of each market price. The weights are based on the volume of output in each market. The price index is $0.604 and is nominally representative of market conditions in 1984. As the production processes change to offset the damage caused by increased UV-B radiation, the

Table 6. Fifty Percent Prediction Interval for PVC Demand (millions of pounds)

Year	Lower Estimate	Point Estimate	Upper Estimate
1980		2,198	
2000	2,440	3,397	4,729
2025	3,874	6,316	10,298
2050	6,071	11,303	21,048
2075	8,910	18,563	38,671

average cost of production will increase. This increase will lead to a corresponding increase in the aggregate price index for the PVC products and is the source of the economic damages estimated for the case study.

Economic Damage Calculations

A hypothesis of the case study is that producers of the PVC products will change the amount of titanium dioxide in the resin compound to maintain the and lifetime and quality of their products. The change in compound formulation will affect production costs in several ways. First, resource costs will be increased as more titanium dioxide is used per pound of plastic produced. In addition, it is believed that the change in the formulation will require more energy to produce and will lead to more frequent replacement of screws and barrels. For the three model plants developed for the case study, an estimated 25% increase in titanium dioxide concentrations in the compound are expected to lead to price increases of 2.67%, 1.67%, and 1.73% for siding, profiles, and pipe, respectively. The aggregate price index increases by 1.86%. Since a 25% increase in titanium dioxide is believed to be the maximum increase that could be tolerated without adversely affecting other attributes of the PVC products, the 1.86% price increase is the maximum price effect that would be observed.

Table 4, shown earlier, describes the relationship between physical damage to PVC products and ozone depletion. Table 7 shows the scenario for ozone depletion assumed for this study. For a given level of ozone depletion, the factor increase in titanium dioxide stabilizer is computed by linear interpolation. The cost increase associated with the interpolated estimate of titanium dioxide concentration is proportional to the cost increase computed for the model plant analysis.

Table 7. Ozone Depletion Estimates

Year	Ozone Depletion (percent)
1985	0.00
1995	0.15
2005	0.62
2015	1.50
2025	2.84
2035	4.66
2045	7.08
2055	10.41
2065	15.44
2075	26.08

Source: EPA estimates.

Forecast values of the price index of PVC products used in building and construction are computed for each year s from the equation:

$$P_{1s} = r_{1s}(1 + C_{1s})p_{1s} + r_{2s}(1 + C_{2s})(p_{1s}(p_{20}/p_{10})) + r_{3s}(1 + C_{3s})\ (p_{1s}(p_{30} / p_{10}))$$

where P_{1s} is an aggregate price index for PVC at time s, with ozone depletion

p_{is} is the price of the ith PVC product at time s

C_{is} is the percent increase in costs for product i associated with a given level of ozone depletion in year s.

r_{is} is the ratio of output in year s for the ith good to total output for PVC in building and construction.

The calculation of future PVC prices with ozone depletion requires several assumptions. First, we assumed that the mix of PVC products is not affected by ozone depletion. Second, we assumed that the relative prices of the three PVC products remain unchanged over time. Third, we assumed that each firm produces the same level of output in each year. This last assumption implies that increases in PVC consumption over time are met by increases in the number of firms in the industry.

The change in economic welfare associated with ozone depletion is computed as the area under the aggregate demand curve between prices P_0 and P_1. For the double-logarithmic demand curve, this can be written as:

$$\Delta w_s = A_s \int_{P_1}^{P_0} P^{b1} \, dP$$

where A_s is a term involving all arguments of the demand curve except the price of PVC and its coefficient. A_s varies over time, since each of the explanatory variables in the demand equation varies over time.

Table 8 summarizes the discounted value of damage with several discount rates. The discounted present value calculation is a convenient way to express values that occur over many time periods. The discounted present value, as of the current year, is defined as follows:

$$DPV = \sum_{i=0}^{T} \frac{\Delta w_i}{(1 + r)^i}$$

where Δw_i is the welfare change in year i, and r is the discount rate.

Table 8. PVC Damage Associated with Ozone Depletion
(Discounted Present Value in Millions of 1984 Dollars)

	Discount Rate (%)			
	0	2	5	10
Low estimate of PVC consumption	$2,440	$ 603	$ 97	$10
Middle estimate of PVC consumption	4,716	1,137	174	17
High estimate of PVC consumption	9,158	2,159	315	27

The choice of an appropriate discount rate is especially difficult because of the extended time horizon for this analysis. If a real rate of 10% is assumed, the preferences of future generations (those actually affected by the ozone depletion) are assigned little importance. On the other hand, a discount rate of 0% implies that the preferences of future generations are to be given the same weight as the preferences of the current generation. The literature provides no clear-cut answer to this dilemma (Lind 1982). Therefore, present values are presented for several discount rates.

The results shown in Table 8 apply to the following set of circumstances:

- All estimates are reported in millions of 1984 dollars
- The factor increase in titanium dioxide concentrations is computed for a zenith angle of 60°
- Firms do not respond to the hypothesized decreases in stratospheric ozone until 10 years after the impact is observed.

Additional research is planned to assess the sensitivity of the results reported in Table 7 to alternative assumptions.

CONCLUSIONS

The conclusions of this case study of PVC damage related to increased UV-B radiation can be summarized as follows:

- An approximate damage function can be developed which relates changes ozone to changes in stabilizer required to maintain product lifetimes at present levels.
- The damage function can be improved by using action spectra, dose-response information, and stabilizer effectiveness information for the polymer/pigment system of interest.
- For a given scenario of ozone depletion, it is estimated that undiscounted, cumulative economic damages are $4.7 billion in 1984 dollars.
- A 50% prediction interval, which accounts for only a portion of the uncertainty present in the analysis, indicates that the undiscounted, accumulated economic damages could range from $2.4 billion to $9.2 billion.

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The Interaction of Photochemical Processes in the Stratosphere and Troposphere

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Recent evidence of a dramatic decrease in stratospheric ozone levels (Farman, Gardiner, and Shanklin 1985), which may be related to the continued use of halocarbons (Cicerone, Walters, and Liu 1983; Craig and Chow 1982) and an increase in methane (Craig and Chow 1982) and N_2O (Weiss 1981) emissions, could forebode a concurrent increase in the transmission of solar ultraviolet radiation to the troposphere. In combination with a general warming of the lower atmosphere through the effect of greenhouse gases and other climatic perturbations (Wang et al. 1986), a greater transmission of ultraviolet radiation will augment the principal energy sources of photochemical reactions in the troposphere and may also lead to an increase in the incidence of skin cancer. To date, the enhancement of specific chemical reaction rates has largely been ignored in tropospheric modeling studies. In this paper, we describe an investigation of the effect of changes in tropospheric temperature and the transmission qualities of the upper atmosphere on incident radiant energy using data obtained from atmospheric and laboratory measurements to estimate future conditions and corresponding changes in tropospheric photochemistry.

Because ozone has a strong ultraviolet absorption band that extends to approximately 340 nm (Inn and Tanaka 1953), the presence of ozone in the upper atmosphere limits the transmission of ultraviolet radiation to the earth's surface. A reduction in stratospheric ozone would, therefore, allow more radiation to penetrate into the troposphere, primarily in the wavelength region between 290 and 320 nm (Bahe et al. 1979). An increase in radiation within this region will enhance the photolysis rates of important tropospheric photochemical reactions, most significantly, the radical-forming photolytic reactions of ozone, formaldehyde, and other aldehydes (National Aeronautics and Space Administration 1985). The free radicals from these reactions initiate and propogate oxidation of organic compounds and result in continued radical production. Such radical chain reactions tend to promote the formation of photochemical smog (Whitten n.d.) and produce the oxidized species critical to the formation of acidic precipitation (Calvert et al. 1985).

The photolysis of ozone to oxygen molecules and $O(^1D)$ atoms has been shown to be energetically feasible below 310 nm and inversely dependent on the density of the atmospheric ozone column (Bahe et al. 1983). This photolytic process is very important in the troposphere because the rapid reaction of $O(^1D)$ with water is a primary source of tropospheric hydroxyl radicals during daylight hours (Crutzen and Fishman 1977). These radicals react rapidly with virtually all gaseous species in the atmosphere (Logan et al. n.d.). The J values for photolytic processes (the apparent first-order rate constants) are defined as $J = \int \sigma_\lambda \phi_\lambda I_\lambda d\lambda$, where (for ozone photolysis to $O(^1D)$).

σ_λ = the ozone absorption cross-section (Bass 1985)

ϕ_λ = the quantum yield of the $O(^1D)$ formation process (National Aeronautics and Space Administration 1985)

I_λ = the surface solar irradiance.

This integral is evaluated over all wavelength intervals for which the product $\sigma\phi I$ is nonzero. When plotted for all significant wavelengths of a given spectral distribution, the resulting curve is known as the photoaction spectrum, and the area under the curve represents the J value for those conditions. Figure 1 shows the photoaction spectrum for ozone-to-$O(^1D)$ photolysis derived from the surface solar data (collected at 300 Dobson[1]) of Bahe et al. (1983). Also shown is the photoaction curve at 200 Dobson, which was extraplated from the measured 300 Dobson solar data using lower resolution, computer-generated 200 Dobson data. The difference in area under the photoaction spectra occurs primarily on the shorter wavelength side because with a 200 Dobson ozone column, additional light is available in this photolytically more efficient region. The difference in rate constants calculated by integration of the two curves is nearly a factor of two greater at 200 Dobson over the entire range of solar zenith angles.

Formaldehyde is also an important species in tropospheric chemistry because it is eventually an oxidation product of almost all organic gases. The most significant chemical reaction is the photolysis of formaldehyde to radical products. J values similar to those for ozone were calculated using the formaldehyde absorption cross sections of the U.S. National Bureau of Standards (Bass 1985) and the quantum yield data summarized by Baulch et al. (1984). These data indicate that 200 Dobson J values should increase by about 20% over the 300 Dobson values for the range of possible zenith angles. The current quantum yield data for both formaldehyde and ozone photolysis appear to produce a smooth curve. However, these data do not have the necessary spectral resolution or accuracy to verify such as assumption. In addition, few high-resolution surface solar spectra exist, and those that do are poorly defined with respect to ozone column density and solar elevation. This lack of resolution limits the precision of the J value calculations and indicates an important area for future research.

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[1] The Dobson unit of measure is the height (in 10 cubic centimeters) that all ozone molecules above the earth's surface would attain if all those molecules existed as pure ozone at standard temperature and pressure.

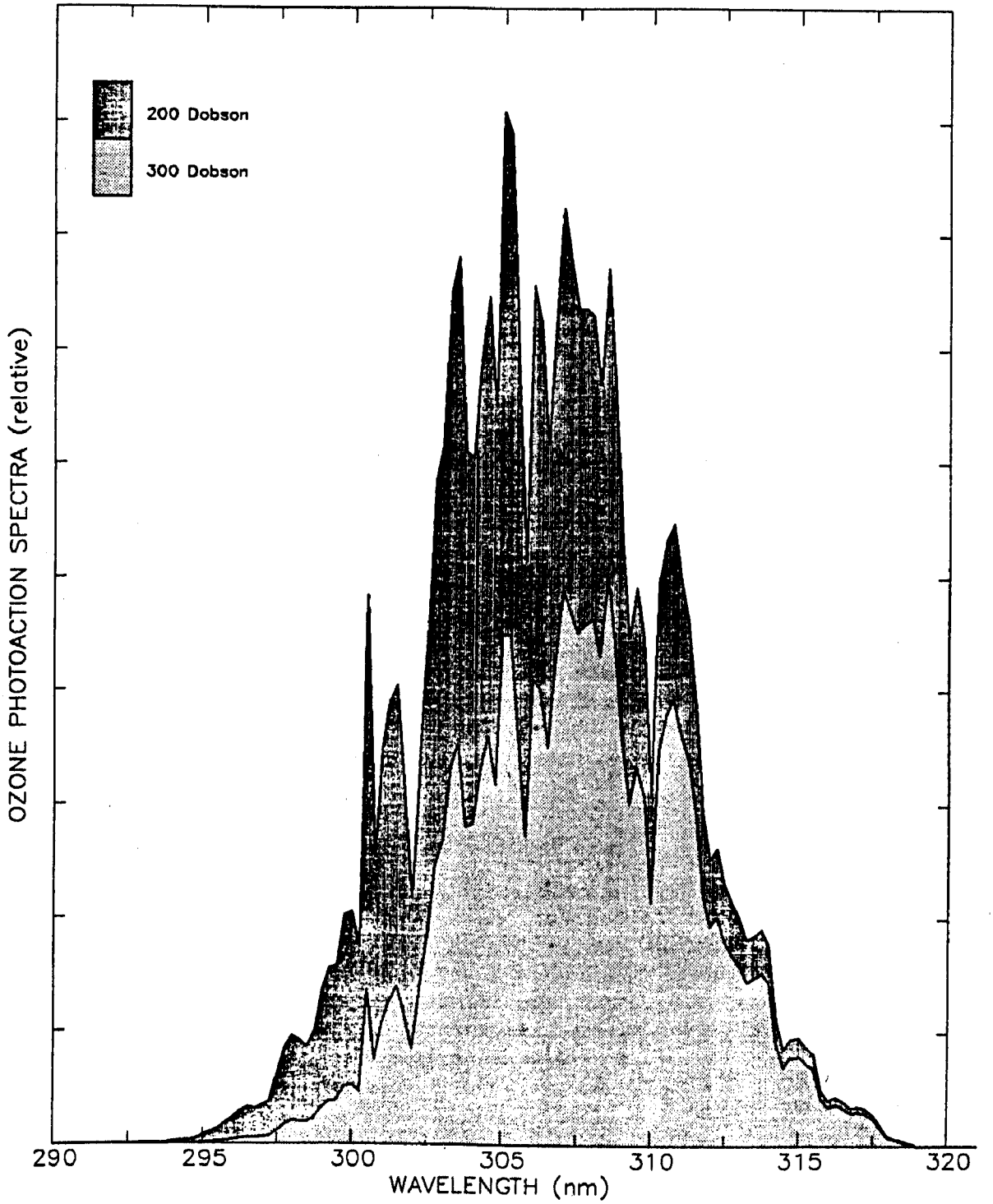

Figure 1. Photoaction Spectra for Ozone Photolysis to $O(^1D)$ and O_2 at 200 and 300 Dobson.

The changes in ultraviolet radiation that could result from stratospheric ozone depletion are unique because they involve specific increases in the short wavelength region of the surface solar spectrum only. Therefore, such changes will cause significant increases in the photolysis rates of only the few species that absorb in the enhanced region, e.g., the radical-forming photolysis reactions of formaldehyde and ozone. Because these reactions are major sources of new gas-phase radicals, the selective increases in those rates will increase the flux in the free radical reaction cycles, resulting in more reactive photochemical conditions in both the free troposphere and the planetary boundary layer. Because hydroxyl radicals provide the dominant sink for a variety of atmospheric compounds (including CO, CH_4, C_2H_6, CH_3Cl, $C4_3CCl_3$, Ch_3Br, H_2S, and SO_2) (Logan et al. n.d.), accurate estimation of hydroxyl radical concentrations is essential to the study of future chemical balance and climatic conditions. Although increased levels of CO and CH_4 will act to suppress hydroxyl radical formation, enhanced photolytic production combined with potentially higher levels of ozone, will counteract this effect.

An increased hydroxyl radical production rate may also diminish the concentrations of some species responsible for stratospheric ozone depletion, thereby providing a feedback mechanism analogous to chemical buffering. However, the magnitude of such a contribution from increased formaldehyde photolysis is uncertain because the formaldehyde mixing ratio is not as clearly defined as that of ozone. Figure 2 illustrates the formaldehyde absorption cross section and surface solar spectrum for the region in which the photoaction spectrum shows significant formation of radical products from formaldehyde photolysis. A correspondence exists between the absorption peaks of formaldehyde and the depressions in measured surface solar irradiance. This correspondence indicates that bin sizes on the order of 0.25 to 0.50 nm may be required to accurately determine formaldehyde J values near the surface. Formaldehyde in the free troposphere could add to this requirement by additional modification of the surface irradiance within the corresponding bands. This phenomenon should be verified with additional high-resolution surface solar data.

The effects of projected spectral changes on near-surface photochemistry will vary depending on the characteristics of each airshed. Airsheds affected by urban emissions have been extensively studied because such emissions significantly increase their concentrations and types of trace gases and their potential for photochemical smog formation. Since the pioneering work of Haagen-Smit (1952), scientists have known that the oxidizing potential of such air masses depends primarily on the concentrations of nitrogen oxides (NO_x) and organic hydrocarbons, as well as on the amount of radiant energy. The impact of variations in ozone column densities and surface temperatures on the chemistry of near-surface air masses can be examined through the use of atmospheric simulation models.[2] Because the chemistry of urban smog formation has

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[2] The urban simulation model used in this work is the OZIPP trajectory model developed for the U.S. Environmental Protection Agency (Hogo and Whitten 1985). This model uses meteorological data to describe the motion of an air mass traveling over urban emission sources to the point at which the highest hourly ozone concentration was observed on the day simulated. Local emission data and a complex gas-phase chemical kinetics mechanism are used to describe the chemical processes within the air mass.

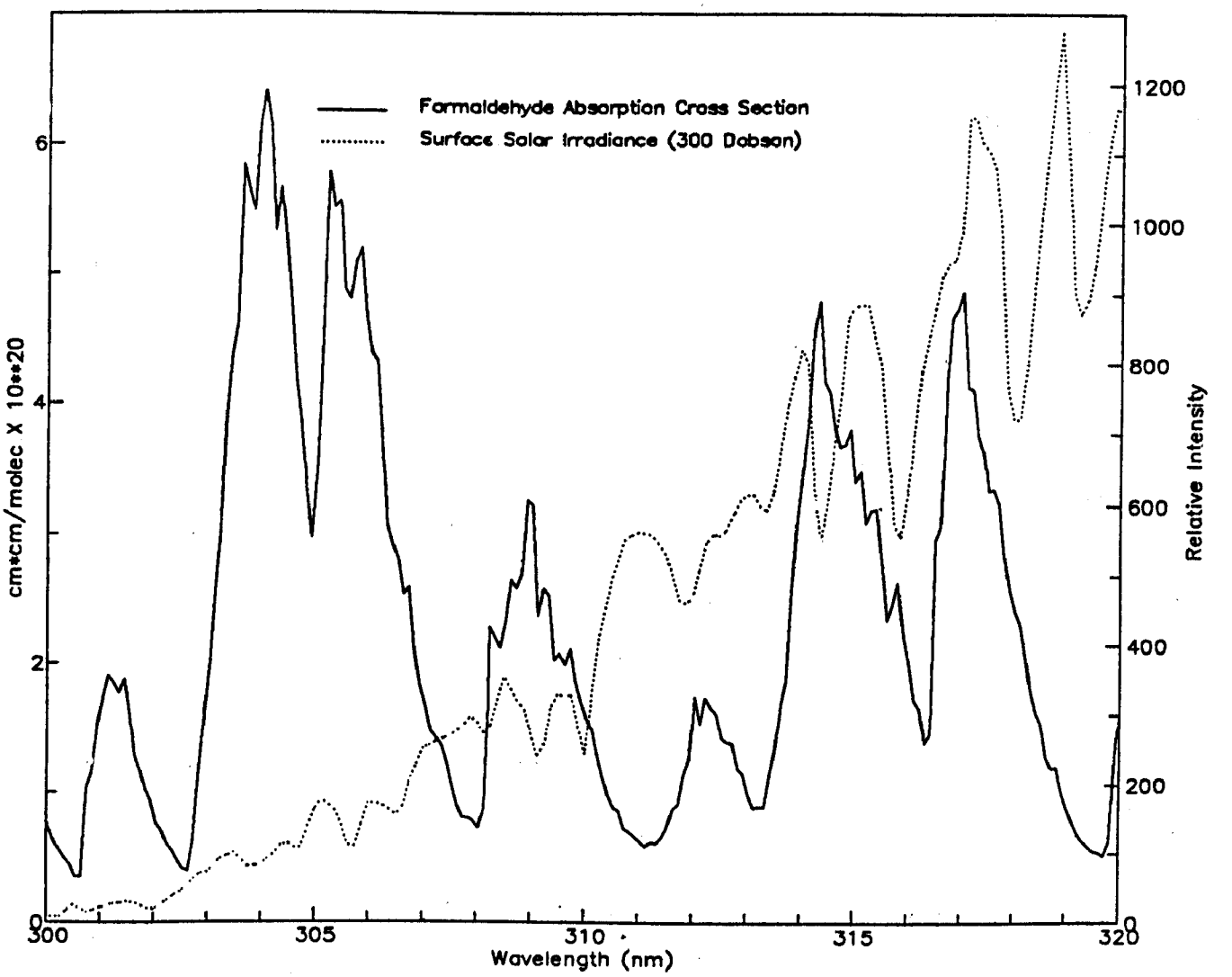

Figure 2. Matched High-resolution for Formaldehyde Absorption Spectra and Measured Surfaced Solar Irradiation.

been extensively studied and many large data collection programs have been carried out, simulations of the photochemistry of this condition are thought to provide a realistic indication of potential impacts.

These models can also be used to estimate the chemical sensitivity to alterations in surface temperatures due to potential changes in concentration of greenhouse gases. Although it is beyond the scope of this discussion to consider the complex perturbations that may alter future surface temperatures, the recent work of Ramanathan et al. (n.d.) and Wang et al. (1986) estimates that these increases will be 1°-3°C by the year 2030. In the work described next, we bracketed this range by assuming a maximum surface temperature variation of 4°C.

We calculated ozone and formaldehyde J values as a function of solar elevation and ozone column density for three urban areas using column densities of 300, 250 and 200 Dobson and surface temperatures of 298 K and 302 K. The test data were selected from data sets for Nashville, Tennessee, Philadelphia, Pennsylvania, and Los Angeles, California. The meteorological conditions and hydrocarbon and NO_x emissions in these three cities differ, as do the maximum ozone concentrations measured in the late afternoon on these days. According to the U.S. National Ambient Air Quality Standard for ozone, air quality on the Nashville test day was nearly in compliance with the standard. The Philadelphia test day was selected to represent urban conditions that require moderate reduction in hydrocarbon emissions to achieve the ozone standard. The Los Angeles test day was selected to examine an extremely reactive region.

Table 1 presents the results of one-day simulations of these three test cases for the Dobson number and temperatures selected. The model consistently predicted higher ozone concentrations with increases in temperature and decreases in Dobson number. For the Los Angeles case, the increases in ozone production were moderate and quite linear with decreasing Dobson number. In the Nashville simulations, the increases were dramatic and the linearity was a function of temperature: At 298 K, the simulated urban ozone tended to increase linearly with a decrease in Dobson number, but at the warmer temperature, simulated ozone concentrations increased more rapidly for the first 50-Dobson unit change than for the second.

The Philadelphia test was performed differently. The hydrocarbon emission data for the original base-case simulation were lowered to the values needed to bring the predicted afternoon ozone maximum into compliance with the federal law. In effect, this scenario represented the condition of future compliance with federal law for 300 Dobson and 298 K. The effects of stratospheric ozone and temperature changes were then simulated for these conditions. The results showed a progressive increase in ozone as the Dobson number declined. In addition, the ozone production occurred much more rapidly at lower Dobson numbers, reaching levels near the final maximum concentration earlier in the day. This finding typifies the simulation results. Although the future scenario conditions resulted in more rapid production of ozone and other oxidants earlier in the simulation period, the maximum predicted ozone concentrations did not reflect such extreme differences, even though lower Dobson numbers and higher temperatures always resulted in more ozone production. This is because ozone production later in the day is often limited because of exhausted hydrocarbon or NO_x concentrations. Because trajectory models, such as the one used here, imply an equivalence in time and distance

Table 1. Ozone concentrations (ppm) predicted for changes in Dobson number and temperature for three cities.

	Ozone Concentrations					
Temperature (K)	298K			302K		
Dobson Number	300	250	200	300	250	200
City						
Los Angeles*	0.288	0.301	0.315	0.306	0.318	0.331
Philadelphia	0.112	0.127	0.149	0.122	0.134	0.159
Nashville	0.130	0.161	0.195	0.146	0.184	0.215

* For Los Angeles, values under 298 K used actual hourly temperatures and values under 302 K are from simulations using those temperatures increased by 5 K.

from the urban center, a rapid occurrence of high ozone concentrations earlier in the day, followed by continued moderate increases, implies that more people will be exposed to episodic levels of ozone. Population density is often greater nearer to the urban center, therefore, the number of people exposed to episodic ozone levels may increase dramatically if future ozone peaks occur earlier in the day.

Our investigation of the key reactions involved in the temperature changes indicated that peroxyacetyl nitrate (PAN) chemistry explains most of the effect in these cases; that is, less PAN exists at higher temperatures because its unimolecular decomposition rate is temperature-dependent. This provides greater amounts of PAN decomposition products (NO_x and the organic peroxyacetyl radical) for use in oxidant-forming reactions. The effects of a 4 K increase in temperature were most pronounced in the Los Angeles and Nashville tests. The effects of a combined temperature increase and reduced Dobson number appear to be always additive, if not synergistic, especially for the Nashville simulations. When a 4 K temperature increase was combined with only a 50-unit Dobson reduction, the simulated peak ozone increased almost 40%.

Enhanced radical flux in urban airsheds should not only increase their ozone-forming potential, but also augment the production of other oxidants such as hydrogen peroxide. In our preliminary tests, the maximum hydrogen peroxide produced for the Philadelphia and Los Angeles cases increased by over an order of magnitude. Additional simulations designed to directly study the effects of future changes on hydrogen peroxide formation should be conducted to verify the magnitude of the processes involved. For the scenarios tested, however, both ozone and hydrogen peroxide concentrations increased in all cases.

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Tropospheric $CH_4/CO/NO_x$: The Next Fifty Years

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ABSTRACT

Previous studies with photochemical models have emphasized the coupling of atmospheric CH_4-CO-OH-NO_x. Recent increases in the concentration of atmospheric methane (CH_4), for example, could be caused by increases in CH_4 sources and/or a decrease in the OH radical that controls the photochemical CH_4 lifetime.

In this paper we use alternate projections of CO and NO_x emissions in a photochemical model to predict tropospheric $CH_4/CO/NO_x$ concentrations over the next fifty years. Simple extrapolation of current CH_4 and CO increases implies that CH_4 will reach 2.9-3.0 ppmv by 2035 and that background CO will double or triple. If we base calculations on projections for combustion CO and NO_x that show a leveling off of CO emissions, there will be less perturbation to background OH and smaller increases in CH_4 and CO over the next fifty years.

INTRODUCTION

Much attention has been given lately to temporal changes in a number of photochemically and radiatively active trace gases. The main topic of Volume 2 is ozone, a gas whose changes over time are driven by perturbations in a large number of trace gases that interact photochemically. One such gas is methane (CH_4), which is of interest for its radiative properties as a greenhouse gas and is of interest photochemically because it helps control abundances of the principal tropospheric oxidant OH and of stratospheric H_2O vapor and HO_x (HO_x = H + OH + HO_2). For example, an increase in CH_4 enhances temperature increases caused by CO_2 (generally regarded as undesirable), but will offset some of the catalytic destruction of stratospheric O_3 caused by reactive odd chlorine and nitrogen (regarded as a good thing).

An example of the mitigating effect of CH_4 on stratospheric O_3 change was described recently by Connell and Wuebbles (1986). Although a catastrophic loss of stratospheric O_3 is predicted in less than 100 years for all CH_4 scenarios considered in their study, there is a significant variation in the evolution of this loss depending on what is assumed for rates of CH_4 changes during the period.

Our purpose here is to discuss changes in atmospheric methane and related changes in tropospheric chemical composition. We give estimates for future CH_4 and offer insight into the complexity of predicting tropospheric change. Methane changes are related to regional and probably global-scale changes in CO, O_3, NO_x, and the free radical OH, which, if anything, are harder to predict than changes in CH_4. These changes strongly influence stratosphere perturbations, although as Whitten and Gery (1986) have shown, coupling between stratosphere and troposphere goes both ways. Stratospheric and climatic changes will ultimately feed back to alter photochemistry in the lower atmosphere.

This paper reviews atmospheric CH_4 perturbations that have already taken place and summarizes tropospheric photochemistry related to CH_4. There is a basic problem in discerning the cause and effect of CH_4 trends because CH_4 is tightly coupled to cycles of other trace gases that may be changing in time, e.g., CO, O_3, NO_x. We also describe results from a photochemical model study interpreting past changes in CH_4 and predicting trends in CH_4 and related gases, $CO/NO_x/O_3$ over the next fifty years. Two types of scenarios for change in $CO/NO_x/OH$ are considered. The first is based on simple extrapolation of recent past CH_4 and CO trends into the future. The second scenario assumes a constant increase in CH_4 sources and smaller increases in CO based on Kavanaugh's (1986) predictions of CO combustion emissions. Finally, since $CH_4/CO/OH$ cycles are coupled to NO_x and tropospheric ozone, we look at changes in these species as well. On a global basis tropospheric O_3, for which there is evidence for a temporal increase (Logan 1985; Bojkov 1986), will probably increase. Changes in shorter lived constituents (e.g., OH, NO_x) are harder to predict because their sources are variable and their geographical distribution will probably change in time with changing patterns of economic activity.

In view of large uncertainties in global budgets of CH_4, CO, and NO_x (Logan et al. 1981; Logan 1983), it is not possible to specify current and future emissions exactly for every geographical region. Rather, we simulate prototypes for various types of environments. One of the specific features of our approach is the use of a one-dimensional model run in a steady-state mode with inputs appropriate for a given year. That is, long-term integration has not been attempted. Furthermore, there is no attempt to simulate the dynamics that allow various regions to communicate with one another, i.e., we do not have a truly global model. Finally, climate changes and perturbations to stratospheric ozone, which may feed back to tropospheric temperature, water vapor, and photodissociation, are not included in the present study.

METHANE OBSERVATIONS AND PHOTOCHEMISTRY: ANALYSIS OF PAST TRENDS

A number of recent studies (Craig and Chou 1982; Khalil and Rasmussen 1985; Stauffer et al. 1985) show that atmospheric CH_4 has been increasing steadily since approximately 1700, after several millenia of constant

concentration. For example, detailed analysis of CH_4 over the past seven or eight years, as measured by Rasmussen and Khalil (1984) at a nonpolluted site along the Oregon coast, reveal seasonal variations in CH_4; once these are taken out there is a definite upward trend. Rowland and coworkers (Blake et al. 1982; Blake and Rowland 1986) present similar evidence from sites around the world, using gas chromatographic analysis of ambient air to determine CH_4 levels. Independent evidence deduced from infrared spectra collected at a European astronomical observatory (Rinsland, Levine, and Miles 1985) shows that CH_4 has been increasing 1% per year for at least 30 years. Over the past century or so CH_4 has roughly doubled, going from 0.8 ppbv to 1.6-1.7 ppbv presently in the Northern Hemisphere; in the Southern Hemisphere where CH_4 sources are fewer background levels are slightly lower.

Why is atmospheric CH_4 increasing? Will it continue to do so and at what rate? Basically, causes for the increase fall into two categories. First, CH_4 sources, all of which are biological in origin (outgassing of oceans and marshes, rice paddies, enteric fermentation of ruminants, termites), are increasing. Second, the photochemical sink limiting the atmospheric lifetime of CH_4, the OH radical, is decreasing.

What controls OH and how do we decide which of these processes is during current CH_4 changes? First of all, the fact that CH_4 is increasing itself suppresses OH and adds CO to the atmosphere (Sze 1977; Chameides, Liu, and Cicerone 1977). This further suppresses OH, methane and CO being the trace gases that react most rapidly with OH. Nonmethane hydrocarbons (NMHC) also react efficiently with OH (Greenberg, Zimmerman, and Chatfield 1985), and further study of their role in OH perturbation is needed. In the present study we assume NMHC are localized and not significant on a global scale and neglect NMHC in our model except for C_2H_6. There is some evidence of a secular increase in CO (Khalil and Rasmussen 1984; Rinsland, Levine, and Miles 1985) although this is harder to establish than increasing CH_4, owing to the shorter lifetime and greater natural variability of CO. OH, a transient species (lifetime approximately 1 s), is even more variable, and so far has been impossible to measure in the troposphere. Additional evidence for diminishing OH may be the detection of a temporal increase in atmospheric C_2H_6 over the past 35 years (Levine, private communication 1986).

It appears that recent CH_4 changes are caused by some combination of both increasing CH_4 sources and decreasing OH. (Thompson and Cicerone 1986a; Khalil and Rasmussen 1985; Levine, Rinsland, and Tennille 1985). Figure 1 shows results from model calculations simulating CH_4, CO, and OH from 1860 to 1985, making several assumptions about the rate of CO change over the past century. Simulations in Figure 1a assume no change in CH_4 sources, whereas in Figure 1b we are able to reproduce CH_4 observations by assuming that CO increases are accompanied by an increase in CH_4 sources.

PREDICTING METHANE, CO, AND OH PERTURBATIONS

Extrapolation of Recent Trends

Predictions of future CH_4 have been published (Chameides, Liu, and Cicerone 1977; Sze 1977), although this is not a simple matter. One reason is the ambiguous nature of changing CH_4 sources and/or perturbed CO-OH as causes of increasing CH_4, as described above. Furthermore, budgets of CH_4 and CO are

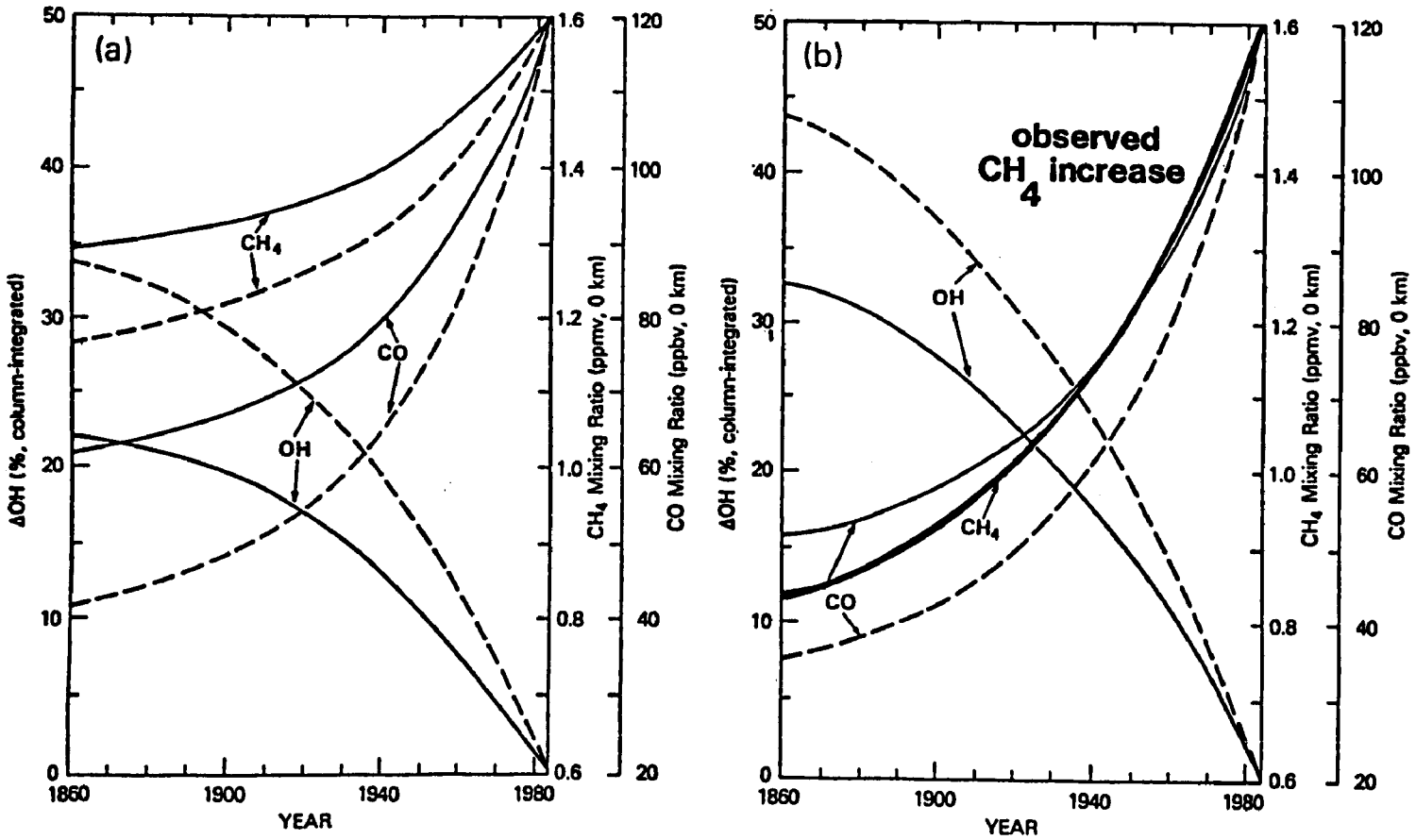

Figure 1. (a) Past methane, CO mixing ratios and fractional change in tropospheric OH (relative to 1985). No change assumed in CH_4 sources from 1860 to 1985. (b) CO mixing ratios and fractional OH loss with observed CH_4 mixing ratios (Khalil and Rasmussen 1985). Observations are matched if an increase in CH_4 sources is assumed (Thompson and Cicerone 1986a).

poorly known at present and predicting them in the future is problematic because they depend to a large extent on human activity. Agricultural practices are subject to change; biomass burning in developing nations plays an important role; economic activity depends heavily on combustion of fossil and nonfossil fuels; there may be switches away from the currently most popular fuels. The issue is further complicated by photochemical coupling of OH and CH_4 changes to tropospheric NO_x, NMHC, and ozone, which also appear to be changing in time (Volz, Smit, and Kley 1985; Bojkov 1986).

One approach to predicting future CH_4 is to extrapolate from recent trends, making certain assumptions about the nature of current CH_4 and CO perturbations (Thompson and Cicerone 1986b). For example, if atmospheric CH_4 over the next 50 years increases at its current rate of 1% per year, it will reach 2.9-3.0 ppmv in 2035 (Figure 2). Concurrent increases in background CO and decreases in OH can be calculated with a photochemical model. In a typical simulation, total CO sources consist of 35% surface flux in 1985, mostly anthropogenic in origin, i.e., fossil fuel combustion and biomass burning; the other 65% is mostly natural, i.e., photochemical oxidation of CH_4 and C_2H_6 and a specified input of CO to simulate the photo-oxidation of NMHC. (See Table 1, Type 1-CO.) A ground-level mixing ratio of 120 ppbv is chosen as typical of nonpolluted northern midlatitudes. Inputs for CO in our calculations simulate both natural and anthropogenic sources with the former taken as constant in time and the latter assumed to increase with world population and industrial growth continuing at their current rates. If combustion CO follows CO_2 (increasing in output approximately 2.5% per year), background CO will increase from 120 ppbv in 1985 to 260 ppbv in 2035 (Figure 2).

Table 1. Parameterization of CO Sources--1985

$S^{total,CO}$ =	Q_{CO} +	$flux_{CO}$ +	S_{NMHC}
Low NO_x: $S_{total} = 1.9 \times 10^{11}$ cm^{-2} s^{-1}			
Type 1-CO	15%	35%	50%
Type 2-CO	15%	65%	20%

Q_{CO} is photochemical source of CO (molec cm^{-2} s^{-1}) from OH oxidation of CH_4 and C_2H_6 as computed by model.

$flux_{CO}$ is model lower boundary condition, representing CO sources introduced at surface (ocean, combustion, plants, etc),

S_{NMHC} is CO source specified at every altitude, simulating oxidation of nonmethane hydrocarbons not included in model.

Perturbations to atmospheric CO and CH_4 are highly sensitive to background NO_x (especially in the boundary layer). The calculations described above are appropriate for simulating background conditions in northern midlatitudes (low NO_x, approximately 25-30 pptv NO_x; surface CO, 100-150 ppbv in 1985). Increasing CH_4 and CO suppress the concentration of OH through their control of the OH lifetime. In our model CO reacts with 80% of the OH, while CH_4 and C_2H_6 react with the remainder. If higher NO_x is assumed at the surface (approximately 1 ppbv, a level characteristic of many continental environments, not strongly polluted but influenced by areas of high combustion), then increasing CH_4 and CO emissions contribute to smog-type reactions forming ozone and OH through ozone photolysis:

$$CO + OH \rightarrow CO_2 + H$$

$$H + O_2 \rightarrow HO_2$$

$$HO_2 + NO_2 \rightarrow NO_2 + OH$$

$$NO_2 + h\nu \rightarrow NO + O(^3P)$$

$$O_2 + O(^3P) + M \rightarrow O_3 + M$$

$$O_3 + h\nu \rightarrow O_2 + O(^1D)$$

$$O(^1D) + H_2O \rightarrow 2OH$$

In the lower troposphere these reactions partially offset mid-troposphere losses of OH to give a lower total column loss than for low NO_x regimes (Hameed, Pinto, and Stewart 1979; Thompson and Cicerone 1986b).

Figure 3a shows OH and O_3 changes corresponding to CH_4 and CO increases illustrated in Figure 2. For low NO_x, surface values of 25-30 pptv, as might be found over unpolluted marine or even continental regions, present day O_3 is approximately 30 ppbv. With CO doubling and CH_4 increasing from 1.6 to 2.9 ppmv over the next fifity years, surface O_3 increases to 36 ppbv, and the loss in total tropospheric OH (integrated from 0-15 km) is 35%. For a region with NO_x approximately 1 ppbv, surface O_3 is 40 ppbv in 1985, increasing to 50 ppbv by 2035; the corresponding loss in OH is only 25% because additional O_3 mitigates OH depletion.

Assuming constant NO while CO increases is not realistic since combustion is a large source of both gases. Consider an environment in transition from a moderately low background NO_x value, 0.1-0.2 ppbv in the past two decades, increasing along with increasing NO_x emissions to 0.48 ppbv by 2035. The historical record of NO_x is not known, but NO_x emissions are certainly increasing (Dignon and Hameed 1985), which may be a more realistic representation of continental environments than assuming constant 1 ppbv NO_x. We refer to these as "transitional" regions. With O_3 prescribed as in

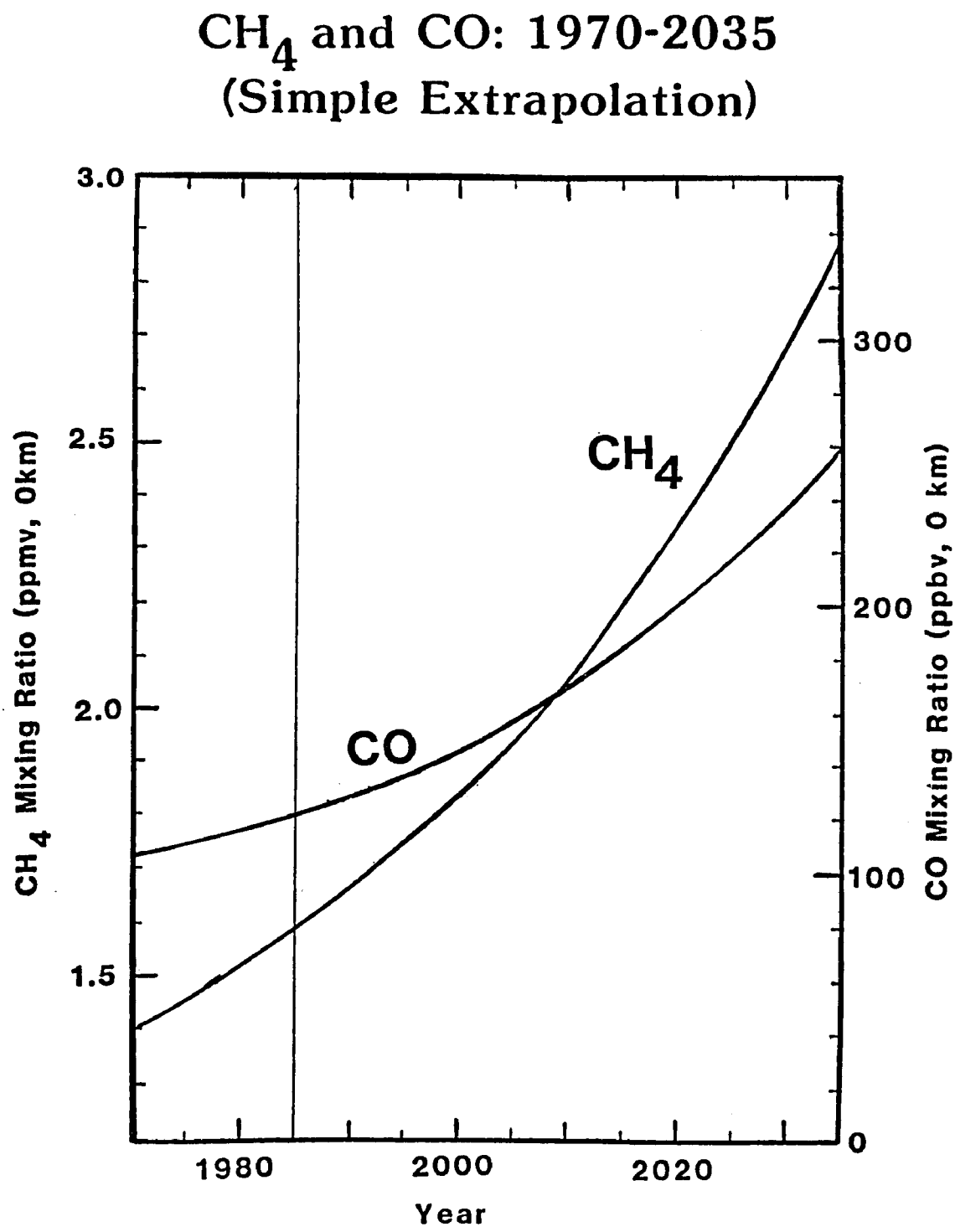

Figure 2. Ground level CH_4 and CO mixing ratios from 1970 to 2035 (Thompson and Cicerone 1986b). Simulations were carried out with a steady-state model at 10-year intervals assuming CH_4 mixing ratio increases 1%/year from 1985 and CO increases are driven by fossil fuel combustion and population growth continuing at current rates of increase. Model conditions are appropriate for the mid-latitude northern hemisphere with low background NO_x (20-25 pptv).

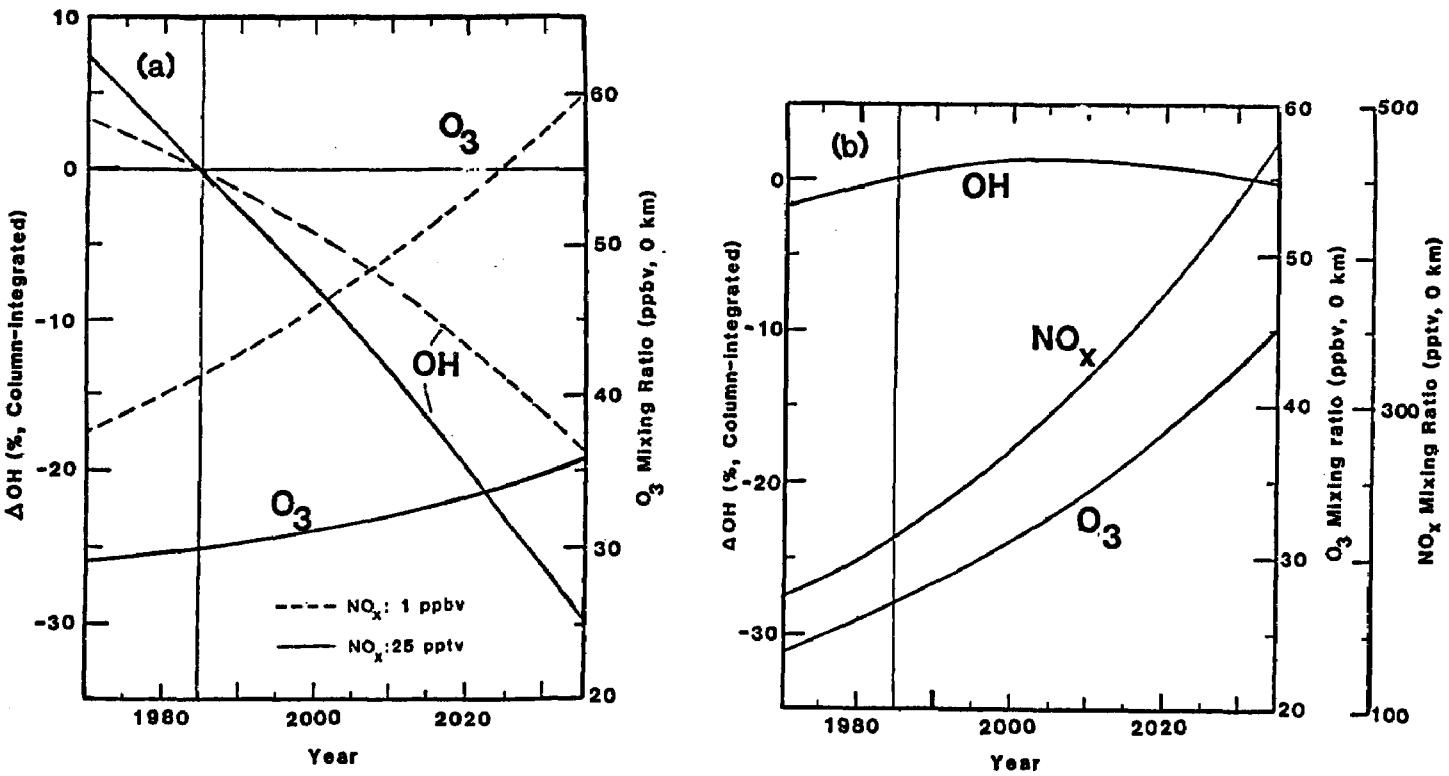

Figure 3. Surface O_3 and percent changes in column-integrated OH (0-15 km) corresponding to CH_4 and CO changes shown in Figure 2 (a) Constant background NO_x. Solid lines calculated with low surface NO_x (25-30 pptv); dashed lines calculated with surface NO_x at 1 ppbv. (b) O_3 and change in OH calculated with CH_4 and CO as in Figure 2 and NO_x as shown.

Figure 3b the corresponding loss in OH is almost negligible. If O_3 is not prescribed explicitly but is calculated self-consistently with changing CH_4, CO, and NO_x, ozone reaches approximately 60 ppbv in 2035 and OH increases slightly.

CH_4/CO/OH Trends from Kavanaugh's CO and NO_x Projections

Predictions of CH_4, CO, and OH based on simple extrapolation of recent CH_4 trends probably give upper limits for CH_4 and CO fifty years from now. Recent evaluation of energy-related combustion sources of CO from 1975 to 2025 (Figure 4a; Kavanaugh 1986) shows that this important part of the CO budget will level off with increasing controls of mobile source emissions in developed nations, slowing population growth, and a shift away from rich CO-producing fuels for domestic energy use (e.g., charcoal bricks in China) in favor of large-scale utilities that burn carbon more completely.

We have performed a series of model calculations of CO and CH_4 based on Kavanaugh's emissions projections for CO, making reasonable assumptions about noncombustion sources of CO. We find that to the degree that CH_4 is currently responding to CO increases and OH decreases, methane increases over the next fifty years will slow down as CO emissions rise more slowly than they have recently.

Detailed analysis of model results shows that if the CO changes illustrated in Figure 1b are realistic, the corresponding CH_4 source change during the past 20 to 30 years is 0.2, 0.4% or 0.6% per year when NO_x is at low levels (25-30 pptv) as observed in the nonpolluted atmosphere (McFarland et al. 1979; Torres 1985). These three cases form the basis for our projections of background CH_4, CO, and OH over the next fifty years. We assume that CH_4 sources continue to increase 0.2%, 0.4%, 0.6% yr^{-1} and we combine this with Kavanaugh's global estimate for the rate of combustion CO increase, assuming that natural CO sources do not change significantly over the period. Details of the scenarios are given in Table 2. The model is run at several specified dates from 1985 to 2035. The two types of CO sources refer to the fraction of the total CO budget at 1985 that is caused by direct surface sources of CO (e.g., fossil fuel combustion, wood- and biomass-burning, lightning-induced forest fires, ocean and plants) that are mostly anthropogenic. Remaining CO comes from CH_4 and C_2H_6 oxidation calculated by the model and from a specified in situ source that represents oxidation of NMHC; the latter is presumed mostly natural in origin, but that fraction coming from anthropogenic sources increases at the rate predicted by Kavanaugh (1986), whose projections are also the basis of changes in the anthropogenic fraction of the flux (model lower boundary condition). The same rates of increase are used in both types of CO models, but the one with only 35% of the CO source being anthropogenic increases more slowly.

Methane mixing ratios calculated with low NO_x from 1985 to 2035 appear in Figure 5a; corresponding CO increases and fractional loss in OH appear in

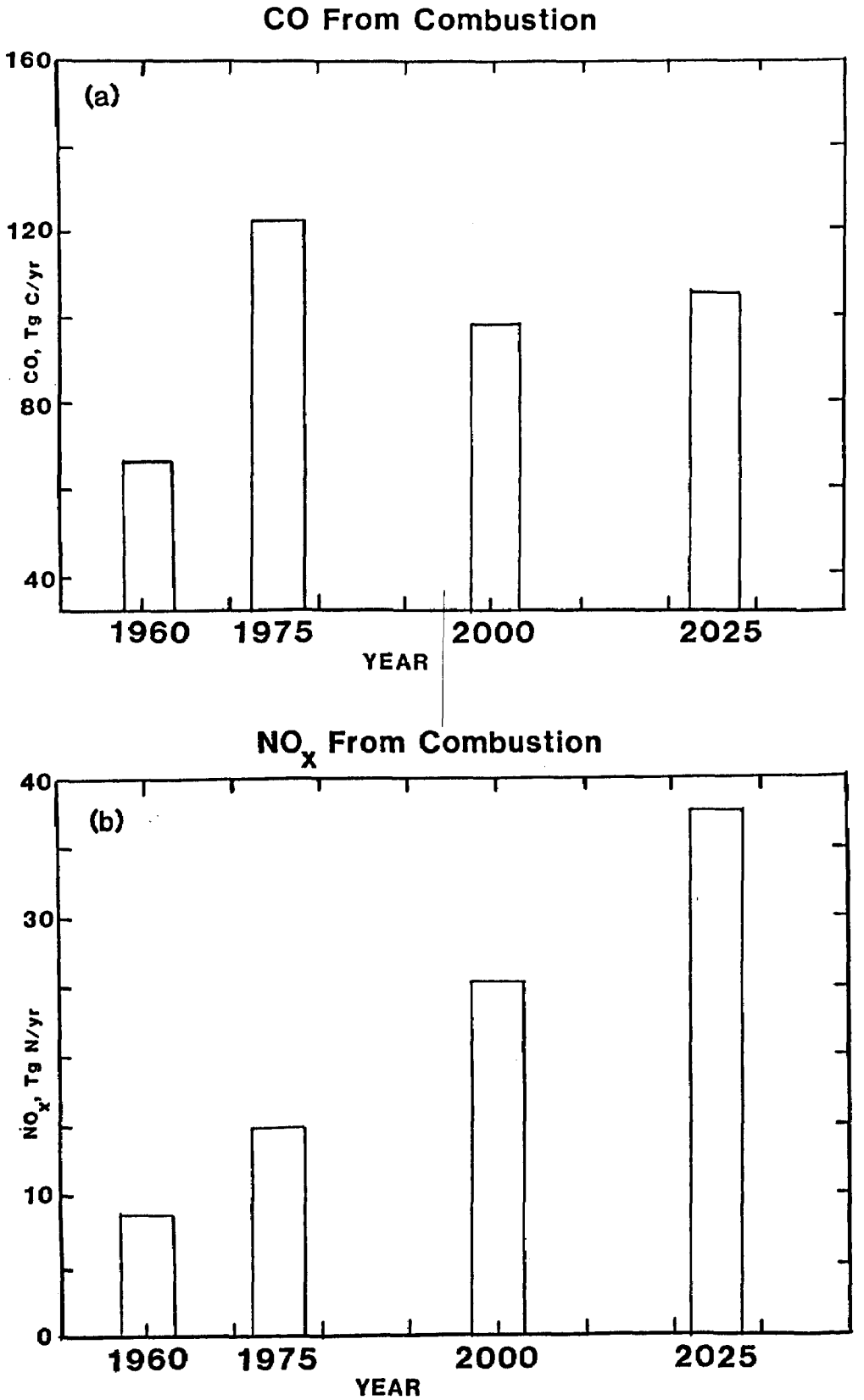

Figure 4. Total global emissions from energy combustion of fossil and non-fossil fuels (Kavanaugh 1986). (a) CO, in Tg C per year; (b) NO_x, in Tg N per year.

Table 2. Scenarios for CH_4, CO Perturbations

Source	Model 1	Model 2	Model 3
CH_4	CH_4 flux** inc. .2%/yr	CH_4 flux inc. .4%/yr	CH_4 flux inc. .6%/yr
CO	Type 2-CO*	Type 1-CO*	Type 1-CO
	increase in combustion CO after Kavanaugh (1986)		

* Type 1-CO: 35% surface flux (mostly anthropogenic) CO sources; Type 2-CO: 65% surface flux CO sources in 1985. See Table 1 and Thompson and Cicerone (1986) for detailed description of CO sources.

Photochemical model (Thompson and Cicerone 1982; 1986b) run at background continental NO_x (1 ppbv) and marine (25-30 pptv) conditions.

** 1985 CH_4 flux: 4.4×10^{10} cm^{-2} s^{-1} (low NO_x); 1.31×10^{11} cm^{-2} s^{-1} (high NO_x). OH column, 0-15 km (1985): 5.4×10^{11} cm^{-2} (low NO_x); 1.15×10^{12} cm^{-2} (high NO_x).

Figure 5b. The 50-year projection for CH_4 is 2.0-2.6 ppmv, appreciably lower than the figure of 2.9 ppmv obtained assuming an indefinite 1% per year increase in mixing ratio. We see that CO changes are much less than the doubling derived from smooth extrapolation of current trends; with less CO there is less perturbation to OH and to CH_4.

Kavanaugh (1986) shows that emissions of NO_x will continue to increase worldwide (Figure 4b). We have considered several NO_x scenarios in our calculations, but because of the short lifetime and localized nature of NO_x, it is difficult to say how NO_x increases will affect background OH. It seems likely in the lower troposphere, at least, that background NO_x, O_3, and OH will increase over large regions of the earth. For example, greatest growth in CO and NO_x will be in China and third world countries. At the same time highly industrialized nations may be controlling emissions of these gases. This implies a shift in global background photochemistry, e.g. oxidant and acid formation, away from the industrialized nations to the developing countries. OH losses from increasing CO and CH_4 (also NMHCs) might be offset by increasing O_3 and NO_x, resulting in slower depletion of global OH.

Model calculations using Kavanaugh's projected changes in CO and NO_x need to be performed on a regional basis, dividing the continents into types of regions according to economic and population growth patterns, assuming little change in NO_x over the oceans. In some regions there will be substantial changes in CO and NO_x simultaneously, in others there will be little NO_x change. In still others there will be nearly constant combustion CO but large increases in NO_x.

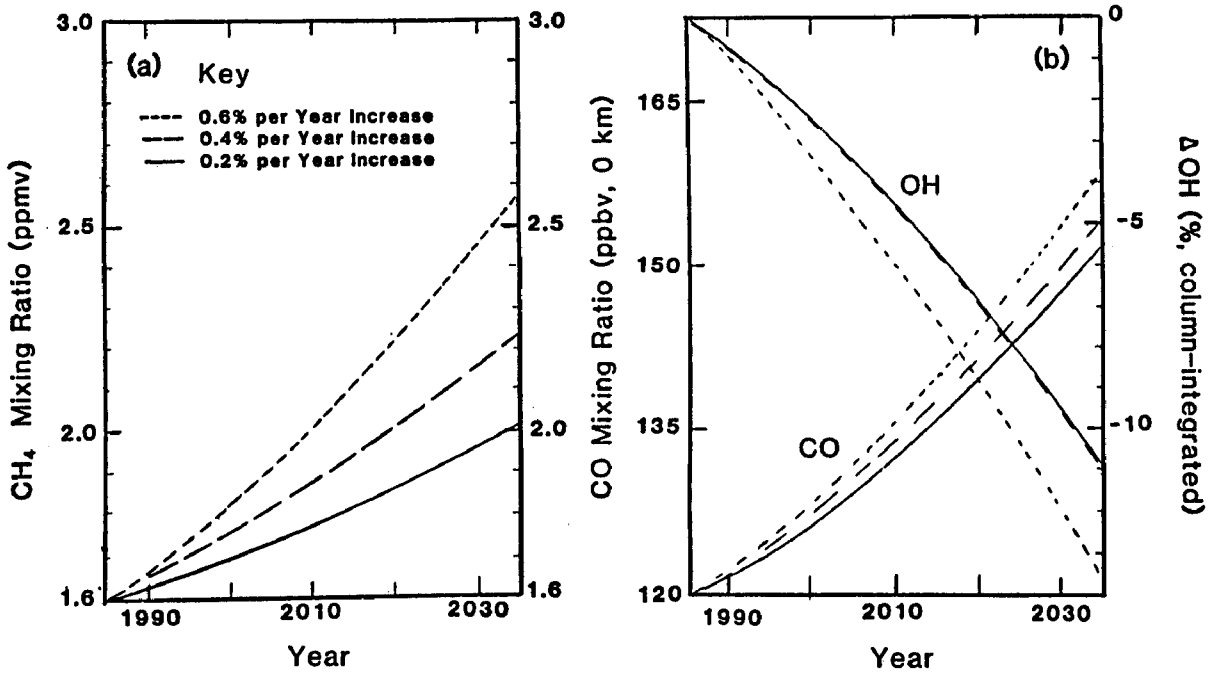

Figure 5. (a) Ground level CH_4 and (b) CO mixing ratios and percent changes in column-integrated tropospheric OH from 1985 to 2035 assuming various rates of increase in CH_4 surface fluxes. Results from Models 1-3 (Table 2).

Global change in OH is a composite of regional changes. We compute global OH and ozone in a greatly oversimplified way, given the limitations of a one-dimensional model. First, we divide the earth into six broad latitude bands; then, we use a model to calculate OH from typical $CH_4/CO/NO_x/O_3$ for continental and marine areas within each band. We add marine and continental OH within each band and take a weighted mean throughout the troposphere. Total mean OH (4.8×10^5 molecules per cubic centimeter) compares well with global OH computed from a similar set of model calculations by Chameides and Tan (1981). To make a very crude estimate of future changes in global OH, we (a) assume that OH changes over the oceans follow the course of OH in constant, low NO_x environments as calculated from the Kavanaugh-based scenario, decreasing 11% from 1985 to 2035 (Figure 5b); and (b) that OH over continental areas follows the course of "transitional" NO_x environments, virtually unchanged from 1985 to 2035 (Figure 3b):

$$[OH(land)]_{1985} \times (1 + \text{frac. change } OH_{2035,\text{transitional } NO_x}) +$$

$$[OH(marine)]_{1985} \times (1 + \text{frac. change } OH_{2035,\text{low } NO_x}) = [OH]_{2035,\text{mean}}$$

The result is 5% to 10% less OH in 2035 than at present. If we apply the formula assuming that marine areas lose OH at the rates shown in Figure 3a, the loss is 18% to 25% after 50 years. These values are different from assuming that Northern Hemisphere midlatitude trends in CH_4, CO, and OH will continue at current rates and are representative of the earth as a whole. It looks as if the recent OH decline deduced from CO/CH_4 perturbations will slow down because CO combustion is leveling off and NO_x combustion will continue to increase. However, increasing NO_x may contribute to substantial increases in tropospheric ozone.

SUMMARY AND CONCLUSIONS

This paper describes photochemical aspects of atmospheric methane perturbations and presents model predictions of CH_4 over the next fifty years. Simple extrapolation of recent CO/OH trends implies a doubling of CO from 1985 to 2035 and CH_4 increasing from 1.6-1.7 to 2.9 ppmv. Depending on background NO_x levels, these CO/CH_4 increases will suppress tropospheric OH 15% to 30%. However, reduced emissions of combustion CO and increasing NO_x (Kavanaugh 1986) maintain higher OH and, given a constant increase in CH_4 sources, lower CH_4 levels are predicted in 2035, i.e., 2-2.5 ppmv.

Strictly speaking, CO and NO_x changes must be considered on a regional basis to calculate perturbations in OH and CH_4, with an appropriate averaging to estimate global changes in OH. The best way to predict future CH_4, CO, and OH is with a multidimensional model that includes NO_x, CO, and CH_4 source changes on a well-resolved spatial grid along with transport of longer lived trace gases. A further uncertainty in our calculations is the neglect of explicit nonmethane hydrocarbon (NMHC) chemistry. NMHC emissions are probably changing in time, and because NMHC produce CO and O_3 photochemically their effects may be complex, working either to destroy or enhance OH. Finally, we note that our study does not include explicit perturbations to CO emissions from photochemical oxidation of vegetative hydrocarbon emissions or biomass burning, both of which may be altered by changing patterns of land use in developing nations.

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