SEASONAL CHANGES IN IMMUNE FUNCTION

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ABSTRACT

    Winter is energetically demanding. Physiological and behavioral adaptations have evolved among nontropical animals to cope with winter because thermoregulatory demands increase when food availability decreases. Seasonal breeding is central within the suite of winter adaptations among small animals. Presumably, reproductive inhibition during winter conserves energy at a time when the odds of producing viable young are low. In addition to the well-studied seasonal cycles of mating and birth, there are also significant seasonal cycles of illness and death among many populations of mammals and birds in the field. Challenging winter conditions, such as low ambient temperatures and decreased food availability, can directly induce death via hypothermia, starvation, or shock. In some cases, survival in demanding winter conditions puts individuals under great physiological stress, defined here as an adaptive process that results in elevated blood levels of glucocorticoids. The stress of coping with energetically demanding conditions can also indirectly cause illness and death by compromising immune function. Presumably, the increased blood concentrations of adrenocortical steroids in response to winter stressors compromise immune function and accelerate catabolic mechanisms in the field, although the physiological effects of elevated glucocorticoids induced by artificial stressors have been investigated primarily in the laboratory. However, recurrent environmental stressors could reduce survival if they evoke persistent glucocorticoid secretion. The working hypothesis of this article is that mechanisms have evolved in some animals to combat seasonal stress-induced immunocompromise as a temporal adaptation to promote survival. Furthermore, we hypothesize that mechanisms have evolved that allow individuals to anticipate periods of immunologically challenging conditions, and to cope with these seasonal health-threatening conditions. The primary environmental cue that permits physiological anticipation of season is the daily photoperiod; however, other environmental factors may interact with photoperiod to affect immune function and disease processes. The evidence for seasonal fluctuations in lymphatic organ size, structure, immune function, and disease processes, and their possible interactions with recurrent environmental stressors, is reviewed.

    Seasonal peaks of lymphatic organ size and structure generally occur in late autumn or early winter and seasonal minima are observed prior to the onset of breeding. Although many of the field data suggest that immune function and disease processes are also enhanced during the winter, the opposite seasonal pattern is also observed in some studies. We propose that compromised immune function may be observed in some populations during particularly harsh winters when stressors override the enhancement of immune function evoked by short day lengths. Because so many factors covary in field studies, assessment of our proposal that photoperiod mediates seasonal changes in immune function requires laboratory studies in which only photoperiod is varied. A review of the effects of photoperiod on immune function in laboratory studies reveals that exposure

to short day lengths enhances immune function in every species examined. Short day exposure in small mammals causes reproductive inhibition and concomitant reduction in plasma levels of prolactin and steroid hormones, as well as alterations in the temporal pattern of pineal melatonin secretion. These hormones affect immune function, and influence the development of opportunistic diseases, including cancer; however, it appears that either prolactin or melatonin secretion is responsible for mediating the effects of photoperiod on immune function. Taken together, day length appears to affect immune function in many species, including animals that typically do not exhibit reproductive responsiveness to day length. These data could have a major impact on understanding the etiology and progression of diseases in humans and nonhuman animals. The clinical significance of these data is also considered.

INTRODUCTION

INDIVIDUALS INHABITING nontropical habitats experience seasonal deterioration and renewal of environmental energy resources. High thermoregulatory requirements for small mammals and birds during the winter typically coincide with low environmental food availability (Blank 1992; Wunder 1992; Wingfield and Farner 1993). The energetic "bottleneck" created by high energy demands during times of low energy availability has led to the evolution of many adaptations that allow individuals to cope with winter. Breeding is energetically expensive, and cessation of breeding activities is central within the suite of winter-coping adaptations observed in boreal and temperate zone species (Bronson and Heideman 1994). Tactics have evolved that permit individuals to maximize the length of the breeding season without jeopardizing survival *vis a vis* energy use. Because seasonal adaptations often require time to develop, the precise timing of behavioral and physiological adaptations necessary to cope with energy shortages is a critical feature of individual reproductive success and subsequent fitness. Mechanisms have evolved to ascertain the time of year precisely in order to phase territorial defense, breeding, molt, migration, and other energetically expensive activities to coincide with peak energy availability and other local conditions that promote survival (Bronson 1989; Moffatt et al. 1993; Wingfield and Farner 1993).

In some cases, physiological and behavioral changes that have an obvious and immediate adaptive function may occur in direct response to environmental factors. For example, food and water may be available only during certain times of the year, and a decrease in the amount of procurable nutrients can lead to reproductive inhibition (Bronson and Heideman 1994; Nelson 1987; Wingfield and Kenagy 1991). These types of environmental factors have been termed the "ultimate factors" underlying seasonality (Baker 1938). Many animals need to forecast the onset or offset of these ultimate factors in order to initiate time-consuming seasonal adaptations. Therefore, seasonally-breeding animals frequently detect and respond to environmental cues that accurately signal, well in advance, the arrival or departure of seasons favoring reproductive success. The cues, or proximate factors (Baker 1938), used to predict environmental change may or may not have direct survival value (e.g., photoperiod), and may or may not be the same as the ultimate factor (e.g., food availability).

Proximate environmental factors that regulate reproductive activities have been classified into four categories (Wingfield 1980; Wingfield and Kenagy 1991): (1) initial predictive cues, (2) supplementary factors, (3) synchronizing and integrating information, and (4) modifying information. Initial predictive cues include environmental factors that stimulate gonadal development; e.g., increasing or decreasing day lengths in birds and ungulates, respectively. Supplementary environmental factors work to fine tune the timing of breeding; factors such as temperature or availability of specific types of food are considered to be supplementary cues. Synchronizing and integrating information includes behavioral interactions that affect reproductive function; these cues are usually stimulatory, but they can also be inhibitory. Finally, modifying factors are essentially unpredictable stressors that act to inhibit reproductive function either by affecting the breeding individuals directly (e.g., a late freeze that limits food availability) or indirectly (e.g., a late freeze that kills the offspring, but does not physically harm the adults).

The most frequently studied proximate, initial predictive, factor is photoperiod (day length), a cue that can serve as a very precise reference for the time of year. Virtually all nontropical species of mammals and birds thus far studied can potentially use the annual cycle of day length to time their breeding season (Goldman and Nelson 1993; Karsch and Moenter 1990; Wingfield and Farner 1993). Presumably, with only two bits of data, length of day and direction of change in the daily photoperiod, an animal could tell precisely the time of year. This information could then be used to anticipate subsequent seasonal environmental changes and initiate or terminate specific seasonal adaptations in order to maintain a positive energy balance.

Maintaining a positive energy balance is required for survival and reproductive success. But other threats to survival must also be met in order for individuals to increase their fitness. Individuals must avoid predators, potentially dangerous attacks by conspecific competitors, and succumbing to pathogens or parasites. In some cases, a marginal energetic balance can weaken animals to the extent that they are very susceptible to disease processes (Berczi 1986). Immunological defense against invading organisms requires cascades of mitotic processes that demand substantial energy (Kelley 1985). In addition, modifying environmental factors that can interrupt breeding, or other conditions perceived as stressful, can compromise immune function and promote opportunistic pathogens and parasites to an extent that leads to premature death (Ader and Cohen 1993; Berczi 1986). Modifying factors are operationally defined as unpredictable (Wingfield and Kenagy 1991), but many potential stressful conditions, such as low ambient temperatures, reduced food availability, migration, overcrowding, lack of cover, or increased predator pressure, can recur on a somewhat predictable, seasonal basis, potentially leading to seasonal changes in population-wide immune function and death (John 1994; Lee and McDonald 1985; Lochmiller et al. 1994; McDonald et al. 1988; Fänge and Silverin 1985). Thus, in addition to the well-studied seasonal cycles of birth, there are also significant, albeit not as well studied, seasonal cycles of illness and death among populations of wild mammals and birds (Descôteaux and Mihok 1986; Lochmiller et al. 1986).

Seasonal fluctuations in immune function and survivorship may not be observed every year or in every population. Some winters may not be perceived as stressful, either because of mild ambient conditions or because energetic coping adaptations succeed in buffering individuals from harsh conditions. Consequently, populations in nature may exhibit compromised, enhanced, or static immune indices during the winter, although we suspect that the literature will be biased in favor of reporting *changes* in immune function. We have summarized the literature on seasonal fluctuations in immune function in the present article, and propose that animals have evolved mechanisms to enhance immune function in order to compensate for compromised immune function induced by the stressors of winter. During mild winters, the immunological compensatory mechanisms should enhance or stabilize immune function; during harsh winters or when environmental conditions elicit sustained glucocorticoid secretion, immune function may become compromised despite the compensatory mechanism(s).

Our working hypothesis is that some animals have evolved mechanisms to combat seasonal stress-induced reductions in immune function as a temporal adaptation to promote survival. We propose that exposure to short day lengths enhances immune function. Many field studies are consistent with this hypothesis; i.e., lymphatic tissue size or immune function are elevated during the winter. In every laboratory study in which only day length is manipulated, immune function is enhanced in short days as compared to animals maintained in long-day-length conditions. Thus, mechanisms have evolved to allow animals to anticipate immunologically-challenging conditions by monitoring photoperiod, and to cope with these seasonal health-threatening conditions by bolstering lymphatic tissue development and immune function directly.

We address the physiological mechanisms underlying the detection of and response to environmental factors that affect immune function and disease processes. We review reports of seasonal changes in lymphatic organ size and structure, immune function and dis-

ease processes, and their possible interactions with recurrent environmental stressors. Most research in seasonality has focused on the role of photoperiod in providing temporal information for breeding (Reiter 1991). Here we assess the physiological sequelae of photoperiod in mediating the seasonal changes in immune function. The effects of short photoperiods on mammals include reduction in blood levels of steroid hormones (in long-day breeders) and prolactin, as well as alterations in the temporal pattern of pineal melatonin secretion (Goldman and Nelson 1993). The effects of these hormones on immune function and opportunistic diseases, including cancer, will also be explored. Individuals of some species do not adjust reproductive function in response to photoperiod, and changes in gonadal steroid hormones or prolactin levels are not detected on a seasonal basis (Nelson 1987; Nelson et al. 1994). Despite the lack of reproductive responsiveness to day length, seasonal changes in nonreproductive responses may be observed, and these changes may be mediated by melatonin. Because alterations in melatonin secretion occur in animals that do not exhibit reproductive responses to day length, including humans, this is an important hormone to study for its clinical implications. Melatonin will receive particular attention because this hormone has been reported to affect immune function and tumorigenesis in several model systems. The clinical relevance of these seasonal fluctuations in lymphatic tissue size and immune function for humans and nonhuman animals will be presented.

SEASONAL BREEDING AND PHOTOPERIODISM

There is an extensive literature on the mechanisms regulating seasonal cycles in reproduction. The principles of seasonality derived from this literature will serve as a basis for the examination of the sparser database directly related to seasonal changes in immune function. The mechanisms that regulate seasonal reproductive changes may be classified under two categories. One set of mechanisms is directly responsible for controlling changes in the reproductive system. For example, changes in the rate or pattern of pituitary hormone secretion are important for "driving" changes in reproductive activity. These

have been called "activational" mechanisms because they generally involve activational effects of hormones (Beach 1975; Goldman and Nelson 1993). A second set of neuroendocrine mechanisms is directly responsible for the timing of seasonal rhythms, and ensuring that they are synchronized to the annual geophysical cycles. For example, the neuroendocrine mechanisms that transduce day length information into melatonin secretion patterns are critical for ultimately translating environmental factors into season-specific target organ responses.

The pineal gland and its primary hormone, melatonin, are involved in mediating the effects of day length on the timing of many seasonal changes in physiology and behavior among mammals (Goldman and Elliott 1988; Goldman 1983). Pinealectomy abolishes photoperiodic responsiveness in virtually all mammalian species examined (Goldman and Nelson 1993). Melatonin is secreted rhythmically by the pineal gland in a circadian fashion, with an extended peak occurring at night (Carter and Goldman 1983a,b; Bittman and Karsch 1984); daylight suppresses melatonin release. Although melatonin secretion can retain its circadian pattern even in constant light conditions (e.g., Honma et al. 1995), the duration of the nocturnal secretion of melatonin is inversely related to the length of day and appears to provide the physiological code for photoperiod (Bartness and Goldman 1989; Illnerova et al. 1985). In other words, a prolonged release of melatonin secretion correlates with a long night (short day). Physiological mechanisms that respond to specific critical day lengths have evolved in populations of individuals, and the critical day lengths are used to time physiological and behavioral processes precisely during the geophysical year. Treatments of animals with melatonin, either by infusions, injections, or implants, that cause an extended peak in blood melatonin levels, result in short-day phenotypic responses (Bartness et al. 1993).

Individuals of other species use endogenous, self-sustaining circannual clocks to phase their seasonal adaptations appropriately. Circannual rhythms have been most thoroughly studied in birds and mammals, but are probably common among long-lived or-

ganisms (Gwinner 1986). Individual ground squirrels, sheep, and deer display rhythms of reproductive activity and inactivity with a period that approximates one year when housed in constant conditions (reviewed in Bronson and Heideman 1994; Goldman and Nelson 1993; Wingfield and Kenagy 1991). For example, when golden-mantled ground squirrels (*Spermophilus lateralis*) are held under constant conditions of temperature and day length in the laboratory, they exhibit recurring cycles of body mass gain and loss, reproductive activity and quiescence, molting of pelage, and hibernation. While ground squirrels housed under constant conditions continue to show normal sequences of annual changes, the timing of these changes gradually becomes asynchronous with the local geophysical season. For these sciurid rodents, the period length of the circannual changes is approximately 320 days (Pengelley and Asmundson 1974). When individuals of species that display prominent circannual reproductive rhythms are maintained in constant conditions, the pattern of pineal melatonin displays an endogenous circadian rhythm (Klein and Weller 1971; Rollag and Niswender 1976). Seasonal changes in pineal melatonin have also been demonstrated in both natural and laboratory photoperiods (Bittman et al. 1982). It has been suggested that changes in environmental photoperiod probably synchronize endogenous circannual rhythms via alterations in the pattern of melatonin rhythm (Karsch et al. 1991). Other animals with strong circannual rhythms, including golden-mantled ground squirrels, sheep, and deer, also rely on photoperiod to entrain their circannual rhythms (e.g., Lee and Zucker 1991; Jackson et al. 1990; Brinklow and Loudon 1990). The extent to which a circannual pattern of melatonin secretion underlies circannual rhythms of physiology and behavior remains an open question (Bartness et al. 1993).

Regardless of the organization of the underlying regulatory mechanisms, there are so-called "long-day" and "short-day" breeders. Long-day breeders are usually birds or short-lived mammals that mate in spring, produce offspring during the summer, and typically stop reproductive activities by autumn. Short-day breeders are usually large mammals such as ungulates; copulation coincides with the short day lengths of autumn, females are pregnant throughout winter, and they bear offspring in the spring. In both cases, the young are produced at the time of year, spring and summer, when food is relatively abundant in the habitat. The role of melatonin in timing seasonal breeding in birds remains unspecified, but the circadian pattern of melatonin secretion closely mimics the mammalian pattern (Hasegawa et al. 1994).

Regardless of the organizing mechanisms underlying seasonal breeding, there are important similarities in the annual endocrine profile. The endocrine sequelae associated with seasonal breeding include elevated blood levels of sex steroid hormones during mating, maximal durations of melatonin secretion during the short day lengths of winter, and minimal blood prolactin concentrations during short day lengths (Reiter 1993). Other endocrine changes associated with seasonal breeding include thyroid hormones (Dahl et al. 1994; Moenter et al. 1991; Vaughan et al. 1994; Chaturvedi et al. 1992; Wingfield and Farner 1993), growth hormone (Vriend et al. 1989; Barenton et al. 1987, 1988), and the glucocorticoids (Tähkä 1978; Gower et al. 1992; Nelson et al. 1996). As detailed below, many of these hormones have both direct and indirect effects upon immune function.

SEASONAL CHANGES IN LYMPHATIC TISSUE DEVELOPMENT

One approach to understanding the interaction among the endocrine, nervous, and immune systems in an ecologically valid way is to explore the functional role of seasonal changes in immune status and the role of photoperiod in mediating these cycles of immune function. As noted above, annual cycles of energy-saving adaptations, including alterations in reproductive morphology, physiology, and behavior, coincide with seasonal fluctuations in the environment (e.g., temperature, photoperiod, and food availability) and have been documented in many avian and mammalian species (Bronson and Heideman 1994; Wingfield and Farner 1993). Animals use environmental cues to anticipate the season of the year and to make physiological or behavioral adjustments (e.g., nest building, territorial defense, and

initiating or terminating gametogenesis). It seems reasonable to expect that animals should adjust their immune status prior to the onset of winter stressors, thereby providing a margin of safety should stress-induced adrenal steroids act to suppress immune function. Although seasonal cycles of antigens, allergens, and pathogens may be superimposed on vertebrate seasonal fluctuations in immune function, the evidence, particularly in laboratory studies, suggests that these effects are modest in comparison to the seasonal changes in the host.

As described in the previous section, the primary endpoint of most research on seasonality is the annual birth cycle in several avian and mammalian species. Animals tend to be born during the spring and summer when food is most plentiful. Although relatively unexamined, there is an equally salient seasonal cycle of illness and death, to the extent that many animals die most frequently during the winter in temperate and boreal regions (John 1994; Lee and McDonald 1985; Lochmiller et al. 1994; McDonald et al. 1988). This may not seem too surprising, because during the winter animals experience low ambient temperatures and low food availability. The challenging winter conditions can *directly* induce death via hypothermia, starvation, or shock (Selye 1956; Sapolsky 1992; Black 1994), and surviving these demanding conditions likely places animals under great physiological stress. The stress of coping with energetically demanding conditions can also *indirectly* cause illness and death by compromising immune function (Lee and McDonald 1985; Ader and Cohen 1993; Andrews et al. 1972; Geller and Christian 1982; Kelley et al. 1982). Stress is operationally defined here as a sustained activation of the hypothalamus-pituitary-adrenocortical axis. Presumably, elevated circulating blood levels of adrenocortical steroids would depress immune function in the wild as they do in the laboratory (Ader and Cohen 1993; Berczi 1986; Black 1994). Of course, not all animals respond to winter with a sustained elevation of adrenocortical activation. Many adaptations have evolved to cope with winter, so that even severe environmental conditions might not evoke glucocorticoid secretion. For some animals, the most stressful time of the year

may not be winter, but might coincide with the establishment of breeding territories, migration, or copulation. Consequently, the field data should reflect this variation; some populations should exhibit higher immune function during the winter and some populations should exhibit higher immune function during the summer. This observation would not necessarily rule out our working hypothesis. Because so many factors can affect immune function, laboratory studies are necessary to assign causation to specific environmental factors. Adaptations that maintain thermoneutrality with minimal stress during the winter would represent one category of successful survival tactics that may have evolved. Again, many animals are presumably so well adapted to winter that winter conditions are not perceived as stressful. Mild winter conditions also may not evoke secretion of adrenocorticoid hormones. Certainly, survival rates within a population are higher during mild winters than during severe winters (Descôteaux and Mihok 1986; Mihok et al. 1985; Krebs and Myers 1980).

Another strategy to increase survival is to enhance immune function prior to the onset of the poor conditions that may compromise immune function. For instance, lymphatic organ size might increase, circulating antibody levels might rise, and immune function might generally improve in autumn before food availability becomes scarce and temperatures drop. Scarcity of food, low ambient temperatures, or other deteriorating winter conditions could act as stressors, cause elevated glucocorticoid levels, and ultimately reduce immune function (MacMurray et al. 1983; Monjan 1981). Thus, animals must maintain a "positive" immune function balance, as well as a positive energy balance to survive winter. Immune function may be curtailed at other times because of energetic incompatibility with other functions or to reduce the possibility of developing an autoimmune reaction. In order to adjust immune function at a particular time of year, animals would have to cue to some environmental factor that reliably predicted the season of the year. Photoperiod would seem an obvious candidate. As noted previously, virtually all studies of seasonality have emphasized the annual changes in adapta-

tions associated with energy use, especially reproduction, mediated by the annual change in photoperiod (Bronson and Heideman 1994). Regression of reproductive organ size and function is commonly observed among species of small birds and mammals, presumably as an adaptation to cope with winter. However, other nonenergy systems must also undergo seasonal adjustments to maximize the odds of survival. Given the severe selection pressures to survive winter, it seems reasonable to suggest that mechanisms to monitor photoperiod in order to anticipate the threat of winter and make changes in immune function have evolved in temperate and boreal animals. Thus lymphatic tissue, in common with reproductive structures, should also exhibit an annual cycle of growth and activity, and regression and quiescence. If the hypothesis that winter stressors suppress lymphatic tissue size and function is true, then immune suppression should be observed during severe winters. However, it should be emphasized again that, in field studies, the absence or reversal of seasonal changes in lymphatic organ size or function does not necessarily rule out the working hypothesis that short photoperiod ought to enhance immune function in the winter. Additional field studies correlating immune function, blood glucocorticoid levels, and environmental conditions will be necessary to test our hypothesis.

Because many factors in addition to photoperiod vary seasonally in the field, laboratory studies can be useful tests of our working hypothesis. By analogy, the demonstration of winter breeding among rodents does not rule out the hypothesis that short days usually inhibit reproductive function. The review of field data provides an opportunity to assess the ecological validity of the hypothesis that photoperiod can mediate changes in immune function. If short days bolster immune function, then laboratory animals should exhibit elevated lymphatic tissue size and function when maintained in short photoperiods. In all reported cases, laboratory studies in which only photoperiod was manipulated resulted in short-day animals exhibiting elevated lymphatic tissue size and function. One laboratory study suggests that the effects of the environment on immune function can be teased

apart; i.e., deer mice held in short days exhibited larger spleen size and higher antibody levels than deer mice maintained in long-day-length conditions (basal conditions). When long-day animals were kept in low temperatures (8°C) their antibody levels were compromised. When short-day animals were maintained in low temperatures, their antibody levels returned to the basal levels of long-day animals housed at room temperatures (Demas and Nelson 1996). Factorial studies in the laboratory may prove very useful in understanding the interaction among factors that may affect immune function, survival, and fitness.

Seasonal cycles in the development, regression, and regeneration of the thymus, spleen, and bursa of Fabricius have been described in many vertebrate species. Logically, it has been assumed, in common with the assumptions associated with seasonal changes in reproductive organ mass, that lymphatic organ size positively correlates with organ function. The seasonal cycles of lymphatic organ size often were recognized well before the immunological functions of these organs were identified. Early during ontogeny of homeothermic vertebrates, lymphocytes develop from stem cells in the bone marrow, and migrate to the thymus (T cells) and bursa tissue (B cells) to mature. The thymus and bursa of Fabricius are the primary lymphatic tissues where T cells and B cells, respectively, develop in birds. Mammals also possess a thymus, where T cells mature, but B cell maturation occurs in a diffuse system called the bursa-equivalent system that, depending on the species and stage of individual development, includes the gut-associated immune tissue, the liver, and bone marrow (Borysenko 1987). By the time of birth or hatching, when antigens are most likely to be first encountered, the T cells and B cells move out from the primary lymphatic tissues to the peripheral, secondary lymphatic organs, viz., the spleen, the gut-associated lymphatic tissue, and the lymph nodes (Golub and Green 1991). The spleen serves mainly as a filter for antigenic particles, and subsequent phagocytosis of antigens, parasitized blood cells, and immune complexes in the blood. The blood may move through the spleen with little filtering or enter the splenic reticulum tissue where macrophages are plentiful (Go-

lub and Green 1991). The pulp tissue of the spleen includes the so-called red and white pulp tissues. The white pulp tissue is the main lymphatic component of the spleen; lymphocyte recirculation, macrophage development from monocytes, and the final stages of lymphocyte differentiation are mediated in the splenic white pulp of birds and mammals (Gorse 1990; Kopp 1990; John 1994). The red pulp tissue is involved in oxygen supply maintenance; erythropoiesis (red blood cell formation) and erythrocyte storage occurs in the red pulp tissue (Seifert 1989; John 1994).

BIRDS

The atrophy observed in the thymus, bursa, and spleen after puberty and the obvious link to seasonal changes in reproductive function, prompted many early hypotheses suggesting that these organs were directly linked to breeding (Aimé 1912; Riddle 1928). For example, the avian thymus was originally thought to provide the "egg envelope" (Riddle 1924); however, additional experiments ruled out this hypothesis because thymectomy did not interfere with the production of normal eggs (Morgan and Grierson 1930; Riddle and Krizenecky 1931). Other investigators hypothesized that the thymus was somehow involved with the onset of puberty because castration caused hypertrophy of the thymus (Hammar 1929; Gregoire 1945). The discovery that the thymus, bursa, and spleen were major components of the immune system, and the subsequent pursuit of molecular analyses of immune function have, for the most part, ignored the molar relationship, and possibly the regulatory interactions, between immune function and the reproductive system until very recently.

Splenic and thymic size has been reported to be minimal in a number of avian species when the gonads were undergoing vernal recrudescence (e.g., Krause 1922; Riddle 1928; Oakeson 1953, 1956; Höhn 1947, 1956; Fänge and Silverin 1985; John 1994). Mallard ducks (*Anas platyrhynchos*), in common with other homeothermic vertebrates, undergo thymic involution at puberty (Höhn 1947). There is also a pronounced regeneration of thymic tissue at the end of each breeding season (midsummer) in both male and female adult mal-

lards; mallard thymic tissue regresses before the autumnal migration (Höhn 1947). The physiological stress associated with migration and breeding was considered incompatible with full thymic size and function (Höhn 1947). Similar observations have been made for house sparrows (*Passer domesticus*) and robins (*Turdus migratorius*) (Höhn 1956).

The reduced relative splenic size of white-crowned sparrows (*Zonotrichia leucophrys gambelii* and *Z. l. nuttalli*) at the beginning of the breeding season in western North America cannot be attributed to the "stress of migration" because both migratory and nonmigratory populations displayed an identical seasonal pattern of splenic development (Oakeson 1953, 1956). Splenic size (corrected for lean body mass) was lowest prior to breeding and highest at the end of the breeding season in white-crowned sparrows. Similarly, migratory pied flycatchers (*Ficedula hypoleuca*) also displayed a seasonal cycle of splenic development with splenic regression observed at the onset of the breeding season in Sweden, and subsequent splenic development exhibited by the adults during incubation and feeding of the hatchlings (Fänge and Silverin 1985). The adaptive significance of the development of the spleen before the autumnal migration has been suggested to reflect an enhancement of immune function, particularly of the young birds after hatching, in advance of winter (Fänge and Silverin 1985). One parsimonious proximate explanation for the seasonal pattern of lymphatic organ development among birds is that the high gonadal steroid levels associated with breeding are incompatible with highly developed lymphatic tissue, but this possibility remains untested.

MAMMALS

The proximate explanation that high gonadal steroid levels are associated with low lymphatic organ weights might also account for some, but not all, of the data concerning seasonal fluctuations in mammalian lymphatic organ size. For example, red-backed mice (*Clethrionomys rutilus*) were wild-trapped near College, Alaska at different times of the year (Sealander and Bickerstaff 1967). Blood samples were taken and stained reticulocytes were counted. Animals were then killed;

spleens and thymuses were removed and weighed. Mean reticulocyte counts varied seasonally, with the main peak occurring in early winter and lesser peaks observed in late winter and midsummer. Thymus weights were largest around February and spleen weights were largest in September and October; the lowest weights for both organs occurred in July (Sealander and Bickerstaff 1967).

Similarly, pine voles (*Microtus pinetorum*) were trapped monthly over one year in southwestern Virginia, and several reproductive and related organs were examined (Valentine and Kirkpatrick 1970). Both thymus and paired adrenal masses were highest in early autumn when reproductive organ masses were declining (Valentine and Kirkpatrick 1970).

Adult and subadult, but not juvenile, cotton rats (*Sigmodon hispidus*) inhabiting central Oklahoma display a seasonal cycle of thymic development and regression. Thymic masses were depressed during the summer among both adults and subadults and were maximal during the winter (Lochmiller et al. 1994). Peak splenic masses and peak numbers of splenocytes were recorded in autumn and late winter, respectively, in cotton rats.

In contrast, short-tailed voles (*Microtus agrestis*), wild-trapped near Oxford, England, exhibited a different seasonal pattern of lymphatic organ size. These voles were captured monthly from October 1958 to October 1959, and then at less regular intervals until August 1960 (Newson 1962). Blood samples were obtained; spleens were removed and weighed. The results suggested seasonal changes in reticulocyte counts and spleen weights, with males having low reticulocyte counts and corresponding spleen weights in the winter, and elevated levels for both reticulocyte count and spleen mass during the summer. These changes, although similar in females, were phase-advanced approximately two months as compared to males. It is not clear if the voles in this population maintained reproductive function throughout the winter.

Seasonal changes in lymphatic tissue have also been noted in hibernating mammals. For example, European ground squirrels (*Citellus citellus L.*) were studied during their first 15 months of development (Shivatcheva and Hadjioloff 1987a,b). In autumn, with the onset of the hibernation period in the field, some animals were housed in a dark room maintained at 7±1°C. The spleen and gut-associated lymphoid tissues of both hibernating and nonhibernating animals were examined, and a circannual rhythm in the morphology of the splenic lymphoid tissue, as well as the lamina propria of the mucosa and the Peyer's patches was reported (Shivatcheva and Hadjioloff 1987a,b). These lymphatic tissues regressed in the autumn in both hibernating and nonhibernating squirrels, but regression was more complete in hibernating animals. Notably, proliferation and hypertrophy of splenic and gut-associated lymphoid tissues were observed in squirrels *prior to* arousal in the spring (Shivatcheva and Hadjioloff 1987a,b). The physiological effects of torpor and hibernation, including the contribution of hormones, on immune function remain unspecified.

REPTILES

Seasonal changes in thymic mass were apparently first reported in turtles (*Clemmys leprosa, Testudo mauritonica*) (Aimé 1912). In turtles, thymic mass was reduced during winter estivation and regenerated in the spring. The thymuses of the turtles continued to enlarge throughout the summer, reaching maximal size in the autumn prior to estivation.

More recently, adult male and female lizards (*Scincus scincus*) were collected monthly from the desert regions of Egypt (Hussein et al. 1979). During the winter, the thymuses of lizards were extremely involuted and thymic cells undifferentiated. Thymuses began to regenerate in the spring, leading to redifferentiation of thymic cells into distinguishable medulla and cortex.

In another related study, adult male and female Colubrid snakes (*Psammophis schokari*) were collected from Egyptian deserts throughout various seasons and the sizes of their lymphatic organs were recorded (El Ridi et al. 1981). During the spring and autumn, the thymuses had well-developed medullae and lymphoid cortices. However, thymuses were involuted in the summer and winter with most of the organ being composed of fibrous tissue and nearly all of the lymphocytes appearing "dead." In winter, splenic red pulp (i.e., eryth-

ropoeisis) was well developed, while the white pulp (i.e., immune function) had only a "few scattered lymphoid aggregates" (El Ridi et al. 1981). During the summer, however, the red pulp was indistinct, with lymphatic tissue regressing in size; the white pulp became completely regressed by August.

SUMMARY

Taken together, the evidence suggests that lymphatic organ mass tends to show seasonal fluctuations. Lymphatic organ mass seems to be greatest among homeothermic vertebrates during the autumn and winter, and lowest during spring and summer. In contrast, lymphatic organ mass seems to be greatest in spring and summer among poikilothermic vertebrates, particularly after breeding activities have been completed. Lymphatic organ mass appears to regress during winter estivation in poikilotherms. Similarly, heterothermic rodents also exhibit lymphatic tissue degeneration during the winter and maximal lymphatic organ development after vernal breeding is completed. Further studies are required to tease apart the effects of photoperiod on lymphatic organ size from the effects of reproductive steroid hormones on these tissues.

SEASONAL CHANGES IN IMMUNE FUNCTION AND DISEASE PREVALENCE

BIRDS

In common with the seasonal pattern of lymphatic organ development among birds, elevated gonadal steroid levels associated with breeding coincide with an increased prevalence of some diseases and reduced immune function. The avian spleen is an important filter against parasites such as avian malaria (John 1994). Many studies have demonstrated a seasonal change in parasite and pathogen prevalence, and the overwhelming evidence suggests a seasonal change in the host, rather than in the parasite (John 1994). For example, house sparrows (*Passer domesticus*) infected with avian malaria (*Plasmodium relictum*) display a relapse of symptoms occurring synchronously throughout a population of infected birds and coincident with the onset of vernal breeding activities (Applegate and Beaudoin

1970). Treatment with corticosterone injections in laboratory-held birds led to increasingly marked relapses from winter to spring, mimicking the spontaneous rate of progression of the disease from January to April among birds in nature (Applegate 1970). Gonadotropin treatment, either alone or in combination with corticosterone, stimulated gonadal development, but did not change the incidence of relapse. These results suggest that gonadal steroids are not involved in the prevalence of avian malaria, but that some other factor associated with breeding may account for the increase in the onset of symptoms. The possibility that immune function is enhanced in birds during the winter to keep parasitic activities minimal remains open (Beaudoin et al. 1971; John 1994). For example, it has been hypothesized that *Plasmodium* infections remain latent during the winter, but that parasitemia becomes evident during vernal migration or breeding (Beaudoin et al. 1971; Alexander and Stimson 1988). Increased prevalence or incidence of relapse of blood protozoa of *Plasmodium, Leucocytozoon, Haemoproteus,* and *Trypanosoma* species has been reported during spring and summer among many avian species (see John 1994 for review).

Ducks infected with a parasite related to avian malaria, *Leucocytozoon,* also show a relapse of symptoms during the spring (Chernin 1952). When day lengths were increased during the winter, the relapse could be hastened. Malaria among humans has also been reported to show increased vernal relapses, but it has been thought that these relapses are owing to a fixed interval of disease progression following autumnal infections (Coatney and Cooper 1948; also see below).

Suppression of immune function during the breeding season has also been reported for birds with viral infections. For example, homing pigeons (*Columba livia*) maintained in seminatural conditions and latently infected with pigeon herpes virus displayed an increased rate of viral shedding when breeding (Vindervogel et al. 1985). Also, infected chickens significantly increased the rate of shedding of laryngotracheitis virus after egg-laying had commenced (Hughes et al. 1989).

MAMMALS: LONG-DAY BREEDERS

Seasonal changes in mammalian immune function and disease prevalence have also been reported. For instance, seasonal variation exists in the ability of bank voles (*Clethrionomys glareous*) to infect larval ticks with Lyme disease (*Borrelia burgdorferi*) (Talleklint et al. 1993). Although larval tick infestations of voles were highest in June and July, nearly 70% of *Borrelia* infections occurred during August and September, and virtually no infections occurred during the winter. Whether these data reflect a seasonal alteration in immune function of the host or reflect the latency to infection from year to year requires further studies.

Cotton rats (*Sigmodon hispidus*) were monitored over 22 months in tallgrass prairie in central Oklahoma (Lochmiller et al. 1994). Animals were killed monthly and, as described above, morphological characteristics of primary and secondary lymphatic tissue were measured. Total peripheral white blood cells (WBC) were counted, and the ability of splenocytes to produce specific antibodies in response to an injection with sheep red blood cells (SRBC) was assessed. Lymphocyte proliferation in response to the mitogens, concanavalin A (Con A) and an extract of pokeweed (*Phytolacca americana*) was also determined (Lochmiller et al. 1994). Total WBC reached minimum values in December 1989, February 1990, July 1990, and February 1991. The highest numbers of plaque-forming cells were recorded in December 1989 and February 1990. In addition to elevated humoral responses, cotton rats trapped in February 1990 also displayed elevated lympho-proliferative responses to Con A and pokeweed, coinciding with increased numbers of total splenocytes harvested (Lochmiller et al. 1994). Although these data strongly suggest a seasonal cycle of immune function that is consistent with photoperiodic mediation, animals captured the following February did not display a similar change in immune function in comparison to the previous year (Lochmiller et al. 1994).

There has been some variation reported among seasonal fluctuations of immune function. For example, circulating immunoglobulin levels were elevated in common European voles (*Microtus arvalis*) that were trapped in the autumn and late winter as compared to summer-trapped voles (Dobrowolska et al. 1974; Dobrowolska and Adamczewska-Andrzejewska 1991). WBC and neutrophil counts followed this pattern in some years, but exhibited a reverse pattern in other years. The population parameters associated with these changes in the seasonal pattern of antibody production remain unspecified. It is possible that animals were producing sex steroid hormones (i.e., engaged in winter breeding) during the winter in some years but not others, and that these steroid hormones affected immune cell numbers.

An antibody scan for eleven common murine viruses revealed a pronounced seasonal fluctuation of infection of Theiler's encephalomyelitis and reovirus-type-3 in meadow voles (*Microtus pennsylvanicus*) (Descôteaux and Mihok 1986). The population of voles under study showed virtually no signs of viral infection when the population was enjoying high survival and breeding success; however, a precipitous decline in numbers during the winter of 1982–1983 was associated with evidence suggesting increased rates of infection of Theiler's encephalomyelitis and reovirus-type-3. These results suggest that social factors, especially as they affect glucocorticoid levels, may also affect immune function in the field. Also, the effects of winter breeding on immune function have not been fully investigated. The significance of opportunistic interactions between microparasites and arvicoline population fluctuations will be discussed below.

In a study of a hibernating species, ground squirrels (*Citellus richardsoni richardsoni*) were trapped during the spring and summer and kept in large cages with temperature maintained at 22 to 24°C and ambient light levels set to match natural photoperiods (Sidky et al. 1972). Three animals per month were immunized with SRBC. Five days after immunization, the animals were bled, sacrificed, and their spleens removed. The blood samples were then tested for the presence of hemagglutinins directed against SRBC. Assays demonstrated that antibody response to SRBC decreased significantly during the winter, reaching the lowest level in January. Spleen cell suspensions were tested for the presence of hemolysin-forming cells by a modification of the PFC test.

PFCs decreased significantly during the winter, reaching the lowest levels in January. The number of nucleated cells per spleen increased during the winter, however, reaching a maximum in January (150% of May values). The lack of winter enhancement of immune function may reflect the fact that these squirrels normally hibernate through the winter. Again, the effects of hibernation on immune function are virtually unknown.

Other patterns of seasonal immune function and disease prevalence have been reported for nonhibernating mammals. For example, outbreaks of European brown hare syndrome (EBHS) displayed a strong seasonal fluctuation among *Lepus europeus* in Sweden with peak occurrence observed during the winter months (Gavier-Widen and Morner 1991). Similarly, rabbit viral hemorrhagic disease (VHD) exhibited a peak incidence during the winter months. Again, the extent to which these animals were engaged in winter breeding was not reported.

Outbred male and female beagle dogs (50 days of age) maintained in open colonies were examined to assess seasonal changes in immune function (Shifrine et al. 1980a). Blood samples were taken from 5 beagles each month, and the serum extracted and pooled within each month. Whole blood lymphocyte proliferation tests were conducted by adding one of two mitogens, either phytohemagglutinin-P (PHA) or concanavalin A (Con A) to the monthly samples. After 5 days, radioactive thymidine was incorporated into the samples, which were allowed to incubate for 18 hours. The results demonstrated a seasonal variation in lymphocyte response, with peak lymphocyte proliferation in June/July and a trough observed in January. The reproductive status of these dogs was not described.

In a follow-up study, blood samples were taken from 32 beagles at various times throughout the year (Shifrine et al. 1980b). A lymphocyte proliferation test was conducted on the samples, in which the samples were mixed with one of two lectins, PHA or Con A, to stimulate lymphocyte activity. Estimated mean times of peak activity in lymphocyte proliferation ranged from 19 July to 2 August for samples mixed with PHA, and 18 July to 15 August for samples mixed with Con A. For both mito-gens, lymphocyte activity levels were significantly higher in summer months as compared to winter months.

MAMMALS: SHORT-DAY BREEDERS

A study of cattle revealed seasonal variation in naturally occurring antibody production against substance J, an antigenic compound detected on the erythrocytes of some cattle (Stone 1956). Blood samples were drawn from cattle monthly and added to culture plates containing substance J. Low antibody titers were present in January, with levels rising thereafter to peak levels in August, coincident with the onset of the breeding season. After this peak, levels began to drop, again returning to a minimum in January. Similarly, the rate of seropositive responses to *Borrelia burgdorferi* showed a seasonal response, with the population infection incidence highest during the summer (up to 23.4%) and lowest during the winter (0% in January) (Isogai et al. 1992).

The seasonal occurrence of the equid herpes virus-4 (EHV-4) in foals was studied on thoroughbred stud farms in New South Wales, Australia (Gilkerson et al. 1994). Nasal swabs were obtained once a month for a year to detect the presence of EHV-4 antibodies. Twenty-six foals were EHV-4 positive, and all of these seropositive animals were discovered in the summer months of January, February, and March (25 in January and March, and 1 in February) (Gilkerson et al. 1994). No seropositive animals were detected during the autumnal breeding season or during the winter months.

REPTILES

Seasonal patterns of immune function in reptiles have also been reported. In the previously cited study of seasonal lymphatic organ development of adult male and female Egyptian Colubrid snakes (*Psammophis schokari*) (El Ridi et al. 1981), animals were injected with rat red blood cells (RRBC), human serum albumin (HSA), or polyvinyl pyrrolidone (PVP). During the spring and autumn, humoral antibody response to HSA, RRBC, and PVP was rapid and strong. Humoral response to these three mitogens in winter, however, was weak. Responses to both RRBC and HSA were also weak in the summer.

In a follow-up study, adult male and female snakes (*P. schokari*) were captured from fields and maintained in cages with lighting and temperature conditions set to match appropriate seasonal levels (Farag and El Ridi 1985). Spleens were surgically removed monthly from a random group of snakes; monocellular suspensions of the lymphatic tissue were made, and viable lymphocytes were counted after staining. Spleen cell lymphocytes were then added to mixed leukocyte cultures (MLC) and proliferative ability was determined. The results demonstrated a strong lympho-proliferative response from June to July and again from October through December. However, lymphocyte proliferation appeared to be "abrogated" at all other times of the year (Farag and El Ridi 1985).

Adult male and female lizards (*Scincus scincus*) were collected from the desert regions of Egypt and injected with RRBC in the laboratory (Hussein et al. 1979). Lizards captured in the winter failed to form humoral antibodies to RRBCs. Antibody responses were also low in the spring, but elevated again during the summer and autumn.

Adult male tortoises (*Mauremys caspica*) were obtained from a supplier in May and maintained under natural light and temperature conditions (Leceta and Zapata 1986). Animals in the summer group were immunized with SRBC at the end of July. Two weeks later, animals were given a second immunization with SRBC. Some of these same animals were transferred to an environment that simulated photoperiod and temperatures of October. Following a two-week acclimation period, they received primary immunization with SRBC mixed with equal volumes of 2-mercaptoethanol (2ME), followed by a secondary immunization 45 days later. Blood samples were taken and blood parameters assessed. After primary immunization, animals in the autumnal conditions had the appearance of 2ME-sensitive antibodies, whereas these antibodies did not appear in the tortoises maintained in simulated summer conditions. The antibodies appeared in both autumn- and summer-tortoises after secondary immunization, however. Splenic plaque-forming cells (PFCs) appeared after primary immunization in autumn-tortoises, but not in summer-tortoises. Splenic

PFCs appeared after secondary immunization in snakes maintained in both simulated seasons, but the level was significantly higher in animals maintained in autumnal conditions (Leceta and Zapata 1986). Taken together, the authors suggested a possible enhancement of immune function in autumn. However, it is not clear that a two-week acclimation period would be sufficient to engage short-day mechanisms. Potentially, the move into a new condition (i.e., from summer to autumnal conditions) was sufficiently stressful to affect immune function positively.

FISH

Individual marine teleost fish (*Sebastiscus marmoratus*) were captured at different times of the year, and maintained in tanks with running sea water at $23 \pm 1°C$ for a two-week acclimation period. At the end of the acclimation period the fish were immunized thrice at two-day intervals; the thymuses were removed and weighed two weeks after immunization (Nakanishi 1986). The thymus organ weights were highest during the winter, and lowest during the summer, but these organ weights were inversely related to antibody formation (Nakanishi 1986). Maintaining male Atlantic salmon (*Salmo salar*) in constant light during the parr-smolt transformation depressed the elevation of resting cortisol levels as compared to salmon maintained in simulated natural photoperiod, but did not affect thymic mass or lymphocyte proliferation (Olsen et al. 1993). Testosterone and cortisol, but not 17β-estradiol or aldosterone, have the potential for compromising plaque-forming cells in chinook salmon (*Oncorhynchus tshawytscha*) (Slater and Schreck 1993).

SUMMARY

In summary, immune function in birds and small mammals appears to be generally compromised and diseases are more prevalent during the breeding season. This pattern seems to be reversed in some cases for larger mammals, as well as for heterothermic mammals and poikilothermic vertebrates. Although there are data from many sources indicating seasonal changes in lymphatic tissue size and morphology, as well as immune function, there is significant variation among dif-

ferent populations of animals. Because so many factors can influence steroid hormone levels and because these factors vary across populations (even within studies among different years), field studies are difficult to compare. Another confounding aspect to field studies is that elevated immune function or lymphatic organ size may reflect either infection (activated immune responses) or boosted immune function (as a precautionary tactic to overcome stress-induced immunosuppression). It is not clear whether the results of a field study reporting suppression of immune function during the winter represents a rejection of our working hypothesis, reveals a combination of a variety of stressors on immune function, or lends support to the hypothesis, because immune function might have been further reduced in the winter without photoperiod-mediated immunoenhancement. Although field studies can be designed to test our hypothesis, discovery of the causative agents and understanding the additive effects of these agents on the immune system currently requires laboratory studies in which one or more factors are altered in an otherwise stable and controlled environment. When only photoperiod has been experimentally manipulated in a controlled environment with uninfected animals, the results clearly indicate that short days are coincident with elevated lymphatic organ mass and immune function. These studies are reviewed in the following section.

PHOTOPERIODIC CHANGES IN IMMUNE FUNCTION

The vast majority of studies of environmental changes on immune function have manipulated ambient photoperiod. To our knowledge, evaluation of photoperiodic changes on immune function has been conducted only in small mammals, primarily rodents (Table 1). Consequently, only rodents are discussed in this section. Studies of photoperiodic influence on the neuroendocrine reproductive axis have indicated that slight photoperiod-induced changes occur in the splenic weight in rats (*Rattus norvegicus*) (Wurtman and Weisel 1969). Laboratory strains of rats are traditionally considered to be reproductively nonresponsive to photoperiodic information (Nel-

TABLE 1
The effects of photoperiod on immune function

Immunological Parameters Measured	Species	Reference
Splenic mass	Norway rats (*Rattus norvegicus*)	1
	Deer mice (*Peromyscus maniculatus*)	2
	Golden hamsters (*Mesocricetus auratus*)	3
Thymic mass	Norway rats (*Rattus norvegicus*)	4
Lymphocyte count	Deer mice (*Peromyscus maniculatus*)	5
Neutrophil count	Deer mice (*Peromyscus maniculatus*)	5
White blood cell count	Deer mice (*Peromyscus maniculatus*)	5
	Common voles (*Microtus arvalis*)	6
Antibody levels	Deer mice (*Peromyscus maniculatus*)	7
Wound healing rates	Deer mice (*Peromyscus maniculatus*)	7

Note: All effects are enhanced in short days.
References: (1) Wurtman & Weisel 1969, (2) Vriend & Lauber 1973, (3) Brainard et al. 1985; Vaughan et al. 1987, (4) Mahmoud et al. 1994, (5) Blom et al. 1994, (6) Dobrowolska & Gromadzka-Ostrowska 1984, (7) Nelson & Blom 1994.

son et al. 1994). Nevertheless, maintaining adult Wistar male rats in constant dark (DD) for 4 weeks increased thymic mass by 315% compared to rats maintained in a photoperiod of twelve hours of light and twelve hours of dark (LD 12:12); most of the increases occurred in the thymic medulla (Mahmoud et al. 1994). The number of thymic lymphocytes was also increased. Rats maintained for 4 weeks in constant bright light (LL) decreased thymic mass 53% as compared to rats maintained in LD 12:12; the reduction in total organ mass represented mainly reductions in the thymic cortex (Mahmoud et al. 1994). Because there are no effects of photoperiod on steroid hormones in male rats (Nelson et al. 1994), these data strongly suggest a direct effect of melatonin on immune function (Mahmoud et al. 1994). Although changes in lymphatic tissue size of rats to LL may have reflected a stress response, maintenance in constant dark also affected lymphatic tissue

mass and does not typically evoke a stress response. Further studies are necessary to tease apart the effects of photoperiod and stress on immune function in "nonphotoperiodic" rats.

Short day lengths also caused an elevation in splenic weight of deer mice (*Peromyscus maniculatus*) (Vriend and Lauber 1973), and Syrian hamsters (*Mesocricetus auratus*) (Brainard et al. 1985). Splenic masses, total splenic lymphocyte numbers, and macrophage counts were significantly higher in hamsters exposed to short photoperiod as compared to animals exposed to long photoperiod (Brainard 1985; Vaughan et al. 1987). However, photoperiod did not affect thymic weight or antibody production in hamsters (Brainard et al. 1985). In all of the studies that manipulated photoperiod reviewed here, activity level, food intake, and body mass had not been controlled (but see Nelson et al. 1995).

Photoperiodic influences on lymphocyte number and total white blood cell count have been reported for deer mice (*Peromyscus maniculatus*) (Blom et al. 1994). Animals maintained in short day lengths (LD 8:16) possessed more white blood cells than animals maintained in long day lengths (LD 16:8); neutrophil number was unaffected by day length in adult female deer mice. In another study on adult deer mice, animals maintained in short days displayed faster healing rates than long day mice (Nelson and Blom 1994).

Prenatal and postnatal photoperiodic effects on immune cells were examined in deer mice gestated in one photoperiodic condition, but cross-fostered to mothers in another photoperiodic condition. Photoperiodic information transmitted from the mother to the young in utero subsequently affected immune function of the pups (Blom et al. 1994). Animals gestated in short day lengths displayed higher immune status throughout life than mice gestated in long days, regardless of the day length in which they were reared (Blom et al. 1994). Lymphocyte numbers, WBC counts, and neutrophil numbers were higher in mice gestated in short days as compared to mice gestated in long day lengths. The pressures on winter survival for adult rodents are often severe and presumably the pressures are even greater on juveniles. Hence, many adaptations have evolved to increase the probabilities of surviving environmental stressors. Initiating winter adaptations in utero by adjusting to the physiological signals provided by the mother responding to short day lengths could enhance survival rates of young born late in the breeding season or during the winter. Enhancement of immune function is another adaptation that may have evolved to increase the odds of survival and eventual reproduction. Because prepubertal animals in short days also displayed elevated immune function, photoperiod appears to produce its effects independently of the gonads.

In another recent study from our laboratory, splenic lymphocyte proliferation in response to Con A was assessed in male deer mice that had been maintained for 8 weeks either in long (LD 16:8) or short (LD 8:16) days (Demas et al. 1996). Splenic lymphocytes from short-day deer mice exhibited greater proliferation in response to Con A as compared to the splenic lymphocytes from long-day animals (Figure 1). These data establish that photoperiod influences immune function.

In another study of immune function in rodents, male common voles (*Microtus arvalis*) were raised in colony rooms under three lighting conditions (LD 6:18, LD 18:6, and LL) (Dobrowolska and Gromadzka-Ostrowska 1984). Animals were randomly removed from the colony, blood samples were obtained, and immune parameters were determined. Voles in short days appeared to have a greater number of white blood cells compared to animals in long day and constant light, although the statistical significance of these differences were not reported by the authors.

Young C57BL/6 mice (*Mus musculus*) were housed in a laboratory environment kept at $22.5 \pm 1°C$ and on an LD 12:12 photoperiod (Brock 1983). Animals were killed between 900–1000 hours at weekly intervals throughout the year, spleens were removed, and splenic cell suspensions were prepared. Viable and nonviable lymphocytes were determined, using fluorescein diacetate and ethidium bromide stains. Cell cultures were then stimulated with the T cell mitogens, phytohemagglutinin (PHT) and concanavalin A (Con A), and a B cell mitogen, lipopolysaccharide (LPS). Peak responses in both lymphocyte
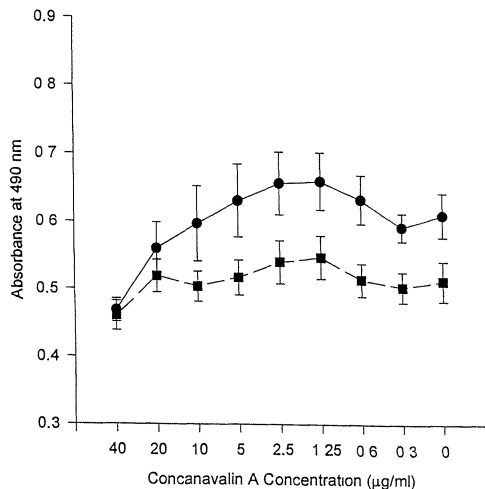
FIGURE 1. SPLENOCYTE PROLIFERATION IN
RESPONSE TO THE MITOGEN
CONCANAVALIN A IN MALE
DEER MICE

Data points are means (± standard error of the
mean). Long days (LD 16:8) are indicated by
squares and short days (LD 8:16) are represented
by circles. For this assay, spleens were removed
under aseptic conditions, splenocytes extracted
and counted, and cells incubated in different con-
centrations of Con A. In response to mitogenic
stimulation, males housed in long or short days
did not differ at high mitogen concentrations. At
lower concentrations of Con A, differences be-
tween males housed in long or short days be-
came apparent.

subpopulations were 2 to 5 times higher in
February through March 1977 and March
through April 1978 than in either of the two
previous Decembers. Summer comparisons
were not reported. It should be noted that
these animals, like laboratory strains of rats,
typically are reproductively nonresponsive to
photoperiod (Nelson 1990). Although the re-
productive systems of these animals may not
respond to day length (or to an endogenous
circannual rhythm), nonreproductive traits
may retain responsiveness to day lengths
(Goldman and Nelson 1993).

In general, short day lengths appear to bol-
ster immune function. The most likely physio-
logical mechanism by which photoperiod af-
fects immune function is via alterations in
steroid hormone levels. The precise mecha-

nisms through which photoperiod interacts
with the endocrine system and exerts influ-
ences on the immune system are unknown.
The presence of receptors both for androgens
in the thymus and for estrogens in the cytosol
of circulating lymphocytes might explain why
these steroid hormones play an important
role in the mediation of immune function
(Grossman 1985; Hall and Goldstein 1984).
Prolactin is also an immunomodulatory hor-
mone, and secretion of prolactin is pro-
foundly affected by day length; short photope-
riods induce reductions in blood prolactin
levels. It is possible that photoperiodic effects
on immune function are mediated via photo-
periodic changes in blood concentrations of
prolactin. The pattern of melatonin secretion
is also altered by photoperiod. It remains pos-
sible that melatonin could act directly on the
immune system. These possibilities will be re-
viewed in the following section.

IMMUNITY AND SEXUAL REPRODUCTION

Immunity and sexual reproduction are
linked on several levels. With regard to immu-
nity, the ability to recognize self from nonself
probably appeared early in the evolution of
multicellular organisms. Most organisms are
susceptible to parasitic and pathogenic infec-
tions, which may greatly affect the fitness of
the host by reducing longevity or reproductive
output, or both (Halliday 1994). Successful
host species have evolved complex defenses
against parasites and pathogens that reduce
the effects of infection on fitness. However,
successful parasite or pathogen species have
evolved mechanisms to neutralize the de-
fenses of hosts. The coevolutionary processes
between parasite and host species have been
well characterized (Lively 1987; Wakelin
1992).

Because pathogenic and parasitic species
generally have high reproductive rates and
short generation times in comparison to those
of host species, infectious agents typically have
a distinct advantage over their host species
(Halliday 1994). These foreign agents can be-
come well adapted to the host during its life-
time, and may be passed on to the progeny of
the host. Thus, asexually-reproducing animals
produce genetically identical offspring to
which pathogens and parasites are already

well adapted. It has been proposed that sexual reproduction arose as an adaptation to reduce parasitism (Hamilton 1982; Hamilton and Zuk 1990; Lively 1987). The production of genetically variable offspring through sexual reproduction reduces the likelihood that pathogens and parasites will be as well suited to the offspring as to the parents.

Sexual reproduction requires union of two gamete types, and for many species this union only occurs when the participating animals come into physical contact. These social contacts often increase the risk of parasitic and pathogenic transmission between individuals (Hoogland 1979; May and Anderson 1987). Recent hypotheses of mate choice and sexual selection center on phenotypic signals of resistance to parasitic infection or low infection (e.g., Hamilton and Zuk 1982; Folstad and Karter 1992; Zuk 1990). From an adaptive functional perspective, it seems reasonable that immune function should be highest during times of social interactions, especially during sexual interactions among species with internal fertilization. Although many studies have reported enhanced immune function when animals are grouped and immunocompromised in isolated animals (reviewed in Barnard et al. 1993), the vast majority of studies have concluded that immune function is often compromised, sometimes drastically so, during the mating season (reviewed in John 1994; Lee and McDonald 1985; Lochmiller et al. 1994; McDonald et al. 1988). Neither the ultimate nor the proximate explanation for reduced immunocompetence during mating has been specified. However, it is possible that the energetic costs of maintaining the vast network of rapidly dividing cell types characteristic of functional avian and mammalian immune systems may be incompatible with the energetic costs of breeding (Kelley 1985).

Obviously, females with internal fertilization must protect against male-transmitted parasites and pathogens during copulation, but they must temper the immunologic response in order to avoid damaging sperm. Blood estrogen levels are typically high when females copulate, and estrogens generally enhance immune function. On the other hand, mitotic activities (Purtilo et al. 1972; Thong et al. 1973) and cell-mediated immunity (Finn et al. 1972; Thong et al. 1973) are reduced during pregnancy, possibly mediated through the actions of progesterone, although skin grafts survive longer in pregnant (Andersen and Munroe 1962), than in nonpregnant women. Thymic involution during pregnancy has been noted in virtually every mammalian species examined (McCruden and Stimson 1991). Mammalian immune function has been hypothesized to be compromised during pregnancy to protect against fetal rejection (Golub and Green 1991). Some diseases are more prevalent, or more severe, during pregnancy, such as poliomyelitis, pneumococcal pneumonia, smallpox, and influenza (Larsen and Galask 1978). Latent parasitic diseases, including malaria, babesiosis, trypanosomiasis, and toxoplasmosis, have been reported to "flare up" during pregnancy (Larsen and Galask 1978). Blood levels of progestins, estrogens, and adrenal corticoids increase during pregnancy (Nelson 1995). Treatment with pharmacological doses of these steroids causes temporary lymphocytopenia, marked involution of lymphatic tissue, as well as suppression of inflammation and allograft responses (Billingham 1986). The consensus appears to be that, during pregnancy, humoral immunity is enhanced and cell-mediated immunity is depressed (McCruden and Stimson 1991).

Androgens, in common with glucocorticosteroid hormones (see below), appear to have immunocompromising properties (Grossman 1984). Because individuals of most species display seasonal fluctuations in reproductive activities, it is reasonable to suspect that seasonal fluctuations in immune function may be mediated by photoperiodic effects on reproductive function and steroidal activities, and that sex steroids and glucocorticoids influence immune function.

HORMONES AND IMMUNE FUNCTION

SEX STEROID INFLUENCES ON IMMUNE FUNCTION

Sex Differences

Darwin (1871) noted that males frequently are shorter-lived than female conspecifics; this observation is particularly true among polygynous species (Nelson 1995). Although there is

evidence that differential environmental and social interactions can account for some of the sex differences in mortality, it is clear that males and females differ in immune function (for reviews, see Grossman 1984; Billingam 1986; McCruden and Stimson 1991; Schuurs and Verheul 1990). For example, when male and female mice are maintained in germ-free environments, the sex difference in longevity vanishes (Gordon et al. 1966).

Sex differences have been reported for several immune indices (Grossman 1984; Billingam 1986; McCruden and Stimson 1991; Schuurs and Verheul 1990). In general, sexually mature females seem to have higher immune activity than male conspecifics. For example, females of many species, including humans, have higher circulating immunoglobulin levels than male conspecifics (Rhodes et al. 1969; Nelson and Steinberg 1987; Astorquiza et al. 1987). Females also mount higher antibody responses after an immunological challenge than males (Schuurs and Verheul 1990). Macrophages from adult female rats produce more interleukin-1 (IL-1) than prepubertal females or adult males (Hu et al. 1988); ovariectomy reduces macrophage IL-1 production in female rats and estrogen replacement therapy reverses this reduction (Hu et al. 1988). Cell-mediated immunity has been reported to be both lower (Inman 1978) and higher (Comsa et al. 1982; Weinstein et al. 1984; Ptak et al. 1988) in female mammals as compared to males. However, virtually all studies indicate that females exhibit higher resistance to tumors and parasites than males (Grossman 1984; Ansar-Ahmed et al. 1985). Many sex differences in immune function appear to be organized early in development and are activated at the time of puberty (Grossman 1984), although some appear only to be activated by peripubertal increases in sex steroid levels (Schuurs and Verheul 1990).

Females are also more resistant than males to many diseases (Billingham 1986; Grossman 1984; Schuurs and Verheul 1990). Among humans, for example, females are less likely than males to contract bacterial meningitis, bacterial septicemia, dysentery, gonorrhea, meningitis, pneumonia, Legionnaire's disease, hepatitis B, rabies, syphilis, tetanus, typhoid, and yellow fever (Billingham 1986). The disadvantages for such superior immunological function and resistance to disease among females include enhanced proclivity for developing autoimmune disease (Billingham 1986; Grossman 1984; Schuurs and Verheul 1990). Women are more likely than men to suffer from systemic lupus erythematosus (9:1), Sjögren's syndrome (10:1), rheumatoid arthritis (3:1 to 7:1), multiple sclerosis (2:1), Graves disease, thyroiditis, and certain forms of diabetes (Billingham 1986; Grossman 1984; Schuurs and Verheul 1990). Animal models for many human autoimmune diseases exist, and hormonal manipulations in these animal models have indicated the involvement of sex steroid hormones in their expression (Raveche et al. 1976). Furthermore, natural hormonal changes in humans (e.g., pregnancy, menopause) have an effect on immune function and some diseases. Generally, estrogens are immunostimulatory, androgens are immunocompromising, and progestins can be either stimulatory or suppressive of immune function.

Androgens

Testosterone generally suppresses immune function. Castration of adult male rodents results in increased immunoglobulin levels, increased humoral and cell-mediated immunity, and increased lymphatic organ size, including thymic, splenic, and lymph nodal masses (Schuurs and Verheul 1990). Castration of male rodents leads to similar immune responses, but they are not equivalent to those of females; this suggests that some of the sex difference in immune function is organized prior to puberty. Treatment of adult castrated males with physiological doses of testosterone restores (i.e., depresses) immune function to precastration levels (Schuur and Verheul 1990; Grossman 1984). Testosterone replacement therapy of castrated or intact male rats or mice significantly suppresses humoral and cell-mediated immunity, as well as thymic mass (Schuur and Verheul 1990; Grossman 1984). Androgen receptors have been identified in thymic tissues, particularly in the epithelial, lymphatic portion of the thymus (McCruden and Stimson 1984; Sasson and Mayer 1981). Androgenic effects on lymphocytes may be in-

direct or through aromatization of androgens to estrogens, because no androgen receptors have been found on circulating lymphocytes (McCruden and Stimson 1991).

Estrogens

In contrast to the pattern of androgen receptor localization, estrogen receptors have been localized in the cytosol of circulating lymphocytes (Danel et al. 1983; Grossman 1984), CD8+ cells (Cohen et al. 1983; Stimson 1988), and thymic cells (Danel et al. 1983; Nilsson et al. 1984; Weusten et al. 1986). Physiological treatment of estrogen, or the estrogen receptor antagonists, tamoxifen and FC-1157a, enhances pokeweed mitogen induced immunoglobulin synthesis of B lymphocytes (Paavonen and Andersson 1985; Sthoeger et al. 1988).

Treatment of intact male or gonadectomized male or female mice and rats with physiological or supraphysiological doses of estrogens increases antibody responses to a variety of T-dependent and T-independent antigens (Inman 1978; Myers and Peterson 1985; Brick et al. 1985). Cyclic exposures to pharmacological doses of estrogens are more effective in boosting antibody formation than chronic estrogen exposure (Schuurs and Verheul 1990). Pharmacological doses of estrogens also suppress cell-mediated immunity (Grossman 1985; Kuhl et al. 1983). Tamoxifen inhibits the effects of estrogens on antibody formation and cell-mediated immunity (Nagy and Berczi 1986). Taken together, the effects of physiological doses of estrogen appear to enhance immune function.

Seasonal Fluctuations

Sex steroid hormone levels are suppressed by short-day exposure in long-day breeders (Bronson 1989). As reviewed above, sex steroid hormones, especially androgens, have been reported to suppress immune function (Grossman 1984; Billingam 1986; McCruden and Stimson 1991; Schuur and Verheul 1990). Thus, short-day enhancement of immune function could be the result of the reduction of steroid hormone suppression of immune function in males. The effects of estrogens, especially at high doses, and progestins on immune function are not as clear-cut. In some

systems, estrogens and progestins enhance immune function; in other situations, estrogens suppress immune function in a manner similar to the immunosuppression evoked by adrenal steroid hormones (Billingam 1986; McCruden and Stimson 1991; Schuur and Verheul 1990; and see below). Because both males and females, as well as pre- and postpubertal animals, have been reported to display short-day enhanced immune function (Blom et al. 1994; Nelson and Blom 1994; Vriend and Lauber 1973; Brainard et al. 1985), reductions in blood steroid concentrations are unlikely to account completely for the photoperiodic effects on immune function, but may play a secondary role. For example, melatonin, prolactin, and sex steroid hormones may interact to mediate the effects of photoperiod on immune function.

NONSTEROID HORMONAL INFLUENCES ON IMMUNE FUNCTION

Exposure to short day lengths reduces blood prolactin levels in every mammalian species thus far examined, including short-day breeders (Goldman and Nelson 1993). Treatment with melatonin in ways that mimic release patterns associated with short day lengths also suppresses blood prolactin titers (Goldman 1983; Bittman and Karsch 1984). Prolactin has pronounced effects upon immune function in a variety of species (reviewed by Bernton et al. 1991, 1992; Reber 1993; Arkins et al. 1993; Matera et al. 1992; Castanon et al. 1992). Generally, prolactin maintains or enhances normal immunological activities, but there are also examples of prolactin compromising immune function, particularly at high or low circulating levels (Reber 1993). Because exposure to short day lengths suppresses circulating prolactin levels, this hormone is a possible candidate for mediating some of the reported seasonal changes in immune function.

There have been reports of prolactin being involved in the development of immune function in rat and mouse pups (Gala and Shevach 1993; Matera et al. 1992; Blom et al. 1994). In one study, rat dams were injected with the dopamine agonist, bromocriptine (CB-154), to produce hypoprolactinemic milk (Gala and Shevach 1993). The relative percentages of

neonate thymic CD4 and CD8 cells, as well as splenic CD4, CD8, and B cells at 5 days of age were decreased when mothers were administered 100 μg of CB-154 (Gala and Shevach 1993).

Hypophysectomy of rats results in compromised humoral and cell-mediated immunity; immune function can be restored by prolactin replacement therapy (Reber 1993). Prolactin elevates the respiratory burst and phagocytosis of peritoneal macrophages from both young and old mice (Chen and Johnson 1993). Prolactin induces resting lymphocytes to divide, and can also affect the magnitude of their response to polyclonal stimuli, as well as influence the effector phase of the immune response, including increased response of natural killer (NK) cells, T cells, and B cells to the mitogenic signals, IL2, PHA, and *Staphylococcus aureus cowan*, respectively (Matera et al. 1992). Membrane-bound prolactin receptors have been reported on lymphocytes (Reber 1993; Bernton et al. 1991, 1992). Furthermore, prolactin-like substances have been identified in mouse splenocytes and human B lymphoblastoid cell lines (Sabharwal et al. 1992; Reber 1993). Cyclosporin A competes directly with prolactin for binding of the lymphatic receptors. It has been proposed that the immunocompromising effects of cyclosporin A may result from interference with a prolactin-like autocrine growth factor for lympho-proliferation (Sabharwal et al. 1992; Reber 1993).

In addition to compromise of immune function from low prolactin concentrations, high levels of circulating prolactin can also cause immunocompromise; lactating females, males treated with prolactin, and clinically hyperprolactinemic humans often exhibit compromised immune function. For example, very high prolactin levels (5 to 10 times the physiological circulating levels) inhibited mitogenic responses (Matera et al. 1992). Elevated blood prolactin levels (physiological range) have been discovered to mediate some autoimmune diseases (McMurray et al. 1994; Reber 1993). For example, flare-ups of systemic lupus erythematosus (SLE) in humans is associated with elevated circulating prolactin levels (McMurray et al. 1994). In the SLE mouse model (NZB/NZW F_1) (B/W), induc-

tion of hyperprolactemia hastens the onset of lupus-like symptoms (McMurray et al. 1994). In males, and especially females, of this mouse strain, increases in anti-DNA, as well as IgG and IgM titers, were observed after the induction of hyperprolactemia. Hyperprolactemia led to increased mortality primarily owing to vasculitis and renal diseases (McMurray 1994).

Suppression of prolactin secretion with CB-154 in rodents results in a decreased immunological response; these diminished responses include delayed-type hypersensitivity, primary antibody response, T cell dependent macrophage activation, and *ex vivo* T lymphocyte and B lymphocyte proliferation. Cyclosporin A and glucocorticoids have the same immunocompromising effects (Bernton et al. 1992). Treatment of mice with drugs that stimulated endogenous prolactin release, or with exogenous ovine prolactin, partially reverses the suppression of lymphocyte proliferation responses to mitogens caused by cyclosporin A or glucocorticoids (Bernton et al. 1992). Thus, short day lengths suppress circulating prolactin levels, and low prolactin levels can potentially compromise both humoral and cell-mediated immunity (Reber 1993). The immunological effects of prolactin might interact with the immunological effects of steroid hormones to mediate seasonal fluctuations in immune function. However, melatonin also has the potential to affect immunity. Studies that have systematically manipulated several hormone levels to examine the subsequent consequences on immune function are required.

MELATONIN INFLUENCES
IMMUNE FUNCTION

The pineal gland and the primary secretory pineal product, melatonin, appear to modulate immune function. Melatonin treatment of both normal and immunocompromised house mice elevates in vitro and in vivo antibody responses (Caroleo et al. 1992). Impaired T-helper cell activities in immunocompromised mice are restored by melatonin treatment (Caroleo et al. 1992). Antigen presentation by splenic macrophages to T cells is also enhanced by melatonin; furthermore, this enhancement coincides with an increase in MHC class II molecules, as well as IL-1 and TNF_α production (Pioli et al. 1993).

Experimental work on the effects of melatonin on immune function remains controversial. For example, in one early paper, neonatal pinealectomy was reported to be ineffective in evoking immune function change (Jankovic et al. 1970). More recently, neonatal pinealectomy has been reported to affect immune response; murine antibody-dependent cellular cytotoxicity (ADCC) was reduced in adults that were pinealectomized before 7 days of age (Vermeulen et al. 1993). ADCC is a lytic process that occurs when lymphocytes bind to specific antibody-coated target cells through receptors for the Fc portion of the IgG molecule expressed on their membrane. The impairment in ADCC appears peripubertally, around 60 days of age, suggesting an involvement of sex steroid hormones (Vermeulen et al. 1993). Pinealectomy also seems to ameliorate collagen II-induced arthritis in mice (Hansson et al. 1993), and has been reported to inhibit humoral immune function and depress bone marrow progenitors for granulocytes and macrophages in mice (Kuci et al. 1988). Additionally, NK cell activity and IL-2 production are reduced in mice after pinealectomy (del Gobbo et al. 1989).

The circadian synthesis and release of melatonin modulate antibody response and alter tumorigenesis. At the normal cellular level, melatonin is believed to affect antimitotic processes as well as cytotoxic activity (Boucek and Alvarez 1970; Poffenbarger and Fuller 1976; Winston et al. 1974). In mice, the circadian synthesis and release of melatonin play a significant immunomodulatory role. When the synthesis of endogenous melatonin is blocked, antibody production is depressed; in contrast, transplantation immunity is not affected by pinealectomy (Maestroni and Pierpaoli 1981; Maestroni et al. 1986). Pharmacological and surgical pinealectomy also modulate other immune parameters including plaque-forming cells and blastogenic responses of spleen cells and thymus cells to various mitogens (Becker et al. 1988; Kuci et al. 1988). Furthermore, elimination of melatonin synthesis by pinealectomy profoundly decreased the proliferation of bone marrow progenitors for granulocytes and macrophages; the night time peak of melatonin completely abolished CFU-MG proliferation (Kuci et al. 1988). In summary, melatonin appears to enhance immune function in most cases. In common with reproductive responses mediated by melatonin, there may be a temporal component to the biological actions of melatonin. The data on the effects of melatonin on immune function in mice, however, are equivocal. Previous research has demonstrated that inbred strains of mice such as C57BL/6, BALB/c, and NZB, strains typically used in the studies reported here, have a genetic defect and are unable to synthesize melatonin (Ebihara et al. 1986; Goto et al. 1994). Thus, the extent to which melatonin possesses universal immune-enhancing properties remains controversial (see Reppert and Weaver 1995; cf. Maestroni and Conti 1991).

TUMORIGENESIS

Melatonin also appears to influence tumor growth (Table 2). Experimental and clinical reports suggest that there is a link between cancer development and pineal function (Bartsch and Bartsch 1981; Lapin and Ebels 1981; Blask 1984). Pinealectomy of adult male rats results in an elevated mitotic index, as well as in an increase in the incorporation of ^{32}P into the DNA of the spleen, small intestines, liver, and adenohypophyses. The pineal has been suggested to cause a deceleration of the cell division of different tissues (Bindoni 1971).

The effects of melatonin on neoplastic cell proliferation depend on the type of neoplastic tissue examined. In general, melatonin appears to inhibit neoplastic cell proliferation in a dose dependent way; that is, cloning efficiency diminishes as melatonin doses increase (Bartsch et al. 1987; Hill and Blask 1988). The data, however, appear to be contradictory. The vast majority of studies suggest that melatonin slows tumor progression or promotion. For example, pinealectomy accelerates the growth of transplanted melanoma in hamsters (Das Gupta and Terz 1967a,b), of transplanted Walker 256 carcinoma in rats (Rodin 1963; Barone and Das Gupta 1970), and of transplanted Yoshida sarcoma in rats (Lapin 1978; Lapin and Frowein 1981). Furthermore, removal of the pineal enhanced the incidence of mammary adenocarcinoma in the rat induced by the chemical carcinogen 9,10-benzanthracene (DMBA), particularly when low

TABLE 2

The effects of pinealectomy and melatonin treatment on cancer

Type of Tumor	Effects of Pinealectomy	Effects of Melatonin Treatment
Transplantable Tumors		
Lewis lung carcinoma		reduced survival time in mice (1)
Walker 256 carcinoma	reduced survival time, increased tumor diameter and tumor metastases (2) increased tumor volume in rats (3)	no effect (4)
Ovarian carcinoma	increased volume of tumor (5)	inhibited in vitro growth of tumor cell lines (6)
Melanoma	stimulated growth metastatic spread and increased tumor volume in hamsters (7)	reversed effects of pinealectomy LD 8:16 compared to LD 14:10 in hamsters (8)
Leukemia		inhibited incidence and growth of tumor (9)
Yoshida tumor	increased tumor growth and decreased survival time (10)	no effect in intact rats and reversed effects of pinealectomy (11)
Chemically-Induced Tumors		
Dimethylaminobenzene-induced hepatocarcinoma	inhibition of tumor growth (12)	
Methylcholanthrene-induced fibrosarcoma	increased rat tumor volume and increased metasteses to lymph nodes (13)	morning treatment decreased and evening treatment increased survival in mice (14)
Estrogen-induced; prolacinoma		inhibited prolactin-secreting pituitary and tumors in rats in vivo and in vitro (15)
N-methylnitrosourea-induced mammary carcinoma	slightly increased tumor rate in rats (16)	slowed tumor growth when present during tumor promotion phase (16)
Dimethylbenzanthracene-induced mammary carcinoma	increased tumor growth rate in rats (17)	suppressed tumor growth in rats and deer mice (17)*
Tumor Lines		
Dunning R3327 rat prostatic adenocarcinoma		inconsistent effects of melatonin, but related to reproductive effects (18)
Human breast cancer line MCF-1		inhibited proliferation of cancer cells in vitro (19)
Erlich's tumor	increased proliferation of tumor cells (20)	stimulated or reduced growth dependent upon time of administration (20)

* Suppressed tumor growth in deer mice housed in LD 8:16.
References: (1) Lapin & Ebel 1967, (2) Rodin 1963, (3) Barone & Das Gupta 1970, (4) Bostelmann et al. 1971, (5) Das Gupta 1968, (6) Leone et al. 1988, (7) Das Gupta & Terz 1967a,b, (8) Stanberry et al. 1983, (9) Buswell 1975, (10) Lapin 1979, (11) Lapin & Frowein 1981, (12) Lacassaque et al. 1969, (13) Barone et al. 1972, (14) Bartsch & Bartsch 1981, (15) Leadem & Burns 1988; Karasek et al. 1988, (16) Blask & Leadem 1987, (17) Tamarkin et al. 1981; Nelson & Blom 1994, (18) Buzzell 1988, (19) Hill & Blask 1988, (20) Billitieri & Bindoni 1969.

doses were used (Tamarkin et al. 1981). However, when high doses of DMBA were used, there was no significant difference between pinealectomized or sham-operated animals in the incidence of mammary neoplasms (Lapin 1979; Aubert et al. 1980). The administration of melatonin to pinealectomized hamsters abolished the effect of pinealectomy on the growth of implanted melanoma (El-Domeiri and Das Gupta 1973). Similarly, tumor development and tumor incidence decreased with the administration of exogenous melatonin to female rats treated with the chemical carcinogen DMBA (Aubert et al. 1980; Tamarkin et al. 1981). The inhibitory effect of melatonin on tumor growth was also demonstrated using a transplantable leukemia in mice (Buswell 1975) and a transplantable mammary tumor in rats (Karmali et al. 1978).

Recently, melatonin has been reported to be effective in protecting against tumor initiation. The chemical carcinogen, safrole, evokes DNA-adduct formation in liver tissue. Both physiological (Tan et al. 1994) and pharmacological (Tan et al. 1993) doses of melatonin coadministered with safrole suppressed DNA-adduct formation in rat liver tissue. A dose-dependent effect of melatonin on DNA-adduct formation was reported, indicating that pharmacological doses (e.g., resulting in a serum melatonin level of 13,950 pg/ml) were extremely effective in preventing modification of DNA in response to safrole exposure (Tan et al. 1994).

In another recent study, adult female deer mice (*Peromyscus maniculatus*) were housed either in long (LD 16:8) or short (LD 8:16) days for 8 weeks, then injected with the chemical carcinogen, 9,10-dimethyl-1, 2-benzanthracene (DMBA), dissolved in dimethyl sulfoxide (DMSO) or with the DMSO vehicle alone (Nelson and Blom 1994). Animals were evaluated weekly for 8 weeks after injection. None of the animals treated with DMSO developed tumors in any of the experiments. Nearly 90% of the long-day deer mice injected with DMBA developed squamous cell carcinoma. None of the short-day deer mice injected with DMBA developed tumors (Nelson and Blom 1994). Small lesions developed at the site of injection; short-day females had less severe lesions and healed faster than long-day females. The role

of estrogens in the photoperiodic responses were evaluated in a follow-up study. Ovariectomized or sham-ovariectomized deer mice received estradiol benzoate replacement therapy or a control procedure in long day lengths for 8 weeks prior to injection of DMBA or DMSO, then were monitored for 8 additional weeks. Females treated with DMBA developed tumors at the same rate regardless of estrogen manipulation. Again, wound healing was faster in short as compared to long days; estrogen did not affect healing rates. In another follow-up study (Nelson and Blom 1994), female deer mice were injected with a slurry of microspheres that contained either bromocriptine (CB154) or were empty. Suppression of prolactin with CB154 decreased tumor incidence from 55.6% to 24% in long-day females 8 weeks after injection with DMBA. However, healing rates were not affected by prolactin manipulations. Silastic capsules that were filled either with melatonin or cholesterol were implanted into long-day female deer mice in another study (Nelson and Blom 1994); 8 weeks later, females received either an injection of DMBA or DMSO, then were monitored for 8 weeks. Approximately 66% of females implanted with cholesterol and injected with DMSO developed histologically-verified tumors. None of the melatonin-implanted mice developed tumors (Nelson and Blom 1994). Melatonin did not affect healing rates. Taken together, these results indicated that photoperiod, mediated by melatonin, and possibly prolactin, can exert a functionally significant effect on immune processes and clinical disease.

This brief review emphasizes that pineal melatonin affects immune function, influencing both humoral and cell-mediated immunity. Most of the available data suggest an anticarcinogenic effect of melatonin, although there are a few studies suggesting a procarcinogenic effect of melatonin (Hill and Blask 1988). Again, the conflicting effects on immune function are reminiscent of the confusion surrounding the anti- and progonadal effects of melatonin on reproduction. The confusion was resolved with the understanding that the timing of melatonin treatment provided the critical cue in organizing reproductive response. It remains possible that the

anticarcinogenic effects of melatonin depend upon a circadian rhythm of tissue responsiveness. Further work on the role of melatonin on the development of cancer is needed.

EFFECTS OF STRESS ON IMMUNE FUNCTION

Many interactions between glucocorticoids and immune cell function have been reported in relation to environmental stress (reviewed in Nakono et al. 1987). However, the mechanisms underlying seasonal changes in stress hormones and immune function have not been elucidated. Adrenocortical hormones, especially glucocorticoids, suppress immune function in both humans and nonhuman animals (Baxter and Forsham 1972; Claman 1972; Hauger 1988; Ader and Cohen 1993; Black 1994; but see Tavadia et al. 1975), although the vast majority of work on the stress response has been conducted with mammals. The role of glucocorticoids in compromising immune function of nonmammals is not clear. Glucocorticoids are released in response to stressful stimuli, and can compromise cellular immune function (Berczi 1986; Levi et al. 1988). Adrenalectomy enhances lymphatic organ masses and B cell activities (del Rey et al. 1984). The precise mechanisms by which the immune system is affected by the hypothalamo-hypophysial-adrenocortical axis (HPA) are unknown, but probably involve cytokine release rates from activated immunological cells (Besedovsky et al. 1981, 1983, 1986; Besedovsky and del Rey 1991). Activation of the HPA axis is not independent of energetic systems; elevated glucocorticoid concentrations suppress anabolic processes and stimulate catabolic processes (Sapolsky 1992). Regardless of mechanism, substantial evidence links glucocorticoids with suppressed immune function.

Again, glucocorticoids are released in response to perceived stressors (Black 1994). Other interactions between glucocorticoids and immune cell function have been reported in relation to environmental stress in laboratory settings (Nakono et al. 1987). Food restriction may be perceived as stressful. For example, poor food quantity and quality can impair synthesis of antibodies (Jose and Good 1973). Similarly, caloric restriction can also affect tumor incidence and growth, as well as other disease processes in naturally-selected species (Tannenbaum 1940; Tannenbaum and Silverstone 1953; Rusch 1944; Visscher et al. 1942). In common with restricted food intake, low ambient temperatures also seem to be perceived as stressful, and can potentially depress immune function (e.g., Claman 1972; MacMurray et al. 1983; Monjan 1981). A detailed account of the interactions between stress and immune function is beyond the scope of this review (for recent reviews see Ader and Cohen 1993; O'Leary 1990; Black 1994), but both artificial and natural stressors evoke glucocorticoid release from the adrenal glands, which interact with the immune system and eventually suppress immune function.

Glucocorticoids have been implicated in several dramatic seasonal cycles in mortality. For instance, the brown marsupial mouse (*Antechinus stuartii*) has a highly synchronized breeding season that lasts about two weeks (Wood 1970). The breeding season is followed in the field by the death of all reproductive males (Woolley 1966; Wood 1970) and, in laboratory studies, by mortality or reproductive senescence (Woolley 1966). Death is the result of hyperactivity of the adrenal glands, apparently owing to the stress of breeding (Wood 1970) and the onset of several opportunistic diseases, including cancer (Lee and McDonald 1985). A similar adrenal mechanism was hypothesized to induce mortality of postspawning salmon; the stress of migration to natal streams was believed to stimulate the oversecretion of adrenal steroids, resulting in death (Robertson and Wexler 1959). However, captive salmon, that do not participate in the strenuous upstream migration, also die immediately after spawning (Robertson and Wexler 1959). Thus, the long-day breeding activities may suppress immune function via adrenocortical stimulation.

Previous studies have demonstrated that environmental stressors elevate blood glucocorticoid levels and that high glucocorticoid levels suppress immune function (Baxter and Forsham 1972; Claman 1972; Hauger 1988; Ader and Cohen 1993; Black 1994; Fauci 1975; Kawate et al. 1981; Besedovsky and del Rey 1991). Winter survival in small animals is hypothesized to require a positive balance between short-day enhanced immune status and

glucocorticoid-induced immunosuppression. This immunosuppression may be due to many factors: overcrowding, increased competition for scarce resources, low temperatures, reduced food availability, increased predator pressure, or lack of cover that causes high blood concentrations of glucocorticoids. Winter breeding with its concomitant elevation of sex steroid hormones may also cause immunodepression (e.g., Lochmiller et al. 1994). Presumably, winter breeding occurs when other environmental stressors such as temperature and food availability are not perceived as severe. The balance of enhanced immune function (i.e., to the point where autoimmune disease becomes a danger) against stress-induced immunosuppression (i.e., to the point where opportunistic pathogens and parasites overwhelm the host) must be met for animals to survive and become reproductively successful. Thus, the mediation of reproductive function and immune function will likely be intertwined (Besedovsky and del Rey 1991). Both ambient temperature and photoperiod were manipulated in another recent study of deer mice in our laboratory. Animals in short days showed regression of their reproductive systems and also displayed significantly higher IgG levels than did those on long days. Animals maintained in both short days and low temperatures displayed IgG levels comparable to mice in long days and mild temperatures. Animals maintained in long days and low temperatures had significantly higher serum corticosterone levels than animals maintained in long days and mild temperatures. These data are also consistent with the working hypothesis that immune values are enhanced in short days to counteract stress-mediated immune suppression in responses to harsh winter conditions (Demas and Nelson 1996) (Figure 2).

Individuals of species that do not rely on photoperiodic cues for timing their breeding may retain responsiveness to photoperiod in order to reap benefits of enhanced immune function at certain times of year, independently of reproductive function. For instance, laboratory strains of mice and rats that do not change reproductive function in response to photoperiod (Nelson 1990; Nelson et al. 1994) exhibit profound changes in immune function in response to photoperiod (Wurt-
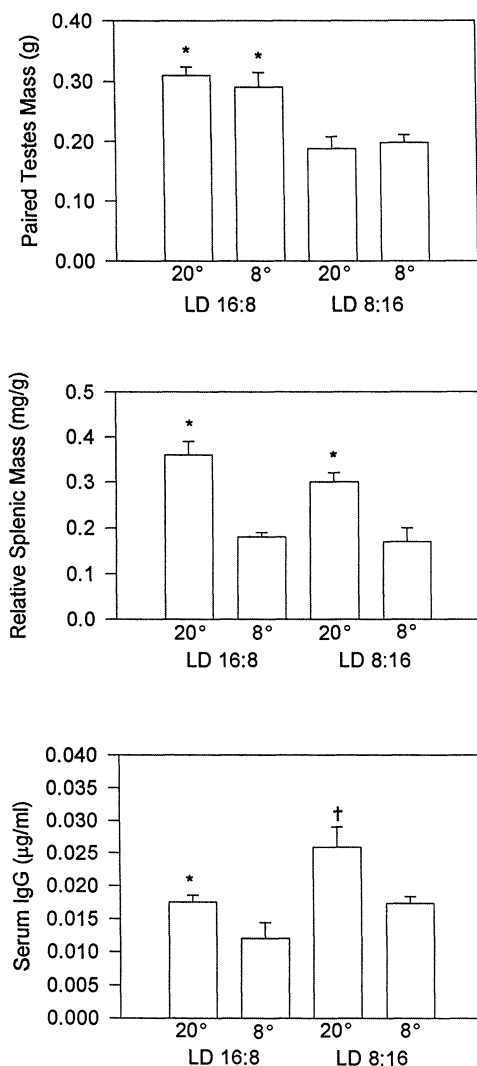

FIGURE 2. ORGAN MASSES AND SERUM IgG
LEVELS OF MALE DEER MICE AT
DIFFERENT TEMPERATURES AND
DAY LENGTHS

Data are means (± standard error of the mean); animals were housed in long (LD 16:8) or short (LD 8:16) days, and mild (20°C) or low (8°C) ambient temperatures. Top panel, paired testes masses (g); middle panel, relative splenic masses (mg/g); bottom panel, serum IgG (μg/ml). Columns with no symbol or sharing the same symbol are statistically equivalent. Columns with different symbols are significantly different at $P < 0.05$.

man and Weisel 1969; Mahmoud et al. 1994). Although there is evidence that photoperiod may exert minor effects on human reproduction (Roenneberg and Aschoff 1990a,b; Bronson 1995), the effects of photoperiodic manipulations on human immune function have not been directly assessed.

CLINICAL SIGNIFICANCE OF SEASONAL CHANGES IN IMMUNE FUNCTION

The question of whether photoperiod may affect human immune function remains open. Seasonal changes in disease prevalence and immune function among humans are well known. Humans living in tropical environments are aware of malaria seasons; in temperate habitats, there is a pronounced cold and influenza season. Although medical researchers have established that low temperatures are apparently unrelated to the clinical symptoms of a cold, the link between low temperatures and the constellation of symptoms associated with the common cold has been known since antiquity. It is perhaps no coincidence that the English word "cold" means both the subjective experience of low temperature *and* the constellation of flu-like symptoms. This dual use of the word is also seen in western European languages, including Dutch, Italian, and Spanish. Not surprisingly, "low ambient temperature" is *frigidus* in Latin, and to catch a cold is *frigus colligere.* This association between low temperatures and influenza is also apparent outside the Latin-influenced languages. Interestingly, the word(s) for flu-like symptoms in Mandarin translate as "hurt by coolness," whereas in Cantonese, the translation for the illness is "hurt by the cold wind."

Thus, humans have been aware of the temporal association of illness with the seasonal change in weather for some time. The increased susceptibility for influenza in the autumn and winter may be owing to increased contact in close quarters, seasonal stress-induced reduction in immune function, or some combination of these two phenomena. Several human diseases have strong to moderate seasonal components, including influenza, malaria, dysentery, measles, asthma, arthritis, and many forms of cancer. Obviously, there are no studies with direct manipulation of photoperiod among humans, but several ob-

servations suggest that photoperiod can affect human immune function. For example, several studies have revealed a latitudinal component to several autoimmune diseases, including rheumatoid arthritis and multiple sclerosis (Rosen et al. 1991). Individuals suffering from seasonal affective disorder (SAD) often exhibit aberrations in their immune cell counts (Rosen et al. 1991). Some patients with SAD display aberrant lymphocyte proliferation in response to a mitogen stimulus (Skwerer et al. 1988). Treatment of the SAD symptoms with bright illumination ameliorates the immunological abnormalities (Skwerer et al. 1988). Total number of circulating NK cells was reduced among SAD patients in another study (Kasper et al. 1991); the reduction was inversely related to the score attained on a test of depression. After bright light therapy, the symptoms of depression ameliorated and the NK cell numbers increased. Furthermore, lymphocyte proliferation in response to a mitogen improved after phototherapy (Kasper et al. 1991). Thus, these studies indicate that immune function is significantly compromised in the winter among patients who suffer from SAD (Rosen et al. 1991).

Another observation that is consistent with a photoperiodic influence on human immune function is the association between latitude and multiple sclerosis (MS) (Davenport 1922; Limburg 1950; Kurtzke 1975, 1980). The prevalence of MS increases at higher latitudes, both north and south (reviewed in Rosen et al. 1991). A consistent correlate with MS is the amount of December solar radiation (Acheson et al. 1960); high numbers of sunny hours in December are associated with low numbers of MS cases in the region (Acheson et al. 1960).

Malaria is a common seasonal disease among humans residing in the tropics (Theander et al. 1990). There are a number of reports of seasonal changes in immune function associated with malaria. As noted previously, malaria has been reported to show increased vernal relapses in humans. Typically, these relapses have been attributed to a fixed interval of disease progression following autumnal (onset of the wet season) infections (Coatney and Cooper 1948). Antigen-induced cellular immune responses to *Plasmodium falciparum* are compromised during acute malaria onset

(Abu-Zeid et al. 1992; Chougnet et al. 1990; Theander et al. 1990). Lymphocyte proliferation responses (against nonmalaria antigens) of healthy individuals were also compromised during the malaria transmission season (Theander et al. 1990). This suggests that immune function might be suppressed during the time of *Plasmodium falciparum* infections.

Seasonal changes in human immune function have also been established in other studies. For example, human subjects from a local VA hospital were examined in one study (MacMurray et al. 1983). T cell and B cell functioning was assessed in 30 healthy subjects; blood samples were obtained during the winter and summer and the lymphocytes extracted. Viable cells were counted and the percentage of viable T cells and B cells was significantly elevated in winter subjects compared to those tested in the summer. In addition, blood samples of patients from five different VA hospitals were tested, revealing significantly higher IgG levels in the winter samples compared to those of summer for four out of five hospitals (MacMurray et al. 1983). However, IgA and IgM levels did not differ significantly across seasons in these studies.

Six blood donors were tested in another study (Bratescu and Teodorescu 1981). The absolute values and percentage of B cells and T cells in the peripheral blood were examined. Blood samples from these donors were tested every two months for a period of one year. The total number of lymphocytes and leukocytes did not vary throughout the year. However, the ratio of B cells to T cells was nearly doubled during the winter months compared to the summer months (Bratescu and Teodorescu 1981). In another study, seasonal changes in murine splenic natural killer cell activity and murine lymphocyte responsiveness to several B and T cell mitogens were reported in healthy subjects, with maximum and minimum responsiveness occurring in the spring and winter, respectively (Pati et al. 1987). Circadian rhythms of circulating T and NK cells have also been reported in healthy subjects (Levi et al. 1988; Bourin et al. 1993) and these rhythms can vary across seasons (Levi et al. 1988).

In the remaining studies, the opposite pattern of immune function was observed—it was elevated during the summer and compromised during the winter. For example, blood samples were drawn from 15 healthy individuals at two time periods each year for two years (Boctor et al. 1989). These time periods were from June to August and from December to February. Lymphocytes were separated, suspended, and examined for proliferative responses to the mitogens PHA, Con A, and PWM. Sixteen hours after initial cell suspension, lymphocyte cells were pulsed with tritiated methyl thymidine and the amount of radioactive thymidine incorporated into newly synthesized DNA was assessed. The lymphoproliferative response to all three mitogens was significantly greater in the summer compared to winter.

In another recent study, forty-five healthy school children, ages 8 to 16 years, were examined (Afoke et al. 1993). Blood samples were drawn from each subject during each season of the year (January, April, July, and October). Peripheral blood lymphocytes were separated and assayed using an E-rosette assay to determine the percentage of viable lymphocytes. Serum IgG, IgA, and IgM concentrations were also determined using a nephelometric immunoassay. The results showed peak CD4+ T cell counts in the spring and the lowest levels in the winter. Total B lymphocytes remained unchanged throughout the seasons. Natural killer cells and macrophages showed significant increases during the autumn and summer seasons, with the lowest levels occurring in the winter. There were no significant seasonal differences in levels of IgG, IgA, or IgM. The authors concluded: "These observations suggest that seasonal variations of some immunological parameters occur in healthy children. This may be an adaptive response to variable climatic and other environmental factors" (Afoke et al. 1993:209).

Aryl hydrocarbon hydroxylase (AHH) activity was measured in cultured lymphocytes taken from 977 donors over a 30-month period (Paigen et al. 1981). Donors were primarily between the ages of 20 to 50 years. Heparinized blood was drawn from each subject; lymphocytes were separated and resuspended in a medium consisting of fetal calf serum, pokeweed mitogen, and phytohemagglutinin

for three days. AHH activity was induced by adding methylcholanthrene 24 hours before measurement. The results demonstrated that maximal induced AHH activity occurs during the late summer and early fall, with minimal activity in the spring and winter.

Human tonsillar lymphocytes were examined from resected tonsils of children 5 to 12 years old from April 1984 to August 1985 (Komada et al. 1989). Lymphocyte proliferation in response to Con A mitogenic stimulation increased during the summer and decreased during the winter months. Identification of the lymphocyte subsets by using monoclonal antibodies suggested that the proportion of T4 and T8 cells also increased in the summer and decreased during the winter (Komada et al. 1989).

Another group of healthy volunteers was monitored for a period of 12 months (Mac-Rury et al. 1992). Leucocyte ascorbic acid levels and serum cholesterol levels were monitored at monthly intervals. Results demonstrated an increase in ascorbic acid and a reduction in cholesterol levels during the summer months. This pattern was reversed during the winter months. Furthermore, when compared to patients with peripheral vascular disease, healthy subjects had significantly higher levels of ascorbic acid than those suffering from vascular disease. The authors suggested that the important role vitamin C has as an antioxidant can reduce free radical damage.

Although there are several reports of seasonal cycles in immune function, the regulation of these cycles has not been investigated thoroughly. Virtually no studies of clinical outcome have been performed when taking season of the year or extrinsic factors that cue for time of year into consideration (but see Holdaway et al. 1990; Lee 1967; Kirkham et

al. 1985). Despite the lack of clinical research investigating the proximate causes of seasonal cycles in immune function and disease susceptibility, it is clear that seasonal environmental factors can influence human immune responses. The environmental conditions during the autumn and winter compromise several immune parameters, including total lymphocyte numbers (Bratescu and Teodorescu 1981) and antibody titers (MacMurray et al. 1983). The interaction among environmental conditions, stress responses, and immune function in humans remains unspecified. Perception of seasonal stressors has the potential of compromising immune function unless countered.

CONCLUSIONS

Seasonally predictable environmental stressors can be anticipated by monitoring photoperiod. In animal studies, short day conditions inevitably lead to enhancement of immune function. Furthermore, several field studies suggest elevated lymphatic organ size and function during the short days of autumn and winter (Lochmiller et al. 1994; Isogai et al. 1992; John 1994). Taken together, these findings support the hypothesis that animals may have evolved mechanisms to predict, adjust, and counter seasonal changes in immune function. Additional integrative studies are required to understand the complex interactions among the endocrine, immune, and nervous systems that result in seasonal fluctuations in reproduction, immune function, and survivorship.

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