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Periodicity of Susceptibility to Pneumococcal Infection: Influence of Light and Adrenocortical Secretions

Abstract. *Circadian periodicity of susceptibility to pneumococcal infection was altered but not abolished in blind or adrenalectomized mice. Serum corticosterone concentrations 24 hours after pneumococcal challenge were greatest in animals challenged at 0400 hours, a time when circulatory corticosterone is lowest; the smallest absolute increase in serum corticosterone followed challenge at 1600 hours.*

A circadian rhythmicity of susceptibility to pneumococcal infection has been documented in mice (*1*). We have elucidated several factors that modulate this rhythmicity and have documented that survival patterns could be altered by environmental lighting conditions (*2*). In contrast, survival periodicity was not altered by changes in feeding or activity of the mice or by administration of penicillin (*2*). Serum corticosterone concentrations of infected mice at 6 and 12 hours after challenge were greatest when infection was initiated at 0400 hours and lowest after infection at 1600 hours (*2*). In this report we describe the effects of blinding on the periodicity of susceptibility to infection, as well as additional attempts to ascertain the role of adrenocortical secretions in modulating periodicity of morality.

Normal or adrenalectomized male mice (16 ± 2 g) of the CD-1 strain (Charles River Laboratories, Wilmington, Massachusetts) were used for all experiments. Normal mice were blinded by bilateral optic enucleation. Blinded and normal mice were conditioned for 21 days in an animal chamber maintained at 24.4°C and lighted from 0600 to 1800 hours. Animals had free access to Wayne Mouse Breeder Blox and water. Adrenalectomized mice were conditioned similarly but were maintained with 0.9 percent sodium chloride for drinking water.

In all experiments, groups of 30 mice were inoculated subcutaneously, the first group at 0800 hours and the others at successive 4-hour intervals ending 24 hours later. *Diplococcus pneumoniae*, type I, A5, was the challenge organism, and either 10^3 organisms of modified virulence or 10^3 fully virulent organisms were inoculated. Virulence of the challenge organism and its preparation by serial dilution have been described (*1*). Counts of viable bacteria before and after injection at each time interval showed a maximum variation of 5 percent in the number of challenge organisms. The inoculum was suspended in 0.5 ml of tryptose phosphate broth, *p*H 7.4. Groups of ten normal or adrenalectomized control mice were inoculated with sterile broth at each challenge time.

In each experiment, mice were checked and deaths were recorded hourly beginning at the time of challenge; mice were observed for 14 days or until all had died. In experiments in which corticosterone concentrations were assessed, mice were killed by cardiac puncture 24 hours after challenge, and the blood obtained was used for measurement of corticosterone. A modification (*3*) of the ultramicromethod for measurement of corticoids as described by Murphy (*4*) was used for corticosterone evaluation.

Rectal temperatures were obtained before inoculation on groups of five mice at each challenge time. Temperatures were obtained by means of a veterinary electronic thermometer (Diagnostic V, Diagnostic, Inc., Indianapolis, Indiana). Temperatures were also recorded on groups of five control mice at each challenge time. These controls were normal mice that had not been blinded or adrenalectomized.

Statistical analysis was performed by the division of biostatistics, Washington University School of Medicine. Survival time was expressed in logarithmic form to facilitate use of mathematical assumptions necessary for analysis of variance. The model equation was

$$X_{ik} = u + t_i + e_{ik}$$

where X_{ik} denotes the logarithm of survival of the kth animal in the ith groups, u is the general mean, t_i is the effect of time, and e_{ik} is the experimental error. For each experiment, individual differences between the groups inoculated at various times were investigated by the multiple range test. The proportion of animals surviving challenge was analyzed separately by chi-square statistics, and the significance of differences in number of survivors in each experimental group was compared by the method of Kimball (*5*).

Results after challenge of blind mice with 10^3 *D. pneumoniae* of modified virulence were evaluated by analysis of variance. No significant differences in survival time were noted, but significant differences in the number of animals surviving challenge were found. The number of survivors after challenge at 0400 hours was significantly less ($P < .05$) than that after challenge at 0800, 1200, 1600, or 2000 hours. The number of survivors at 2400 hours was significantly less ($P < .05$) than at 0800 hours. Mean rectal temperatures of blind mice infected at 1600 hours were significantly less ($P < .01$) than those of any other group.

Analysis of variance revealed a significant difference ($P < .05$) in the survival pattern after challenge of adrenalectomized mice. The mean survival time of animals challenged at 2400 hours was significantly less ($P < .05$) than that for animals challenged at 1200 hours. In addition, mean survival time of the group challenged at 2000 hours was significantly less than that for animals challenged at 0800, 1200, or 1600 hours ($P < .05$). No significant differences in rectal temperatures were noted among the groups of animals.

The mean corticosterone concentra-

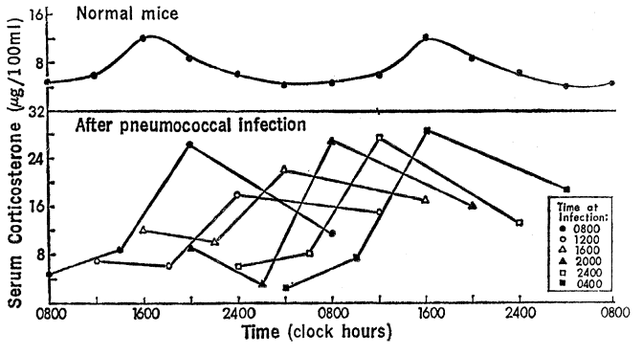

Fig. 1. Serum corticosterone concentrations as a function of time of challenge. Mean corticosterone concentrations are shown for groups of ten normal uninfected mice (top) and for groups of 30 mice at 6, 12, and 24 hours after pneumococcal infection.

tions 6, 12, and 24 hours after challenge of each group of mice and those for normal animals are illustrated in Fig. 1. At 24 hours after infection, corticosterone concentrations compared to those of control animals were significantly increased ($P < .01$ to $< .0005$) in all groups except that challenged at 0800 hours. The greatest relative increase and the highest absolute corticosterone concentrations at 24 hours were noted in animals challenged at 0400 hours, a time when circulating corticosterone is lowest.

Specific mechanisms for the observed periodicity of susceptibility of mice to pneumococcal infection are not fully understood. Environmental light is important in synchronizing the periodicity of host response to infection (*1*).

Interrelation between time of challenge, the virulence of the challenge organism, lighting conditions, the animals' ability to perceive light, and adrenocortical secretions are shown in Table 1. As shown previously (*1*) regardless of the dose of *D. pneumoniae* inoculated or virulence of the challenge organism, mice conditioned for 21 days in an animal chamber lighted from 0600 to 1800 hours and challenged at 0400 hours survived longer than animals inoculated at any other time of day (Table 1).

When mice were conditioned for 21 days in continuous light (Table 1, line 8) or darkness (Table 1, line 9), the pattern of susceptibility to infection was altered but not abolished. In this study, visual perception of light was necessary for entrainment of survival periodicity; however, periodicity of susceptibility to infection persisted even in the absence of this entraining variable.

When normal mice were housed under the lighting conditions used for the study of infection in blind mice, longest survival and the greatest number of survivors followed challenge at 0400 hours, whereas shortest survival followed challenge at 1600 hours (Table 1). Peak rectal temperatures in normal mice maintained in an animal chamber lighted from 0600 to 1800 hours for 21 days were at 1600 hours. In contrast, rectal temperatures of blind mice conditioned similarly were lowest at 1600 hours. Haus *et al.* (*6*), in longitudinal studies of blind mice, demonstrated a gradual shift in the time of maximum and minimum body temperatures but temperature periodicity, an endogenous rhythm, was not abolished at 21 days. The finding that periodicity of susceptibility to infection is shifted in phase but not abolished by blinding suggests that rhythmicity of mortality is also an endogenous rhythm.

Wongwiwat *et al.* (*7*) reported that adrenalectomy abolished the periodicity of susceptibility of mice to pneumococcal infection; however, they challenged adrenalectomized mice only at 0400 and 1600 hours. In the present study, periodicity of susceptibility to pneumococcal infection was preserved in adrenalectomized mice, but longest survival followed challenge at 1200 hours whereas shortest survival followed challenge at 2000 hours. Survivals after challenge of adrenalectomized mice at 1600 and 0400 hours were not significantly different. Thus, corticosterone secretion appeared to be an entraining influence but was not required for expression of survival periodicity.

Adrenalectomized mice were challenged with 10^3 *D. pneumoniae* of modified virulence. Despite the use of an

Table 1. Maximum and minimum survival or maximum and minimum number of survivors after pneumococcal challenge of mice at different times of day. Results are shown for a variety of experimental conditions. Time of day refers to time of inoculation. Food (0800–1600) indicates that food and water were available during this period; PCN indicates that aqueous penicillin G (one dose, 1000 units intraperitoneally) was given 12 hours after pneumococcal challenge; Adrex indicates use of adrenalectomized mice. Duration of survival: ● maximum; ○ minimum. Number of survivors: ▲ maximum; △ minimum.

Light (hours)	Conditioning period before inoculation (days)	Inoculum	Other conditions	Time of day					
				2400	0400	0800	1200	1600	2000
0600–1800	21	10^1 virulent			●			○	
0600–1800	21	10^2 virulent			●			○	
0600–1800	21	10^4 virulent			●			○	
0600–1800	21	10^6 virulent			●			○	
0600–1800	21	10^2 attenuated			●▲			○△	
1800–0600	10	10^4 attenuated		○				●	
1800–0600	21	10^3 attenuated			○			●	
0000–2400	21	10^4 attenuated			○	●			
0	21	10^4 virulent		●			○		
0600–1800	21	10^2 virulent	Food 0800–1600		●			○	
0600–1800	21	10^2 virulent	PCN		●				○
0600–1800	21	10^4 virulent	Blind		○	●			
0600–1800	21	10^2 attenuated	Blind		△	▲			
0600–1800	3	10^3 virulent	Adrex				●		○

organism of modified virulence the mean survival time of animals in each group was significantly less ($P < .01$) than the mean survival time of normal mice challenged previously with 10^8 fully virulent *D. pneumoniae* (*1*). Thus, the capacity to elaborate corticosterone appears to be an important factor in host survival, as suggested previously (*8*).

Rhythmicity of adrenocortical secretion has been documented in many animal species. The mouse adrenal cortex is most responsive to adrenocorticotrophic hormone at a time (0400 hours) when circulating corticosterone in lowest, and conversely, minimal response accompanies stimulation when circulating corticosterone is greatest (1600 hours) (*9*). As shown in Fig. 1, maximal corticosterone elaboration follows infectious challenge at 0400 hours, whereas challenge at 1600 hours evokes relatively little increase in corticosterone concentration compared to values in control mice sampled at the same time of day. Longest survival follows challenge at 0400 hours, whereas disease is most devastating after challenge at 1600 hours. These results support the observations of Beisel *et al.* (*10*) and suggest that the endogenous steroid responses to infectious challenge may be anabolic in type and an important factor in survival.

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Complete Amino Acid Sequence of the Mu Heavy Chain of a Human IgM Immunoglobulin

Abstract. *The amino acid sequence of the μ chain of a human IgM immunoglobulin, including the location of all disulfide bridges and oligosaccharides, has been determined. The homology of the constant regions of immunoglobulin μ, γ, α, and ε heavy chains reveals evolutionary relationships and suggests that two genes code for each heavy chain.*

Because of the importance of immunoglobulin M (IgM) as the first antibody formed in the newborn animal and in the primary immune response, and because of its role in certain autoimmune diseases, we have determined the amino acid sequence and the carbohydrate composition of a human macroglobulin with a covalent molecular weight of 950,000 (*1*). The protein sequenced (Ou) is from a patient with macroglobulinemia and serves as a model for structural study of IgM antibodies just as Bence Jones proteins have been models for light chains and myeloma globulins have been models for IgG antibodies (*2*). Protein Ou is comprised of a pair of κ light chains disulfide-bonded to a pair of μ heavy chains to give a monomeric subunit of 190,000 molecular weight. Five such subunits are joined through an intersubunit bridge on each μ chain to form a pentamer (*3*). Because of this structural symmetry, the primary structure of the entire polymer could be established by amino acid sequence determination of the κ light chain and of the μ heavy chain and by identification of all of the disulfide bridges in each chain. We have reported the animo acid sequence of the κ light chain (*4*) and of the Fabμ fragment, which comprises the light chain and the Fd′ portion of the μ chain (*5*); we have also given extensive sequence data on parts of the remainder of the μ chain (*6*) and on the location and composition of the five oligosaccharides C1 to C5 (*7*). We now report the sequence of the 576 residues in this μ chain and the location of all disulfide bridges and oligosaccharides. This is the longest continuous sequence for a single polypeptide chain yet recorded.

Structural study of IgM is facilitated by limited cleavage of the polymer by incubation with trypsin for 4 hours at 60°C (*8*). This yields two fragments, Fabμ and Fcμ, and also some peptides excised from the portion of the μ chain between Fabμ and Fcμ (Fig. 1). Fabμ consists of the NH_2-terminal portion of the μ heavy chain (the first 213

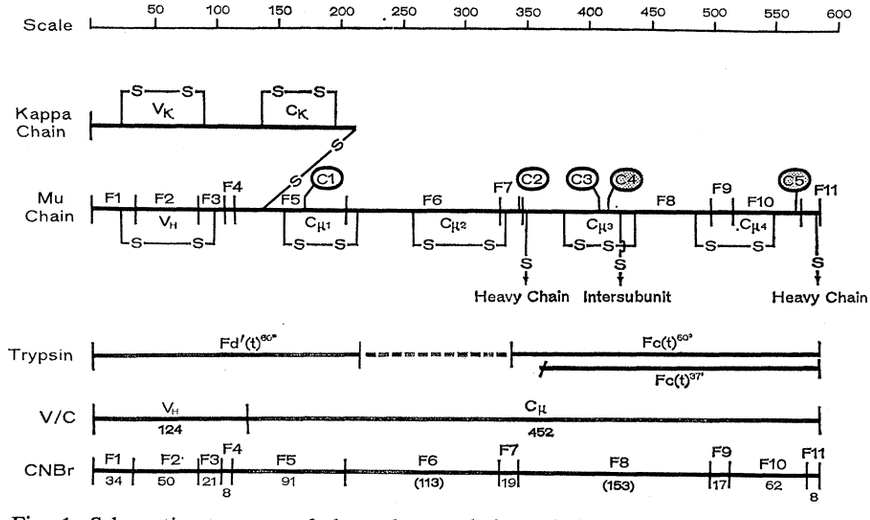

Fig. 1. Schematic structure of the μ heavy chain and the κ light chain of IgM Ou showing (i) the interchain and intrachain disulfide bridges, (ii) the two homology regions of the light chain ($V\kappa$ and $C\kappa$), and (iii) the five homology regions of the heavy chain (V_H and $C\mu 1$ to $C\mu 4$). The figure for the μ chain also shows the location of the five oligosaccharides (C1 to C5), the points of cleavage by trypsin with the respective fragments (Fd′ and Fc), the sites of cleavage by CNBr and the respective fragments (F1 to F11), and the point of division between the variable and constant regions (V_H and $C\mu$). The scale and the numbers indicate the number of amino acid residues in each chain and fragment.