1B. The uncertainties in these ratios are substantial, perhaps around 25 percent. The plasma characteristics implied by our [OII] measurements are thus entirely consistent with those derived from [SII] observations. We are not yet prepared to report an absolute brightness for the [OII] emission, but estimate that it is comparable in strength to the [SII] 6716 and 6731 Å lines, probably less than 100 rayleighs.

The source of the oxygen around Jupiter is at present ambiguous. Although Io is clearly the source of much of the heavy ion plasma in the inner Jovian magnetosphere (*5*), a potential nearby source of large amounts of oxygen is water-covered Europa (*12*). Lanzerotti *et al.* (*13*) have discussed the sputtering of water by energetic charged particles from Europa and the outer Galilean satellites, and Pilcher (*14*) has discussed the mechanisms by which water might be removed from the surface of Io. Wu *et al.* (*15*) have noted that ultraviolet emission detected around Europa from the Pioneer 10 spacecraft may indicate the presence of clouds of atomic oxygen and hydrogen. However, there are no grounds to rule out Io as an oxygen source as well. We are therefore left with an ambiguity that we hope to resolve by means of spacecraft data and further ground-based observations.

Note added in proof: The presence of OII around Jupiter was confirmed by the Voyager 1 plasma experiment (*16*), although far-ultraviolet emissions characteristic of this ion were not seen from the spacecraft (*17*). The apparent presence of large quantities of SO_2 on Io (*18*) indicate that Io is an important source of magnetospheric oxygen.

C. B. PILCHER
J. S. MORGAN

Institute for Astronomy,
University of Hawaii, Honolulu 96822

References and Notes

1. R. A. Brown, in *Exploration of the Planetary System, IAU Symposium No. 65*, A. Woszczyk and C. Iwaniszewska, Eds. (Reidel, Dordrecht, Netherlands, 1974), pp. 527–531; for additional references see C. B. Pilcher and W. V. Schempp, *Icarus* **38**, 1 (1979).
2. L. Trafton, *Nature (London)* **258**, 690 (1975).
3. I. Kupo, Y. Mekler, A. Eviatar, *Astrophys. J.* **205**, L51 (1976).
4. D. L. Judge, R. W. Carlson, F.-M. Wu, V. G. Hartmann, in *Jupiter*, T. Gehrels. Ed. (Univ. of Arizona Press, Tucson, 1976), pp. 1068–1101.
5. The concentration of neutral sodium and potassium around Io demonstrates clearly that Io is the source of these elements; see D. L. Matson, B. A. Goldberg, T. V. Johnson, R. W. Carlson, *Science* **199**, 531 (1978); F. J. Murcray and R. Goody, *Astrophys. J.* **226**, 327 (1978); and Judge *et al.* in (*4*). The hypothesis of an Io-based sulfur source is given strong support by the Voyager imaging data [B. A. Smith *et al.*, *Science* **204**, 951 (1979)].
6. By overlapping spectral orders we were able to obtain simultaneous measurements of these lines and two pairs of forbidden transitions of singly ionized sulfur, those at wavelengths of 6716 and 6731 Å and 4068 and 4076 Å. The latter had not been previously detected around Jupiter. We will report these observations in a later publication.
7. The translational Earth-Jupiter Doppler shift at these wavelengths at the time of the 15 February 1979 (UT) observations was only + 0.14 Å. In comparison, the corotational Doppler shift at 6 R_J is 0.94 Å.
8. C. B. Pilcher, *Bull. Am. Astron. Soc.* **10**, 579 (1978); J. S. Morgan and C. B. Pilcher, *ibid.*, p. 579.
9. R. A. Brown, *Astrophys. J.* **206**, L179 (1976).
10. ———, *ibid.* **224**, L97 (1978).
11. D. E. Osterbrock, *Astrophysics of Gaseous Nebulae* (Freeman, San Francisco, 1974), p. 112. The labels in the figure on this page have been reversed; they are correct in the caption.
12. C. B. Pilcher, S. T. Ridgway, T. B. McCord, *Science* **178**, 1087 (1972); U. Fink, N. H. Dekkers, H. P. Larson, *Astrophys. J.* **179**, L155 (1973)
13. L. J. Lanzerotti, W. L. Brown, J. M. Poate, W. M. Augustyniak, *Geophys. Res. Lett.* **5**, 155 (1978).
14. C. B. Pilcher, *Icarus* **37**, 559 (1979).
15. F.-M. Wu, D. L. Judge, R. W. Carlson, *Astrophys. J.* **225**, 325 (1978).
16. H. S. Bridge *et al.*, *Science* **204**, 987 (1979).
17. A. L. Broadfoot *et al.*, *ibid.*, p. 979.
18. R. Hanel *et al.*, paper presented at American Geophysical Union meeting, Washington, D.C. (1979); F. P. Fanale *et al.*, *Nature (London)*, in press; B. Hapke, in preparation; D. B. Nash and R. M. Nelson, in preparation; W. D. Smythe, in preparation.
19. This work was supported in part by NASA grant NGL 12-001-057.

10 April 1979

Host Defense Against *Neisseria meningitidis* Requires a Complement-Dependent Bactericidal Activity

Abstract. *Some individuals, with severe or recurrent infection with* Neisseria *species, have been identified as lacking a component in the terminal attack sequence of complement (complement components 5 to 9). The relevance of the terminal attack sequence to various phases of host defense was tested with the use of the C-11 strain of meningococci and human serum genetically deficient in complement component 8 (C8-D). The C8-D serum was comparable to normal serum in supporting ingestion and intracellular killing by leukocytes but was not bactericidal in the fluid phase unless reconstituted with C8. Thus, serum complement-dependent bactericidal activity may be especially critical for the host's defense against invasive* Neisseria *species.*

The classic in vitro and epidemiological studies of Goldschneider *et al.* (*1*) established that antibody was essential for immunity to *Neisseria meningitidis*. Although a complement-dependent bactericidal reaction was used to conveniently measure antibody to meningococci, a requisite role for the complement system in the host defense against *Neisseria* was never established. Recently, unusually severe, chronic, or recurrent infections with *Neisseria* species have been recorded in association with deficiencies in components of the terminal complement sequence, notably C5 (*2*), C6 (*3*), C7 (*4*), and C8 (*5*). These human deficiencies of components C5 to C8 provided the first evidence that the terminal attack sequence of complement might be critical for the host's defense against pathogenic *Neisseria*. Our studies were designed to determine the mechanism by which the terminal complement sequence mediates activity against *Neisseria*: phagocytic ingestion, intracellular killing, or fluid phase bactericidal activity.

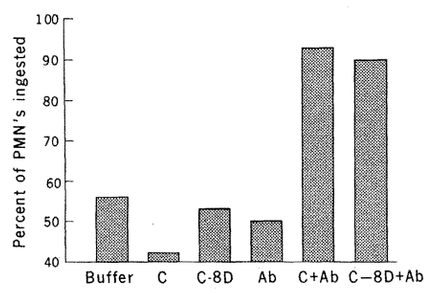

Fig. 1. Effect of opsonizing meningococci with specific antibody (Ab) and either normal (C) or C8-deficient serum (C8-D) as a complement source. The percentage of polymorphonuclear leukocytes (PMN's) having ingested one or more bacteria is plotted on the ordinate.

Group C (C-11) meningococci grown to log phase in Müller-Hinton broth, human peripheral blood leukocytes isolated from dextran-sedimented blood, the immunoglobulin G fraction of human antiserum to group C, and both normal and C8-deficient (C8-D) human serum were utilized in these studies. The normal serum was selected to be without detectable bactericidal antibody but to have an intact complement system. In order to evaluate the capacity of C8-D serum to support ingestion, meningococci in the log phase of growth (5×10^7 cells) were opsonized in a 0.2-ml reaction mixture containing normal or C8-D serum diluted 1:5 in RPMI 1640 (Microbiological Associates) supplemented with 0.4 percent bovine serum albumin and 5 m*M* $MgCl_2$, with or without the addition of 78 μg of the antibody fraction. After incubation at 37°C for 30 minutes, 2.5×10^7 leukocytes were added to each reaction mixture. The tubes were further incubated in an atmosphere of 5 percent CO_2 and 95 percent air for 60 minutes on a gyro-

0036-8075/79/0720-0298$00.50/0

shaker. The cells were collected by centrifugation, washed, and smeared on slides, which were then stained with 1 percent methylene blue and randomly coded. One hundred polymorphonuclear leukocytes (PMN's) from duplicate reaction mixtures were counted under code, and the number of PMN's that had ingested one or more bacteria was determined. In terms of supporting opsonization, there was no significant difference between the C8-D and normal serum (Fig. 1). Although C3b is the critical complement ligand for promoting phagocytic ingestion, it was not known whether the intramembranous insertion of the terminal complement attack sequence into the membrane of *Neisseria* might facilitate ingestion. Our results do not suggest this.

Next, the necessity of the terminal attack sequence for intraphagocyte killing was examined. Reaction mixtures of 4.0 ml containing diluted (1:4) normal or C8-D serum, 0.4 mg of antibody fraction, and 4×10^6 bacteria were incubated for 30 minutes at 37°C on a gyroshaker in an atmosphere of 5 percent CO_2 and 95 percent air. After samples were removed for assay of viable bacteria at the end of the opsonization phase, leukocytes (2.5×10^7) were added to the reaction mixtures and the incubation continued. After 60 minutes, the reaction mixtures were centrifuged, the supernatants sampled for viable bacteria, and the pelleted cells cultured for quantitation of bacteria adherent to the leukocytes. The remaining cells were lysed with water and then cultured to quantitate viable intracellular bacteria. A control reaction tube containing only buffer, heated serum, and bacteria showed that the number of viable bacteria remained constant over the course of the experiment. All the bacteria that interacted with normal serum were killed before the leuckocytes were added. In the case of C8-D serum, there was no killing during opsonization, but 60 minutes after the additon of leukocytes 1.6 $\log_{10}$ bacteria were killed. Less than 1 percent of surviving bacteria were within or adherent to the leukocytes. Although a direct comparison of intracellular killing of C8-D- and normal serum-opsonized meningococci cannot be made since normal serum is bactericidal in the absence of phagocytes, phagocytic killing of 1.6 $\log_{10}$ is of a similar magnitude reported for gonococci (*6*) and pneumococci (*7*) in comparable assays with normal serum. Our data support the conclusion that the terminal complement sequence is not necessary for the intracellular killing of meningococci.

The final experiment was done to establish whether killing of the meningococcus in the absence of phagocytes depends on an intact terminal complement sequence. From 0 to 25 units of functionally purified C8 (Cordis) were added to 0.2-ml reaction mixtures containing diluted (1:8) C8-D serum, 4 μg of antibody fraction, and 2×10^3 bacteria. After a 30-minute incubation period at 37°C, the mixtures were diluted and portions were plated for colony counting. Bactericidal activity did not occur in C8-D serum (Fig. 2), but could be restored in a dose-related manner by the addition of exogenous C8.

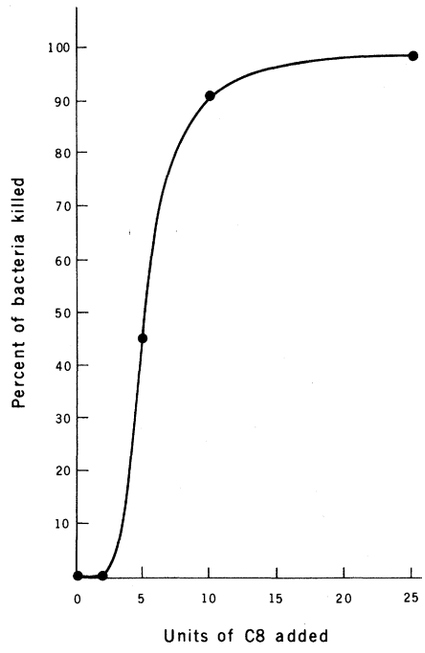

Fig. 2. Reconstitution of C8-deficient serum in the bactericidal assay by the addition of functionally purified C8. Percentage of the initial bacterial inoculum killed is plotted on the ordinate; the units of C8 added are plotted on the abscissa.

Many (*2–5*), though not all (*8*), patients with deficiencies in terminal complement have shown a susceptibility to *Neisseria* infections, suggesting that the terminal complement components may have a role in the host's defense against these pathogens. Previous studies showed that opsonization by C8-D serum allowed for normal ingestion of yeast and normal intracellular killing of *Staphylococcus aureus* (*5*). However, a unique capacity of the terminal complement components is the self-assembly of a macromolecular complex that can insert itself into susceptible lipid membrane (*9*). Since neither yeast nor *S. aureus* have such lipid membrane exposed, it was critical to study the ingestion and killing of an agent with an exposed susceptible membrane. Although most Gram-negative bacteria would fit this criterion, a *Neisseria* species was studied because of the unique association of these pathogens with deficiencies in terminal complement. It seemed possible that a lesion in the bacteria's lipid membrane might affect phagocytic ingestion, perhaps by allowing more C3b fixation or by changing the net charge of the membrane; but ingestion of meningococci was normal in C8-D serum. Similarly, the terminal complement lesion might affect the intracellular fate of the ingested bacteria. Other workers have found that complement can enhance the intracellular killing of *S. aureus* (*10*), although the role of the terminal complement components in this process could not be assessed. Our data show that the effect of complement on the intracellular fate of *Neisseria* does not depend on the terminal complement sequence, and, therefore, the membrane lesion must not be critical for killing inside the phagocyte. With the reservation inherent in extrapolating from a laboratory strain of meningococci to the wild-type strains, our data suggest that the only role of the terminal complement components in the host's defense against meningococci is to provide for extraphagocytic bactericidal activity.

ANNE NICHOLSON
Department of Medicine,
Harvard Medical School,
Boston, Massachusetts 02115
IRWIN H. LEPOW*
Department of Medicine,
University of Connecticut,
Farmington 06032

References and Notes

1. I. Goldschneider, E. C. Gotschlich, M. S. Artenstein, *J. Exp. Med.* **129**, 1307 and 1327 (1969).
2. R. Synderman, M. C. Pike, V. Szatolowicz, L. M. Meadows, *Clin. Res.* **25**, 368A (1977).
3. D. Lim, A. Gewurz, T. F. Lint, M. Ghaze, B. Sepheri, H. Gewurz, *J. Pediatr.* **89**, 42 (1976); J. P. Leddy, M. Frank, T. Gaither, M. R. Klemperer, *J. Clin. Invest.* **53**, 544 (1974).
4. T. J. Lee, P. D. Utsinger, R. Synderman, W. J. Yount, P. F. Sparling, *J. Infect. Dis.* **138**, 359 (1978).
5. B. H. Petersen, J. A. Graham, G. F. Brooks, *J. Clin. Invest.* **57**, 283 (1976).
6. P. J. Watt, *J. Med. Microbiol.* **3**, 501 (1970).
7. R. B. Johnson, Jr., M. R. Klemperer, C. A. Alper, F. S. Rosen, *J. Exp. Med.* **129**, 1275 (1969).
8. S. I. Rosenfeld, M. E. Kelly, J. P. Leddy, *J. Clin. Invest.* **57**, 1626 (1976); J. T. Boyer, E. P. Gall, M. E. Norman, U. R. Nilsson, T. S. Zimmerman, *ibid.* **56**, 905 (1975); B. Wellek and W. Opferkuch, *Clin. Exp. Immunol.* **19**, 223 (1975); J. M. Delâge, P. Bergeron, J. Simard, G. Lehner-Netsch, E. Prochazka, *J. Clin. Invest.* **60**, 1061 (1977); T. F. Lint, H. J. Zeitz, D. Scott, J. R. Malkinson, H. Gewurz, *Clin. Res.* **26**, 714A (1978).
9. C. H. Hammer, A. Nicholson, M. M. Mayer *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5076 (1975); C. H. Hammer, M. L. Shin, A. S. Abramovitz, M. M. Mayer, *J. Immunol.* **119**, 1 (1977).
10. I. W. Li and S. Mudd, *J. Immunol.* **94**, 452 (1965).
11. We thank F. S. Rosen and C. A. Alper for C8-D serum, other supplies, and facilities that were provided by a grant from NIH (AIO5877) to F. S. Rosen.
* Present address, Sterling-Winthrop Research Institute, Rensselaer, N.Y. 12144.

7 March 1979