during the CF tones. The two neurons strongly responded to simultaneous delivery of two sounds corresponding to the second and third harmonics in the CF component of the orientation sound and Doppler-shifted echoes, but poorly responded to each of them alone. One of them did not respond to noise bursts with broad bands. These two types of neurons, which responded better or selectively to a certain combination of two information-bearing elements in biologically significant sounds, have, to the best of my knowledge, not yet been reported in any other animals (*18*).

It is probably a common feature that amplitude spectra of acoustic signals, which would change with time, are represented by spatiotemporal patterns of activities of cortical auditory neurons, but it should be noted that the auditory cortices contain the neurons that are specialized for responding to a particular acoustic signal frequently used by the animal.

NOBUO SUGA

Department of Biology, Washington University, St. Louis, Missouri 63130

References and Notes

1. The frequency of the CF component can be slightly different among *Pteronotus parnellii rubiginosus* living in different parts of Central America [H.-U. Schnitzler, *Z. Vgl. Physiol.* **68**, 25 (1970); Grinnell (*2*)].
2. A. D. Grinnell, *ibid.*, p. 117.
3. N. Suga, J. A. Simmons, T. Shimozawa, *J. Exp. Biol.* **61**, 379 (1974). See also the studies on the lateral lemniscal evoked potential by Grinnell (*2*).
4. N. Suga and P. H.-S. Jen, *Science* **194**, 542 (1976).
5. G. Pollak, O. W. Henson, Jr., A. Novick, *ibid.* **176**, 66 (1972).
6. N. Suga, J. A. Simmons, P. H.-S. Jen, *J. Exp. Biol.* **63**, 161 (1975); N. Suga and P. H.-S. Jen, *ibid.*, in press.
7. A. Gordon and N. Suga, unpublished results.
8. A. R. Tunturi, *Am. J. Physiol.* **168**, 712 (1952).
9. N. Suga, *J. Physiol. (London)* **198**, 51 (1968); *ibid.* **200**, 555 (1969).
10. ———, in *Basic Mechanisms in Hearing*, A. R. Møller, Ed. (Academic Press, New York, 1973), pp. 675–742.
11. Unlike auditory nerve fibers, a majority of cortical auditory neurons show nonmonotonic impulse-count functions. In extremely nonmonotonic functions, excitatory responses disappear when a stimulus amplitude increases beyond a certain level or upper threshold. The upper threshold is due to lateral inhibition (*10, 12*). In some neurons an upper threshold appeared at all frequencies of excitatory sounds, so that their excitatory areas (that is, the area above an excitatory threshold curve) were closed at the top. It should be noted that upper-threshold neurons have specific amplitude selectivity. They are therefore different from auditory nerve fibers which show impulse-count functions that fit the Weber-Fechner function or power function.
12. N. Suga, *J. Physiol. (London)* **181**, 671 (1965).
13. D. H. Hubel and T. N. Wiesel, *ibid.* **160**, 106 (1962); V. B. Mountcastle, *J. Neurophysiol.* **20**, 408 (1957).
14. E. G. Jones histologically examined the auditory cortex of the mustache bat. The auditory cortex is 0.7 to 0.9 mm thick at the Doppler-shifted-CF processing area.
15. Oblique penetrations to the cortical surface gave rise to data that further confirmed the tonotopic and amplitopic representation in the Doppler-shifted-CF processing area (N. Suga and T. Manabe, unpublished results).
16. A. D. Grinnell, *J. Physiol. (London)* **167**, 38 (1963).
17. Specialization of auditory neurons of *Myotis lucifugus* has been studied in terms of responses to the three basic types of acoustic signals used by various types of animals: CF tones, FM sounds, and noise bursts. In these studies CF-, FM-, and noise-specialized neurons have been found, each of which selectively responds to one of the above three types of signals. Such specificity of the neurons to the signals does not change with stimulus level (it is level-tolerant) and the lack of response of these neurons to the other two types of signals is due to inhibition—that is, the "rejection mode" operating in the nervous system (*10*). These two things are the minimum requirements for neurons to be called specialized neurons or feature detectors. The term specialized neurons implies that the neurons express the output of a neural circuit operating as a filter.
18. Neurons that selectively responded to a particular type of communication sound have been found in primates [Z. Wollberg and J. D. Newman, *Science* **175**, 212 (1972)]. However, it has not yet been clarified whether these neurons responded only to a particular combination or combinations of components in their communication sounds.
19. Supported by NSF grant BMS75-17077. I thank R. W. Coles and E. G. Jones for comments and J. Jaeger for assistance in our auditory laboratory. I also thank A. Gordon for his assistance during some of the experiments.

13 September 1976; revised 12 November 1976

Babesia rodhaini: Requirement of Complement for Penetration of Human Erythrocytes

Abstract. *A system has been developed in vitro in which human red cells, in the presence of fresh human (or rat) serum, are parasitized by the hemosporidian protozoan* Babesia rodhaini. *The ability of* B. rodhaini *to penetrate red cells depends on factors of the alternative complement pathway (properdin and factor B) as well as ionic magnesium and the third (C3) and the fifth (C5) components of complement. These data indicate a novel mechanism by which a parasite is able to utilize the complement system. The data are in accord with and further amplify earlier observations that demonstrated a requirement for complement in the development of babesial infection in rats.*

Species of *Babesia* parasitize a wide variety of mammals (*1*). Scattered cases of human infection have been reported (*2*), and the most recent outbreak involved seven patients on Nantucket Island (*3, 4*). Clinically, the disease is similar to malaria; patients usually experience headache, chills, fever, and myalgia (*4, 5*). Like other protozoa such as plasmodia, *B. rodhaini* causes numerous pathophysiologic alterations in the host, including a transient glomerulonephritis with glomerular deposits of immunoglobulin G (IgG) and the third component of complement (C3), hypocomplementemia, proteinuria, hepatosplenomegaly, severe anemia, and thrombocytopenia (*6, 7*). Babesiosis differs from human malaria in that the organism causing the former shows no evidence of sexual reproduction (*8*), and primary infection induces complement depression which correlates linearly with the degree of parasitemia. In humans with relapsing malaria (caused by *Plasmodium vivax*), the cyclic paroxysmal release of parasites is associated with a transient drop in complement levels; however, primary infection only occasionally shows this pattern (*7, 9*).

Previous studies have demonstrated that rats maintained on a magnesium-deficient diet were afforded some degree of protection against *B. rodhaini* (*10*). Furthermore, rats were less susceptible to red cell parasitization after treatment with cobra venom factor, an agent known to cause inactivation of the complement system. Sodium flufenemate, which irreversibly inactivates C3, also blocked development of the infection (*11*). Finally, mice deficient in both C3 and the fifth (C5) component of complement failed to develop babesial infections (*11*). The evidence suggested that an intact complement system was essential for development of babesial parasitemia. Using a newly developed system of babesial infection in vitro, we investigated the role of alternative pathway and terminal complement components in the development of the infection. As will be demonstrated, penetration of human erythrocytes by the parasite requires the presence in serum of properdin, factor B, C3, C5, and magnesium ions.

Complete removal of factor B of the alternative (properdin) complement pathway from 10 ml of fresh normal human serum was accomplished by affinity column chromatography on 3 g of Sepharose 4B (with activated CNBr; Pharmacia Fine Chemicals, Uppsala) covalently linked to 8.5 mg of antibody to human factor B. The factor B antibodies were separated from 10 ml of whole rabbit serum containing specific antibody by affinity column chromatography on 3 g of CNBr-activated Sepharose 4B containing 4 mg of purified factor B. Antiserums had been prepared by a general immunization regimen described elsewhere (*12*); the factor B antigen (C3 proactivator) was purified as described previously (*13*). To prevent inadvertent immune ac-

tivation of the classical complement system, as well as nonspecific protein adsorption to the column matrix, appropriate volumes of saturated NaCl (room temperature) and 0.5*M* ethylenediaminetetraacetate (EDTA), *p*H 7.6, were added to ice-cold human serum to attain final concentrations of 0.3*M* NaCl and 0.01*M* EDTA. After equilibration for 1 hour at cold-room temperature, the serum was applied to the column containing the antibody to factor B, and was eluted with Veronal buffer containing NaCl (VBS) to a final ionic strength 0.3 and 0.01*M* EDTA. The serum eluate (RB) was pooled, dialyzed twice against a 200-volume excess of VBS at 4°C, centrifuged at 20,000 rev/min for 30 minutes at 4°C, and stored frozen at −70°C in small portions. No factor B was detected in the RB preparation by the Laurell rocket technique which was conducted by appropriate modification (antibody concentration and field polarity) of the method described elsewhere (*14*) and by immunoelectrophoresis (*15*). As expected, zymosan (5 mg/ml, 37°C, 45 minutes) activation of the alternative pathway occurred only in factor B–reconstituted RB. Classical complement components were unaffected by this procedure because essentially similar CH_{50} values (where CH_{50} is the amount of complement that lyses 50 percent of a standardized number of sensitized red cells) were obtained in RB and in a sample of normal human serum treated similarly except for the omission of the affinity column step (pseudo RB). The RB and "pseudo RB" contained similar concentrations of properdin as assessed by the Laurell technique (*14*).

Subsequent to saturation with human serum of the available insolubilized antibody to factor B, the affinity column was washed thoroughly with buffer until the optical density (at 280 nm) stabilized at 0.020. Phosphate buffer (*p*H 7.4, ionic strength 0.15), containing 3.5*M* KSCN effectively released factor B from the column (*13*). The protein was dialyzed four times against a 100-volume excess of VBS, centrifuged at 15,000 rev/min for 30 minutes at 2° to 4°C and stored at −70°C. Approximately 4 mg of factor B were obtained from 25 ml of starting serum. The resulting affinity-purified factor B migrated with normal β mobility on immunoelectrophoresis and it reconstituted RB with respect to zymosan activation of the alternative pathway as assessed by immunoelectrophoretic cleavage of C3. Specifically, one part of factor B purified by ionic exchange column chromatography (*13*) was added to three parts of RB to obtain a final concentration of 243 μg/ml, and one part of affinity-purified factor B was added to two parts of RB to obtain a final concentration of 134 μg/ml. The magnesium ion concentration was $7.5 \times 10^{-4}M$. Incubation with zymosan (5 mg/ml) at 37°C for 45 minutes resulted in cleavage of C3. The substitution of buffer for factor B resulted in the conversion of trace amounts of C3.

Properdin and properdin-deficient serum (RP) were prepared as previously described (*16*). The RP contained < 8 percent of normal properdin levels as determined by solid phase radioimmunoassay. In each experiment the factor to be reconstituted (C3, factor B, or properdin) was added to approximate physiologic concentrations in serum. Serums treated with KSCN or KBr were prepared as described earlier (*17*). The KSCN serum (deficient in C3, C4, and C5) showed no measurable hemolytic activity (CH_{50}), while the KBr serum (relatively deficient in C3 and C4) showed 10 percent of the hemolytic activity of control normal human serum. Serum was rendered C4 deficient by treatment with 0.003*M* hydrazine at 37°C for 60 minutes followed by exhaustive dialysis in the cold. Cobra venom factor (CoF) was purified from crude venom (*Naja naja*) in a one-step procedure involving ion exchange chromatography. The active fraction was concentrated such that one unit of CoF contained 0.0004 μg of protein (*18*).

To functionally inactivate the alternative pathway, normal human serum was incubated (37°C, 5 minutes) with CoF (40 μg/ml) or zymosan (3 mg/ml) prior to testing under assay conditions. Zymosan was removed by centrifugation at 4°C for 30 minutes at 4000 rev/min. Control serum was handled identically, but without addition of CoF or zymosan. Where indicated, ethylene glycol bis(aminoethyl ether)-*N*, *N'*-tetraacetate (EGTA) or EDTA was added to human serum as previously described (*19*). Serum from a patient with partial lipodystrophy (PLD), an acquired C3 deficiency (*20*), was also used. This serum was devoid of whole hemolytic activity (CH_{50}), and by immunochemical analysis contained < 100 μg of C3 per milliliter. Mouse serum deficient in C5 was obtained from the

Table 1. Results of various serum treatments on parasitization. Each incubation mixture contained human red cells and parasites. To these were added 100 μl of fresh human serum, treated serum, or purified complement components. Three determinations (A, B, and C) were made in each experiment.

Incubation mixture	Degree of parasitemia (parasites per 1000 red cells)*		
	A	B	C
No serum	0	0	0
Fresh normal human serum (100 μl)	100	84	107
Treatment of serum:			
EGTA (0.008*M*)	80	74	
EDTA (0.01*M*)	0	0	0
Hydrazine (0.003*M*)	60	53	82
Zymosan (3 mg/ml)	0	0	0
C3 inactivator (cobra factor, 16 μl)	0	0	0
KSCN (0.1*M*)	0	0	0
KBr (0.1*M*)	30	24	10
Heated serum			
56°C, 30 minutes	0	0	0
Restored with C3 (1.2 mg/ml)	20	15	7
Restored with C3 and C3 proactivator (factor B, 200 μg/ml)	100	110	70
C3 only	0	0	0
C3 and C3 proactivator	0	0	0
Specifically deficient serums			
C3 deficient (PLD)	0	0	0
C3 deficient, restored with C3 (1.2 mg/ml)	80	87	
Properdin deficient (RP)	0	0	
Properdin deficient, restored with properdin (25 μg/ml)	93	80	
Factor B–deficient serums			
Pseudo RB	97	90	
Factor B deficient (RB)	0	3	
Factor B deficient, restored with factor B (200 μg/ml)	78	70	
C5-deficient mouse serum ($B_{10}D_2$ "old line")	23	20	
C5-sufficient mouse serum ($B_{10}D_2$ "new line")	72	68	

*After 6 hours of incubation.

$B_{10}D_2$ "old line" strain. Fresh heparinized (10 unit/ml) human red cells were obtained from healthy O+ donors and washed twice in Veronal buffer (VB). Minimal essential medium was prepared as described (*21*) except that it was diluted tenfold with VB, and stearic acid was omitted. Infected red cells in blood were collected by cardiac puncture from rats harboring parasitemias ranging between 40 and 60 percent. The red cells were washed twice and resuspended to 2×10^{10} cell/ml in VB. The cells were sonicated in a 40-ml glass tube by the Branson Sonicator at 70 watts for approximately 40 seconds to obtain between 75 and 80 percent hemolysis as determined spectrophotometrically. Released parasites were then separated as previously described (*22*) and resuspended in 1.5 ml of VB. To each well of a 16-mm flat-bottom microtiter tissue culture plate was added 50 μl of fresh normal human red cells (approximately 2×10^8), 50 μl of minimal essential medium, 100 μl of test serum, and 50 μl of the parasite suspension (containing approximately 2×10^8 organisms). The mixtures (which were prepared in duplicate) were allowed to incubate with gentle rocking for 6 hours at 30°C in a 2 percent CO_2 atmosphere. At the end of the incubation period slides of each well were prepared in duplicate and coded, stained with Giemsa reagent, and counted for incidence of parasitemia (red cells containing parasites).

The results are presented in Table 1. Magnesium ions were required in serum for red cell parasitization. Chelation of Ca^{2+} and Mg^{2+} by EDTA completely blocked invasion of red cells, but EGTA, which preferentially chelated Ca^{2+} and not Mg^{2+}, permitted parasitization. Parasitization was impaired in C3-depleted serum (PLD, KSCN, or KBr serum). The PLD serum fortified with purified C3 (1.2 mg/ml) permitted parasitization near control levels. The KBr serum (with measurable but low hemolytic activity) permitted low levels of parasitization, while KSCN-treated serum (with no measurable hemolytic activity) failed completely to sustain penetration of red cells by parasites. No attempt was made to reconstitute the inactivated complement components in these two serums. Hydrazine-treated serum showed diminished activity presumably because of partial inactivation of hydrazine-sensitive C3. Serum that had been heat treated (56°C, 30 minutes) to deplete factor B did not permit parasitization; however, upon reconstitution with factor B and C3 (but not with C3 alone), parasitization of red cells was restored. Properdin-deficient and factor B–deficient serum (affinity chromatography) permitted parasitization of red cells only upon restoration of the missing components. Mouse serum deficient in C5 supported minimal invasion compared to C5-sufficient normal mouse serum.

From the data it can be concluded that ionic magnesium, properdin, factor B, C3, and, to a lesser extent, C5 are required for optimal parasitization of human red cells by the babesial parasite. By substituting rat red cells and serum in the assay system in vitro, similar results to the human system have been obtained. Rat serums treated with KBr, KSCN, heat (56°C, 30 minutes), zymosan, EDTA, and EGTA were also studied. The results were comparable with those obtained when similarly treated human serum and red cells were employed.

The findings are consistent with earlier experiments in vivo which indicated that magnesium deficiency and treatment with CoF conferred protection against babesial infection (*10, 11*). The mechanism of entry by *B. rodhaini* into the red cell remains unclear, although it apparently differs from that observed for the parasitization of macrophages by *Trypanosoma cruzi* (*23*) or red cells by plasmodia (*21*). Analogous to the Duffy receptor in malaria, a membrane component, perhaps the C3b receptor of the red cells, might be utilized by babesia to facilitate penetration into the red cell. It could be postulated that the babesial organism is able to activate the alternative complement pathway resulting in the uptake onto the red cell of C3b and additional complement components. One possibility is that this permits adherence of the babesial organism to the altered red cell through interaction of a C3b receptor on the surface of the babesial parasite; alternatively, the red cell membrane could be modified by interaction with later complement components (C5 to C9) such that the red cell is more susceptible to penetration by the parasite.

It is also possible that a babesial antigen is absorbed onto the surface of the red cell, and that this leads to interaction with antibody, fixation of complement, and alteration of the red cell membrane which permits penetration by the parasite. That some method of fixation of C3 to the red cell membrane is important is suggested by the observation (*24*) that sheep cells conjugated with C3 (EAC143), but not E, EA, EAC1, or EAC14, will permit penetration without exogenous complement under the incubation conditions described herein. It has already been shown by other investigators (*25*) that serum from rats acutely infected with *B. rodhaini* contains an antigen that fixes to the red cell membrane, causing anemia as well as reducing the time required for onset of parasitemia and increasing the severity of the parasitemia. Unfortunately, serum complement levels were not measured in that study (*25*), but it is possible that the antigen is a product of the parasite and that it plays a role in the fixation of complement to the red cell membrane.

Morphologically, the merozoites of *Babesia rodhaini*, *Plasmodium berghei yoelii*, and *P. gallinaceum* are similar (*8, 26*). They bud similarly, have comparable pellicles and, presumably, attach to red cells with similar orientation. It is possible that only limited consumption of the alternative pathway proteins occurs during the attachment, orientation, and interiorization phases of parasitization. This is suggested in babesiosis because there is only slight depletion of alternative pathway activity (*9*) and the principle mechanism of the hypocomplementemia is depletion of the classical complement components C1 to C9 by immune complexes. Further work is needed to define the mechanism of attachment and interiorization. However, the demonstration of the requirement of complement (C3, C5, and the proteins of the alternative pathway) for successful parasitization of human red cells represents a new and subtle utilization of the body's humoral "defenses" by the babesial parasite.

WILLIAM E. CHAPMAN
PETER A. WARD
Department of Pathology, University of Connecticut Health Center, Farmington 06032

References and Notes

1. E. V. L. Soulsby, *Helminths, Arthropods, and Protozoa of Domesticated Animals* (Williams & Wilkins, Baltimore, ed. 6, 1968).
2. K. A. Western, B. D. Benson, N. N. Gleason *et al.*, *N. Engl. J. Med.* **283**, 854 (1970); J. E. P. Fitzpatrick, C. C. Kennedy, M. G. McGeown, D. G. Oreopolos, H. H. Robertson, M. A. O. Soyannwo, *Nature (London)* **217**, 861 (1968); Z. Skrabalod and Z. Deanovic, *Doc. Med. Geogr. Trop.* **9**, 11 (1957).
3. G. R. Healy, A. Speilman, N. Gleason, *Science* **192**, 479 (1976); A. E. Anderson, P. B. Cassaday, G. R. Healy, *Am. J. Clin. Pathol.* **62**, 612 (1974).
4. J. F. Desforges and F. Quimby, *N. Engl. J. Med.* **295**, 103 (1976).
5. P. J. D. Van Peenen and J. F. Duncan, *Bull. Wildl. Dis. Assoc.* **4**, 3 (1968).
6. C. R. Annable and P. A. Ward, *J. Immunol.* **112**, 1 (1974).
7. F. A. Neva, W. A. Howard, R. H. Glen, W. A. Krotoski, A. A. Gam, W. E. Collins, J. P. Atkinson, M. M. Frank, *J. Clin. Invest.* **54**, 451 (1974).
8. J. P. Krier, S. M. Gravely, T. M. Seed, R. Smucker, R. M. Pfister, *Ann. Trop. Med. Parasitol.* **26**, 9 (1975).
9. N. R. Cooper and R. J. Fogel, *Mil. Med.* **131**,

Immunol. **116**, 1284 (1976).
10. B. B. Norman, R. R. Johnson, E. W. Jones, *Fed. Proc.* **24**, 2544 (abstr.) (1970).
11. C. R. Annable, *ibid.* **31**, 2480 (abstr.) (1972); N. Aoki and K. T. VonKaulla, *Proc. Soc. Exp. Biol. Med.* **130**, 101 (1969).
12. J. O. Minta, I. Goodkofsky, I. H. Lepow, *Immunochemistry* **10**, 341 (1973).
13. O. Götze and H. J. Müller-Eberhard, *J. Exp. Med.* **134** (Suppl.), 90s (1971); J. Chapitis and I. H. Lepow, in preparation.
14. M. A. Schrager, *et al., Clin. Immmunol. Immunopathol.* **5**, 258 (1976).
258 (1976).
15. J. J. Scheidegger, *Int. Arch. Allergy Appl. Immunol.* **7**, 103 (1955).
16. L. Pillemer, L. Blum, I. H. Lepow, L. Wurz, E. W. Todd, *J. Exp. Med.* **103**, 1 (1956).
17. A. P. Dalmasso and H. J. Müller-Eberhard, *J. Immunol.* **97**, 680 (1966).
18. S. Ruddy, L. G. Hunsicker, K. F. Austen. *ibid.* **108**, 657 (1972).
19. T. A. E. Platts-Mills and K. Ishiaka, *ibid.* **113**, 348 (1974).
20. C. A. Alper, K. J. Bloch, F. S. Rosen, *N. Engl. J. Med.* **288**, 601 (1973).
21. L. H. Miller, S. J. Mason, J. A. Dvorak, M. H. McGinnis, I. K. Rothman, *Science* **189**, 562 (1975).
22. S. M. Gravely and J. P. Kreier, *Ann. Trop. Med. Parasitol.* **25**, 198 (1974).
23. N. Nogueira and Z. Cohn, *J. Exp. Med.* **143**, 1402 (1976).
24. R. Jack and P. A. Ward, unpublished observations.
25. K. H. Sibinovic, R. Milar, M. Ristic, H. W. Cox, *Ann. Trop. Med. Parasitol.* **63**, 327 (1969).
26. R. Ladda, M. Aikawa, H. Sprinz, *J. Parasitol.* **55**, 633 (1969).
27. Supported in part by grant AI 09651 from the National Institutes of Health. Serum from the patient with PLD was kindly supplied by R. McLean. The properdin content of RP was determined by J. Chapitis. Purified factor B and the antiserum to that factor were prepared in I. H. Lepow's laboratory.

23 August 1976; revised 15 November 1976

N-Nitrosodiethanolamine in Synthetic Cutting Fluids: A Part-Per-Hundred Impurity

Abstract. N-*nitrosodiethanolamine has been found to be present at a concentration of 0.02 to 3 percent in several brands of synthetic cutting fluids. Its identity was confirmed by three independent techniques: (i) by measuring the retention times on two different high-performance liquid-chromatography columns, (ii) by dehydration to* N-*nitrosomorpholine, and (iii) by preparation of the* O-*methyl ether derivative.*

Cutting fluids are widely used to reduce the temperature of the metal-tool interface during metal cutting and grinding. The majority of cutting fluids used in the United States are synthetic; they contain up to 45 percent triethanolamine and 18 percent sodium nitrite and have *p*H values in the range 9.0 to 11.0. Lijinsky *et al.* (*1*) demonstrated that triethanolamine could readily be nitrosated to form *N*-nitrosodiethanolamine (NDElA). Using a model system, Zingmark and Rappe (*2*) recently showed that triethanolamine would be expected to undergo in vivo nitrosation under simulated gastric conditions. *N*-Nitrosodiethanolamine is of interest because it has been shown to produce liver tumors in rats (*3*). We report here on its presence in relatively larger amounts as an impurity in commercial cutting fluids.

Cutting fluids were obtained commercially in the Boston area. Authentic NDElA was prepared as described by Druckrey *et al.* (*3*). The identity of the compound was confirmed by chemical ionization mass spectrometry and electron impact high-resolution mass spectrometry. A high-pressure liquid chromatograph–thermal energy analyzer (HPLC-TEA) was constructed from a high-pressure pump (Waters model 6000A), an injector (Waters model U6K), a column (Waters), and a TEA detector (Thermo Electron model 502) (*4*, *5*).

Cutting fluid was chromatographed as purchased without any treatment on a μ Porasil column (Waters), using as a solvent system 50 percent hexane and 50 percent acetone at a flow rate of 2 ml/min. A peak eluting at the retention time of NDElA was determined. Since no cleanup was used, the possibility of artifact formation was discounted. Because crude fluid rapidly degraded the column, routine analyses were carried out after first extracting with ethyl acetate in the presence of magnesium sulfate. The extract was filtered through sodium sulfate and then injected onto the HPLC-TEA (*6*). The results are presented in Table 1.

In order to confirm the identity of the peak eluting at the retention time of NDElA, the compound was isolated (*7*) and studied by three independent techniques.

1) High-pressure liquid chromatography. The isolated sample was injected onto an HPLC-ultraviolet spectrometer or HPLC-TEA by using either a μ Bondapak NH_2 or a μ Porasil column (*8*). With both systems we always observed a material eluting at the same retention time as authentic NDElA.

Table 1. Concentration of *N*-nitrosodiethanolamine in several brands of synthetic cutting fluids.

Brand	NDElA (%)
A	2.99
B	1.04
C	0.42
D	0.25
E	0.18
F	0.06
G	0.06
H	0.02

2) Dehydration. The isolated sample, when dehydrated with concentrated sulfuric acid (96 percent) at 155°C for 2.5 hours, yielded *N*-nitrosomorpholine as the dehydration product. *N*-Nitrosomorpholine was detected by combined gas chromatography and high-resolution mass spectroscopy (*9*). The isolated sample did not give an *N*-nitrosomorpholine peak before dehydration.

3) Derivative formation. The *O*-methyl ether derivative of the isolated sample was prepared by methylation with methyl iodide, with sodium hydride as a catalyst (*10*). The methylated sample was distilled and the distillate extracted with methylene chloride. The concentrated extract was then introduced into a gas chromatograph fitted with a Coulson electrolytic conductivity detector in the nitrogen mode (*11*). The presence of the NDElA derivative in the isolated sample was confirmed when the retention time was compared with that of the *O*-methyl ether derivative of authentic NDElA.

The cutting fluids that we tested represent only a small fraction of the total number of commercial brands that are available. On the basis of results reported here, we expect that most cutting fluids that contain triethanolamine or diethanolamine and nitrite as additives will be contaminated with NDElA. To assess the magnitude of the problem, we recommend screening all brands of synthetic cutting fluids.

The *N*-nitrosamine content of cutting fluids is about 40 times higher than the nitrosamine contamination in some herbicides (*12*) and 10^7 times higher than that found in foodstuffs preserved with nitrites (*13*). Persons who use cutting fluids could well be exposed to nitrosamine. Even though the fluids are diluted 10 to 100 times before use, the amount of the nitrosamine present is such that it may pose a carcinogenic hazard to all users.

In the past 30 years, cutting oils have been frequently cited as related to cancer of the scrotum among machine operators (*14*, *15*). Several cutting oils were found to be carcinogenic to laboratory animals (*15*, *16*), and compounds such as polycyclic aromatic hydrocarbons and heterocyclic compounds were believed to be the carcinogens (*17*). Over the past 20 to 30 years, synthetic formulations have replaced the original mineral oils. Almost all of the "cutting oils" used today are of the synthetic variety. The synthetic cutting fluids described here have been in