Table 2. Powder x-ray diffraction pattern for plasma-produced boron crystals of hexagonal platelet habit, with CuKα radiation; s=strong intensity; m=medium; w=weak; vw=very weak; vvw=very, very weak.

d(Å)	I	d(Å)	I
4.1990	s	1.8201	w
3.7600	w	1.6662	vw
3.3684	s	1.5398	w
2.5230	vw	1.3745	w
2.4626	vw	1.2526	vw
2.2899	vw	1.1959	vw
2.2382	vvw	1.1752	vvw
2.1247	vvw	0.8507	vvw
2.0229	m	0.8489	vvw
1.8639	vvw		

particles through the plasma, taking care not to contaminate the sample while handling. By this treatment the typical spheroidal particle became more spherical through a rounding of faces and an increased beveling of edges. However, no more of the distinctively shaped crystals formed. The powder pattern of the bulk product material contained no lines extraneous to the β-rhombohedral pattern after the reprocessing.

Whether the plasma is responsible for other effects in addition to supplying heat, for example, electromagnetic influences, we do not know. Certainly the thermal gradient within the plasma is immense, and the quench of particles falling from the plasma is severe because of their small heat capacity. Environments experienced by different particles falling through the same plasma may be quite varied. We have obtained all of the distinctive habits from product samples on occasion. More generally, one or two types were considerably more numerous than the others. We have discovered no underlying cause for the variation, but it seems to depend on the sample, repeated runs with any given sample being reproducible. With our instrument the particle size must be less than roughly 150 μm in diameter in order for decisive thermal effects to occur. Variation in instrument power, plasmoid volume, or other operating conditions might conceivably change this limitation.

DON B. SULLENGER
KENNETH D. PHIPPS
PYRTLE W. SEABAUGH
CLAUDE R. HUDGENS
Monsanto Research Corporation, Mound Laboratory, Miamisburg, Ohio
DONALD E. SANDS
Department of Chemistry, University of Kentucky, Lexington 40506
JOSEPH S. CANTRELL
Department of Chemistry, Miami University, Oxford, Ohio 45056

References and Notes

1. J. L. Hoard, R. E. Hughes, D. E. Sands, *J. Amer. Chem. Soc.* **80**, 4507 (1958); B. F. Decker and J. S. Kasper, *Acta Crystallogr.* **12**, 503 (1959).
2. C. P. Talley, S. La Placa, B. Post, *Acta Crystallogr.* **13**, 270 (1960).
3. R. E. Hughes, C. H. L. Kennard, D. B. Sullenger, H. A. Weakliem, D. E. Sands, J. L. Hoard, *J. Amer. Chem. Soc.* **85**, 361 (1963).
4. M. A. Rollier, *Proc. Int. Congr. Pure Appl. Chem. 11th London* **3**, 935 (1947) (published 1953); St. v. Náray-Szabó and C. W. Tobias, *J. Amer. Chem. Soc.* **77**, 241 (1955); L. Gorski, *Phys. Status Solidi* **3**, 316 (1963); R. H. Wentorf, Jr., *Science* **147**, 49 (1965).
5. B. Post, in *Boron, Metallo-Boron Compounds, and Boranes*, R. M. Adams, Ed. (Interscience, New York, 1964), p. 301, and references therein; A. E. Newkirk, *ibid.*, p. 233, and references therein.
6. J. L. Hoard and R. E. Hughes, in *The Chemistry of Boron and Its Compounds*, E. L. Muetterties, Ed. (Wiley, New York, 1967), p. 25, and references therein.
7. T. B. Reed, *J. Appl. Phys.* **32**, 821 (1961); *ibid.* **34**, 2266 (1963).
8. H. J. Hedger and A. R. Hall, *Powder Met. No. 8*, 65 (1961); D. W. Sturge and R. T. Smith, *U.S. At. Energy Comm. Res. Develop. Rep. NP-10941* (December 1961); L. V. Jones, D. Ofte, P. A. Tucker, L. J. Wittenberg, *U.S. At. Energy Comm. Res. Develop. Rep. TID 7641* (July 1963); D. B. Sullenger and K. D. Phipps, *U.S. At. Energy Comm. Res. Develop. Rep. MLM-1205* (11 December 1964); H. Meyer, *Ber. Deut. Keram. Ges.* **41**, 112 (1964).
9. D. T. Goldman and J. R. Rosser, *Chart of the Nuclides* (General Electric Company, Schenectady, ed. 9, 1966).
10. F. Halla and R. Weill, *Z. Kristallogr.* **101**, 435 (1939); J. A. Kohn, G. Katz, A. A. Giardini, *ibid.* **111**, 53 (1958); J. A. Kohn, in *Boron-Synthesis, Structure, and Properties*, J. A. Kohn, W. F. Nye, G. K. Gaulé, Eds. (Plenum Press, New York, 1960), p. 75; J. A. Kohn and D. W. Eckart, *Z. Kristallogr.* **116**, 134 (1961).
11. C. F. Cline and D. E. Sands, *Nature* **185**, 456 (1960); R. F. Adamsky, *Acta Crystallogr.* **11**, 744 (1958); N. F. Yannoni, thesis, Boston University (1961).
12. S. M. Richards and J. S. Kasper, paper M-6 presented at the American Crystallographic Association Meeting, Gatlinburg, Tennessee, 1965.
13. H. A. Eick, *Inorg. Chem.* **4**, 1237 (1965).
14. ———, *Amer. Doc. Auxiliary Publications Project No. 8307* (Photoduplication Service, Library of Congress, Washington, D.C.).
15. We are grateful to Dr. S. M. Richards, who supplied us with the experimental powder pattern for YB_{66} and the atomic positional parameters for that structure as refined from intensity data from a single crystal.
16. P. K. Smith, thesis, University of Kansas (1965); ——— and P. W. Gilles, *J. Inorg. Nucl. Chem.* **26**, 1465 (1964).
17. We acknowledge the technical assistance of G. L. Downs, J. D. Braun, M. J. Savitski, R. R. Eckstein, J. R. Henes, M. Mowchan, D. L. Roesch, and Y. G. Ishida. Mound Laboratory is operated by Monsanto Research Corporation for the U.S. Atomic Energy Commission under contract AT-1-33-GEN-53.

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Cytotoxic Effects of Leukocytes Triggered by Complement Bound to Target Cells

Abstract. *Chromium-51–labeled chicken erythrocytes (E), treated with rabbit anti-Forssman antibody (A) and the first four (C1–4) or the first seven (C1–7) components of human complement (C), released isotope upon exposure to human leukocytes. Isotope release from EAC1–7 cells proceeded more rapidly and was more extensive than that from EAC1–3 cells. Lysis of these cells was suppressed by pretreatment of leukocytes with antimycin A. Monocyte-enriched leukocyte preparations affected both types of target cell-complement intermediates, whereas purified lymphocytes lysed EAC1–7 cells but not EAC1–3 cells.*

Cell-mediated cytotoxic reactions are operative in delayed hypersensitivity, homograft rejection, and in certain autoimmune diseases. Cytolytic effects of purified lymphocytes may be initiated in vitro by mixing lymphocytes from (i) sensitized donors with antigen-bearing target cells (*1*), (ii) normal donors with target cells in the presence of humoral antibodies to target cell antigens (*2*), and (iii) normal donors with target cells in the presence of lymphocyte stimulants such as phytohemagglutinin (*3*). Phagocytosis does not appear to play a role in these reactions. Similarly, macrophages from sensitized donors (*4*) or monocytes in combination with antibody (*5*) may damage in vitro a variety of target cells by a nonphagocytic mechanism.

Although complement (C) has been reported to potentiate the cytotoxic action of sensitized lymphocytes in a few cases (*6*), it is generally assumed that C is not essential for cell-mediated cytotoxic reactions (*1*). The present experiments were performed to ascertain whether complement, bound to target cells, renders these cells susceptible to cell-mediated lysis.

Chicken erythrocytes were used as target cells (*7*) which were sensitized with four hemolysin units (*8*) of a rabbit antiserum to boiled sheep stromata (*9*). The antibody was primarily of the 19*S* variety and readily cross-reacted with chicken erythrocytes. Intermediate complexes consisting of chicken erythrocytes (E), rabbit antibody (A), and human complement components 1–7 (C1–C7) were prepared essentially as in previous studies in which sheep erythrocytes were utilized. We prepared EAC1,4 with mac-

Table 1. Lysis of complement-target cell complexes by complement components and by two different fetal calf serum preparations.

Complex	C2–9	C3–9	C5–9	C8,9	FCS*	FCS fraction
EAC1,4	100†				0	0
EAC1,4,2‡		100			0	0
EAC1,4,2,3			100		0	0
EAC1,4,2,3,5,6,7				95	35	0

* Fetal calf serum (5 percent by volume), heated 90 minutes at 56°C. † Numbers indicate percent lysis of 3×10^7 cells in 30 minutes at 32°C. ‡ C2 was used exclusively in oxidized form.

romolecular C1 and sufficient purified C4 to achieve uptake of 6000 to 8000 C4 molecules per cell (*10*). We obtained EAC1,4,2 by treatment of EAC1,4 with an excess of isolated, oxidized C2 (*11*). This complex was incubated with isolated C3 to produce EAC1,4,2,3 (EAC1–3) with 5,000 to 25,000 bound C3 molecules per cell (*12*). By treatment with isolated C5 (*13*) and purified C6 and C7 (*14*), this complex was converted to EAC1,-4,2,3,5,6,7 (EAC1–7). The hemolytic reactivity of these complement-cell complexes is shown in Table 1.

The erythrocyte complexes were suspended in tris-buffered (*p*H 7.8) Hanks balanced salt solution (TH) (*9*) and the concentration was adjusted to 1.5×10^8 cell/ml. We incubated 0.1-ml aliquots of the various suspensions at 37°C for 30 minutes with an equal volume of ^{51}Cr-sodium chromate (50 to 100 μc) (*9*). They were washed in TH containing 0.1 percent gelatin and were suspended in Parker's medium 199 (*9*) containing 5 percent heated (56°C, 90 minutes) fetal calf serum (FCS) (*9*) or a fetal calf serum fraction (see below). Neither labeling with ^{51}Cr nor subsequent incubation at 37°C for prolonged periods adversely affected the hemolytic reactivity of the erythrocyte complexes. All complexes were stable except EAC1,4,^{oxy}2, which decayed with a half-life of 200 minutes (*11*).

Leukocytes from 100 ml of defibrinated human blood were isolated by sedimentation through gelatin (*15*), washed, and suspended in Parker's medium 199 containing 5 percent heated FCS or FCS-fraction. Half a milliliter of the leukocyte suspension containing 3.0 to 4.0 $\times$ 10^6 viable lymphocytes and 0.5 ml of the ^{51}Cr-labeled erythrocyte-complement complexes (1×10^5 cells) were mixed and incubated in duplicates under sterile conditions. In controls, the leukocytes were replaced by 10^6 untreated and unlabeled chicken erythrocytes (*7*).

Incubation of the various erythrocyte preparations with an excess of leukocytes in the presence of 5 percent heat-inactivated FCS gave the following results. Isotope release from E, EA, and EAC1,4 cells did not exceed 12 percent within 21 hours. The leukocyte-free controls showed the same low degree of isotope release. The small amount of antibody on the surface of the target cells thus did not result in stimulation and ensuing cytotoxicity of leukocytes observed previously under different experimental conditions (*2*). Within 21 hours EAC1–3 and EAC1–7 cells released 41 to 89 percent of the isotope, respectively, suggesting that appreciable cell damage had been caused by the leukocytes. However, the EAC1–7 cells in the leukocyte-free controls had undergone 82 percent lysis. If there was a leukocyte effect on EAC1–7 cells, it was masked by the high background lysis in the controls, which after 1 and 5 hours was already 43 and 73 percent, respectively. The data suggested that the heated FCS contained residual complement activity. Subsequent testing showed that all of the five different batches of heated FCS used lysed EAC1–7 cells to a significant degree within 30 minutes (Table 1) and thus contained C8 and C9 activity even after 90 minutes of heating at 56°C, which is widely thought to be sufficient to inactivate heat-labile complement components.

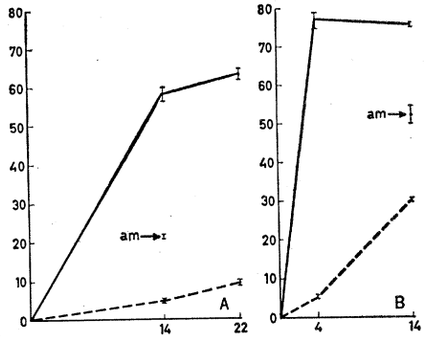

Fig. 1. Percentage of isotope release (ordinate) from EAC1,4,2,3 (A) or EAC1,4,2,-3,5,6,7 (B) after various hours of incubation (abscissa), with leukocytes (solid lines) or in leukocyte-free controls containing an excess of unlabeled and untreated chicken erythrocytes (broken lines). Vertical lines give the range of isotope release obtained in independent duplicate incubations. *am*: Isotope release obtained with leukocytes pretreated with antimycin A. (Ten milliliters of Parker's medium 199, supplemented with $10^{-2}M$ glutamate and containing $10^{-4}M$ antimycin A and 7×10^7 lymphocytes, were incubated for 35 minutes at 37°C in an atmosphere of 95 percent air and 5 percent CO_2. The cells were then washed, stained with Trypan blue for viability, and used as usual. For further details see (*16*).

For all subsequent experiments a fraction of fetal calf serum was used which had been depleted of C8 and C9 by chromatography. Twenty-five milliliters of FCS was applied to a 45- by 9.5-cm column of carboxymethyl cellulose, which was equilibrated with phosphate buffer, *p*H 6.0, ionic strength = 0.1. Under these conditions, C8 and C9 were adsorbed to the cellulose, while 98 percent of the serum protein was eluted with the starting buffer. The eluted protein was concentrated by pressure dialysis to 25 ml, dialyzed against physiological saline, passed through a bacterial filter, and heated for 90 minutes at 56°C. As seen in Table 1, last column, the FCS-fraction did not cause detectable lysis of any of the target cell complexes used.

Figure 1A shows the time course of isotope release from EAC1–3 cells incubated with leukocytes in the presence of C8- and C9-depleted FCS-fraction. Approximately 60 percent isotope release (= 75 percent lysis) was reached within 14 hours. Even after 22 hours of incubation the isotope release in the leukocyte-free control was very low. Pretreatment of the leukocytes with antimycin A (*9, 16*) clearly reduced their cytotoxic capacity (Fig. 1A) without affecting their viability, as determined by Trypan blue staining. Since antimycin A inhibits respiration of leukocytes, the results suggest that their cytotoxic effect on EAC1–3 cells is an energy-requiring process. The kinetics of the reaction recorded in Fig. 1A are similar to the kinetics which are characteristic for the three different lymphocyte-mediated cytolytic reactions outlined in the introduction. The possibility is thus raised that target cell bound C3 represents an additional pathway of inducing a cell-mediated cytotoxic reaction.

Experiments with fractionated leukocytes (*17*) showed that monocyte-rich preparations (*18*) were strongly cyto-

toxic for EAC1–3 cells. In contrast, purified lymphocytes (*19*) exhibited a strikingly reduced cytotoxicity. These data would argue for the monocyte being the cell type responsible for the observed cytotoxic effect. However, the participation of lymphocytes in the cytotoxic reaction should not be excluded at this stage of our investigations, particularly in view of the observation (*20*) that lymphocyte transformation induced by antigen or by "mixed culture" conditions was strongly diminished when purified lymphocytes were used. It is conceivable that the cytotoxic capacity of unfractionated leukocyte preparations was due mainly to lymphocytes which were triggered into action by a small number of other leukocytes. The monocyte-rich preparations contained up to 30 percent small lymphocytes and the unfractionated leukocytes were usually more potent than the purified cell preparations.

Figure 1B illustrates the effect of lymphocyte-rich leukocytes on EAC1–7 cells. The leukocytes came from the same batch of cells used for the experiment represented in Fig. 1A. Almost all cells were lysed within 4 hours. Thus, kinetically, this reaction is different from the usual lymphocyte-mediated cytotoxic reactions. The target cells were more labile than EAC1–3 cells, since they lost 30 percent of their isotope upon prolonged incubation even in the absence of leukocytes. However, the lability of EAC1–7 cells was not a constant phenomenon; the isotope release in leukocyte-free controls varied from 8 to 30 percent within 14 hours. Pretreatment of leukocytes with antimycin A led to a strong reduction of lysis (Fig. 1B). EAC1–7 cells were efficiently lysed both by glass bead purified lymphocytes (*19*) and by the monocyte-enriched preparations (*18*). Thus, lymphocytes, and perhaps also monocytes, appear to be effector cells in this cytotoxic reaction. The effect of these cells on EAC1–7 resembles that of C8, which is capable of lysing EAC1–7 cells in the absence of C9. Characteristically, this reaction is considerably slower than that mediated by C8 and C9. One hypothesis, which is presently being investigated, proposes that lymphocytes and perhaps some other leukocytes lyse EAC1–7 cells by releasing C8 or C8 and C9. This assumption finds some support in results obtained with leukocytes pretreated with rabbit antiserum to purified human C8 (*21*). This treatment inhibited their phytohemagglutinin-induced cytotoxic effect on complement- and antibody-free chicken erythrocytes. Rabbit antiserum to human C1q, C2, C3, or C4 had no such effects, while anti-C5 had a weak inhibitory effect.

Regardless of the exact mechanism underlying our observations, we conclude that complement may participate in cell-mediated cytotoxic reactions. The results underline the importance of the complement system as a link between humoral and cellular immune reactions of a tissue-damaging nature.

PETER PERLMANN
HEDVIG PERLMANN
Wenner-Gren Institute for Experimental Biology, University of Stockholm, Stockholm, Sweden
HANS J. MÜLLER-EBERHARD
JORGE A. MANNI
Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

References and Notes

1. D. B. Wilson and R. E. Billingham, *Advan. Immunol.* **7**, 189 (1967); K. T. Brunner *et al.*, *Immunology* **14**, 181 (1968).
2. P. Perlmann and G. Holm, in *Mechanism of Inflammation Induced by Immune Reactions*, P. A. Miescher and P. Grabar, Eds. (Schwabe, Basel, 1968), p. 325; P. Perlmann and G. Holm, in *Biological Recognition Processes*, R. T. Smith, P. A. Miescher, R. A. Good, Eds. (Univ. of Florida Press, Gainesville, in press); P. Perlmann, H. Perlmann, J. Wasserman, T. Packalén, *Int. Arch. Allergy Appl. Immunol.*, in press.
3. G. Holm and P. Perlmann, *J. Exp. Med.* **125**, 721 (1967); N. H. Ruddle and B. H. Waksman, *Science* **157**, 1060 (1967).
4. G. Granger and R. S. Weiser, *Science* **151**, 97 (1966).
5. L. LoBuglio, R. S. Cotran, J. H. Jandl, *ibid.* **158**, 1582 (1967).
6. A. Govaerts, *J. Immunol.* **85**, 516 (1960); P. Perlmann and O. Broberger, *J. Exp. Med.* **117**, 717 (1963).
7. P. Perlmann, H. Perlmann, G. Holm, *Science* **160**, 306 (1968).
8. M. M. Mayer, in *Experimental Immunochemistry*, E. A. Kabat and M. M. Mayer, Eds. (Thomas, Springfield, Ill., 1961), p. 133.
9. The rabbit anti-sheep hemolysin was purchased from Baltimore Biological Laboratories, Baltimore. Hanks balanced salt solution, Parker's medium 199, and antibiotics were obtained from Microbiological Associates, Bethesda, Md. The fetal calf serum (CS 1192, lot 162) was from Colorado Serum Co., Denver, and serum PHA-M (S 12667) from Difco Laboratories, Detroit. Antimycin A was purchased from Sigma Chemical Co., St. Louis, and ^{51}Cr-sodium chromate (specific activity 100 to 260 c/g of Cr) from New England Nuclear Corp., Boston.
10. N. R. Cooper and H. J. Müller-Eberhard, *Immunochemistry* **5**, 155 (1968).
11. M. J. Polley and H. J. Müller-Eberhard, *J. Exp. Med.* **128**, 533 (1968).
12. H. J. Müller-Eberhard, A. P. Dalmasso, M. A. Calcott, *ibid.* **123**, 33 (1966).
13. U. R. Nilsson and H. J. Müller-Eberhard, *ibid.* **122**, 277 (1965).
14. ———, *Immunology* **13**, 101 (1967).
15. G. Holm and P. Perlmann, *ibid.* **12**, 525
16. G. Holm, *Exp. Cell Res.* **48**, 334 (1967); ——— and P. Perlmann, in *Advances in Transplantation*, J. Dausset, J. Hamburger, G. Mathé, Eds. (Munksgaard, Copenhagen, 1968), p. 155.
17. Details are in preparation for publication elsewhere.
18. W. E. Bennett and Z. A. Cohn, *J. Exp. Med.* **123**, 145 (1966).
19. Y. Rabinowitz, *Blood* **23**, 811 (1964).
20. N. R. Ling, *Lymphocyte Stimulation* (North-Holland, Amsterdam, 1968); W. McFarland, D. M. Heilman, J. F. Moorhead, *J. Exp. Med.* **124**, 851 (1966); J. Gordon, *Proc. Soc. Exp. Biol. Med.* **127**, 30 (1968); W. McFarland, in *Proceedings of the Third Leukocyte Culture Conference, Iowa City, Iowa, 1967*, W. O. Rieke, Ed. (Appleton, Century, Crofts, New York, in press); E. M. Hersh and J. E. Harris, *ibid.*
21. J. A. Manni and H. J. Müller-Eberhard, *Fed. Proc.* **27**, 479 (1968).
22. P.P. is supported by a travel grant, 2032-24, from the Swedish Natural Science Research Council. J.A.M. is supported by USPHS training grant GM683. This work was supported by USPHS grant AI-07007, American Heart Association, Inc. grant 65-G-166, and AEC contract AT(04-3)-730. This is publication No. 306 from the department of experimental pathology, Scripps Clinic and Research Foundation, La Jolla, California. We thank Dr. R. Dutton for helpful discussions and generous supply of material.

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Green Sea Turtles: A Discrete Simulation of Density-Dependent Population Regulation

Abstract. *Field data on the nesting of the green sea turtle were used to construct a stochastic model. This model was simulated by use of a digital computer language simscript (Monte Carlo) to determine the relation between the percentage of nests destroyed and the size of the turtle population. Nest destruction is dependent on population density and provides a mechanism to regulate population size.*

During a study on the ecology of the green sea turtle *Chelonia mydas* (L.) on the Great Barrier Reef we observed that, where the density of the population was high, the nesting turtles frequently destroyed their eggs. All turtles were tagged, and we kept a complete record of the movement of every turtle for 5 weeks. Furthermore, every nesting turtle was observed over a 14-week period in each of four successive years. These data formed the basis for constructing a stochastic model.

Turtles come ashore after dark, usually around high tide, and nest in a narrow zone above the spring high-tide mark in the outer limits of the vegetation. Turtles first dig a body pit about