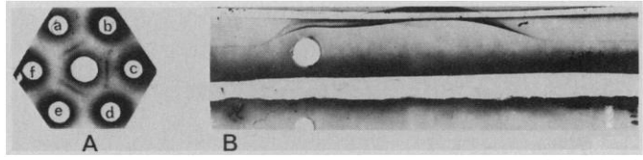
8. D. Bindra, *Psychol. Rev.* **81**, 199 (1974); S. S. Steiner, B. Beer, M. M. Shaffer, *Science* **163**, 90 (1969); M. B. Cantor, *ibid.* **174**, 610 (1971).
9. After stabilization of amphetamine self-administration the animals were tested in the present paradigm with no previous apomorphine experience. They were placed in the self-administration chambers 24 hours after their last amphetamine session and were given two experimenter-administered "priming" injections of apomorphine (0.5 mg/kg per injection); then they were allowed to self-administer apomorphine at this dose on a continuous reinforcement schedule for 4 hours.
10. B. L. Baxter, M. I. Gluckman, L. Stein, R. A. Scerni, *Pharmacol. Biochem. Behav.* **2**, 387 (1974); R. A. Yokel and R. A. Wise (*4*).
11. R. A. Yokel and R. A. Wise, in preparation.
12. Saccharin intake tests followed 16 hours of water deprivation, in the same paradigm as was used in the amphetamine study.
13. In fact it is already known that when compound stimuli are associated with injections of poison, the taste elements of the compound are readily associated with the aversive effects of the poison, but auditory and visual elements are not. Conversely, auditory and visual elements are associated with the aversive effects of footshock, while taste elements are not [Garcia and Ervin (*6*)].
14. Supported by the Non-Medical Use of Drugs Directorate of Canada. We thank Smith Kline & French for the amphetamine, Merck Frosst for the apomorphine, and Jane Stewart and Barry Hoffer for comments on the manuscript.
* Present address: Department of Pharmacology, University of Cincinnati, Cincinnati, Ohio.
9 January 1976

Cobra Venom Factor: Evidence for Its Being Altered Cobra C3 (the Third Component of Complement)

Abstract. *Evidence is presented that cobra venom factor, the anticomplementary protein in* Naja naja *venom, is modified cobra C3 (the third component of complement). Antiserum to the cobra venom factor cross reacts with human C3. A protein in cobra serum reacts strongly with antiserum to the venom factor and the former protein, like human C3, is converted by incubation of cobra serum with endotoxin, hydrazine, or simple storage at 37°C. Incubation of cobra venom factor with cobra serum destroys the C3 cleaving activity of the venom factor in human serum, whereas human C3b inactivator is ineffective. Thus, the cobra venom factor appears to be a form of C3 (perhaps C3b); its potent action in human serum probably derives from its lack of sensitivity to human C3b inactivator.*

Interest in the anticomplementary activity of snake venom spans virtually the entire history of the study of complement itself. A medical officer in the American Army, Captain C. B. Ewing, observed in 1894 that venom from some poisonous snakes destroyed the bactericidal activity of serum (*1*). Around the turn of the century, Flexner and Noguchi (*2*) demonstrated that these venoms acted by destroying complement activity in vitro. Ritz, in 1912 (*3*), showed that snake venom did not destroy either the first or second components of complement, the only components known at that time, and he therefore defined a third component. Some 50 years later, when it had become evident that this "third component" was complex and, in fact, consisted of several different proteins, Klein and Wellensieck (*4*) demonstrated that the attack by venom was directed against what we now call C3. Nelson (*5*) and Müller-Eberhard and his colleagues (*6*) characterized and isolated from snake venom the protein (cobra venom factor or CoF) which induced C3 cleavage and showed that this attack on C3 was not direct but required at least one normal human serum protein. Factor B of the properdin system (*7–9*) was shown to be required for the CoF-mediated attack on C3 (*9, 10*). There was, initially, controversy about whether a complex was formed between factor B and CoF. Götze and Müller-Eberhard obtained evidence that the purified proteins formed an equimolar complex (*9*), but neither we (*11*) nor Hunsicker *et al.* (*10*) could show such a complex. There is now evidence that a complex of CoF and factor B does form in the presence of factor D (*12*). In whole serum only a small fraction of the factor B is involved (*11*). There is thus no CoF-binding protein distinct from factor B as previously postulated by us and by Hunsicker and co-workers. The identification of a positive feedback loop within the properdin or alternative pathway of complement activation, triggered by C3b (*13*) prompted Lachmann and Nicol (*14*) to draw an analogy between the action of purified CoF and human C3b. We now report studies which strongly suggest that CoF is, in fact, an altered form of cobra C3.

A potent antiserum to isolated CoF from the venom of *Naja naja*, the Asiatic hooded cobra, reacted, on Ouchterlony analysis, with normal human serum and highly purified C3 but not with C3-deficient serum (*15*) (Fig. 1). To rule out the possibility that human serum and C3 in particular had somehow contaminated the CoF preparation used as antigen, normal human serum was placed in one trough of an immunoelectrophoresis slide (Fig. 1), and antiserum to CoF was placed in the opposite trough and allowed to diffuse against purified CoF subjected to electrophoresis from the center well. The faint line produced by the human serum reacting with the antiserum to CoF was not continuous, but fused with the very strong arc due to reaction of CoF and its antiserum, indicating that the reaction with human C3 was induced by antibody to CoF and not by a contaminating antibody. Further evidence that this reaction did not result from antibody to a contaminating human antigen is that, after the antiserum to CoF was absorbed with lyophilized whole cobra ven-



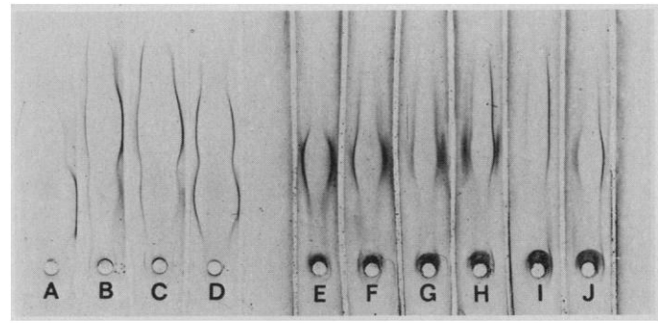


Fig. 1 (left). (A) Ouchterlony analysis in agarose gel in 0.05*M* barbital buffer (*p*H 8.6) containing $10^{-2}M$ disodium ethylenediaminetetraacetate. The central well contained rabbit antiserum to CoF. Peripheral wells a, c, and e contained normal human serum with a C3 concentration of 150 mg/100 ml. Wells b, d, and f were filled with serums from patients with diminished C3 serum concentrations (4, 30, and < 0.25 mg/100 ml, respectively). Reactions were observed only with the normal serum. (B) In immunoelectrophoresis performed in agarose gel, purified CoF was placed in the central well and subjected to electrophoresis. The upper trough was filled with rabbit antiserum to CoF and the lower trough with normal human serum. After diffusion was complete, the gel was washed, dried, and stained. Fig. 2 (right). Immunoelectrophoresis of human serum developed with antiserum to human C3 (left-hand series) and cobra serum developed with antiserum to CoF (right-hand series). The samples placed in the antigen wells were as follows: (A) normal human serum (NHS); (B) NHS incubated with *Escherichia coli* 026 : B6 endotoxin (1 mg/ml) for 30 minutes at 37°C; (C) conditions as in (B) except incubation for 1 hour; (D) NHS incubated alone for 1 hour at 37°C; (E) cobra serum (CS); (F) CS incubated with endotoxin at 0.2 mg/ml for 30 minutes at 37°C; (G) conditions as in (F), except endotoxin concentration was 1 mg/ml; (H) CS incubated with endotoxin at 0.2 mg/ml for 1 hour at 37°C; (I) conditions as in (H) except endotoxin concentration was 1 mg/ml; (J) CS incubated alone for 1 hour at 37°C.

om, the reaction in gel with the human serum was abolished.

The antiserum to CoF was reacted in immunoelectrophoresis with whole *Naja naja* serum, and a strong reaction was observed with a β-mobility protein in the cobra serum. The material in the cobra serum converted to some extent to a more rapidly migrating protein on incubation at 37°C for 1 hour. However, this conversion was much more marked on incubation of the snake serum with endotoxin (*16*) (Fig. 2). Neither zymosan nor antigen-antibody precipitate made from rabbit antibody had an effect on this protein in cobra serum when incubated at 2 mg of precipitate per milliliter of cobra serum. Incubation of cobra serum with 0.15*M* hydrazine for 30 minutes at 37°C produced conversion of about half of the cross-reacting material to a more slowly migrating protein.

Immunofixation (*17*) of whole cobra serum, whole cobra venom, and purified CoF (*18*) is shown in Fig. 3. The protein in cobra serum forms a single, sharp band, whereas CoF either in whole venom or in the isolated state produces a series of electrophoretically slower bands all of which are antigenically identical.

These observations suggest that CoF is closely related to a cobra serum protein with many of the characteristics of C3. The electrophoretic appearance of CoF compared with that of the native material in serum suggests that the material in the venom has been altered, and perhaps acted on by proteolytic enzymes.

Incubation of cobra serum with CoF at 10 μg/ml at 37°C for 30 minutes did not result in increased conversion of the CoF-reactive material of the snake serum, although, as expected, CoF at 10 μg/ml induced marked C3 conversion in human serum, as judged by immunoelectrophoresis. When cobra serum was incubated with ^{125}I-labeled CoF for 3 hours at 37°C (*19*), the labeled CoF was converted to more rapidly migrating material on prolonged agarose gel electrophoresis and radioautography (Fig. 3). Incubation with purified human C3b inactivator or buffer produced no alteration in the electrophoretic appearance of CoF. Furthermore, incubation of CoF at 10 μg/ml with cobra serum at 37°C for 1 hour destroyed the ability of the mixture to induce C3 conversion in human serum. Similar incubation of CoF with barbital-buffered isotonic saline containing $1.5 \times 10^{-4}M$ Ca^{2+} and $10^{-3}M$ Mg^{2+} and 1 percent human serum albumin, or a solution of purified human C3b inactivator (*20*) at 10 μg/ml, did not destroy its ability to convert C3 in human serum. When a mixture of equal parts of cobra serum and human serum were incubated at 37°C for 30 minutes, the extent of human C3 conversion was similar to that of human serum incubated alone.

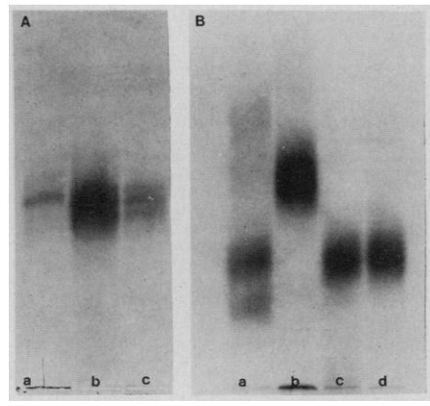


Fig. 3. (A) Immunofixation patterns developed after prolonged agarose gel electrophoresis with antiserum to CoF of (a) cobra serum, (b) isolated CoF, and (c) whole cobra venom. (B) Radioautograph after prolonged agarose gel electrophoresis of mixtures of ^{125}I-labeled CoF and (a) normal human serum, (b) cobra serum, (c) cobra venom, and (d) barbital-buffered saline with Mg^{2+} and Ca^{2+} containing 1 percent human serum albumin. All mixtures were incubated for 3 hours at 37°C prior to electrophoresis. The slower band in (a) represents a complex between CoF and Bb (*12*). The most rapid band is unidentified. The mobility of CoF in (c) and (d) is the same as that of untreated CoF. The native mobility of CoF disappeared on incubation with cobra serum and one or two bands with more rapid mobility appeared.

These observations suggest that CoF is a form of cobra C3 (perhaps the analog of human C3b) which has the active site for forming the alternative pathway convertase in human serum. Its very potent activity would, therefore, derive from its lack of susceptibility to the human C3b inactivator. On the other hand, its failure to convert cobra serum C3 and its conversion in cobra serum suggest its susceptibility to an inactivating mechanism in cobra serum which might be analogous to the human C3b inactivator.

The fact that CoF is probably cobra C3b or a fragment of C3 very similar to C3b explains the observation that CoF incubated with serum from a patient homozygous for C3 deficiency results in normal C5 inactivation and normal passive hemolysis of unsensitized guinea pig erythrocytes (*21*) since activated C3 is being supplied to the C3-deficient serum. The observations in this report also supply a reasonable explanation for the otherwise enigmatic highly specific interaction of a snake venom protein with mammalian serum, an enigma that has been with us for the past 80 years.

C. A. ALPER
D. BALAVITCH
Center for Blood Research, Boston, Massachusetts 02115

References and Notes

1. C. B. Ewing, *Lancet* **1894-I**, 1236 (1894).
2. S. Flexner and H. Noguchi, *J. Exp. Med.* **6**, 277 (1902).
3. H. Ritz, *Z. Immunitaetsforsch. Allerg. Klin. Immunol.* **13**, 62 (1912).
4. P. G. Klein and H. J. Wellensieck, *Immunology* **8**, 590 (1965).
5. R. A. Nelson, Jr., *Surv. Ophthalmol.* **11**, 498 (1966).
6. H. J. Müller-Eberhard *et al.*, *Arch. Pathol.* **82**, 205 (1966).
7. L. Blum, L. Pillemer, I. H. Lepow, *Z. Immunitaetsforsch. Allerg. Klin. Immunol.* **118**, 349 (1959).
8. T. Boenish and C. A. Alper, *Biochim. Biophys. Acta* **221**, 529 (1970).
9. O. Götze and H. Müller-Eberhard, *J. Exp. Med.* **134**, 90s (1971).
10. L. G. Hunsicker, S. Ruddy, K. F. Austen, *J. Immunol.* **110**, 128 (1973).
11. C. A. Alper, I. Goodkofsky, I. H. Lepow, *J. Exp. Med.* **137**, 424 (1973).
12. N. R. Cooper, *ibid.*, p. 451; W. Vogt, L. Dieminger, R. Lynen, G. Schmidt, *Hoppe-Seylers Z. Physiol. Chem.* **355**, 171 (1974).
13. H. J. Müller-Eberhard and O. Götze, *J. Exp. Med.* **135**, 1003 (1972).
14. P. J. Lachmann and P. Nicol, *Lancet* **1973-I**, 465 (1973).
15. Ö. Ouchterlony, *Acta Pathol. Microbiol. Scand.* **32**, 231 (1953). The observed reaction with human C3 also occurred in gels made up in 0.15*M* NaCl with phosphate at *p*H 7.4.
16. The endotoxin was provided by Dr. T. P. Stossel.
17. C. A. Alper and A. M. Johnson, *Vox Sang.* **17**, 445 (1969).
18. M. Ballow and C. G. Cochrane, *J. Immunol.* **103**, 944 (1969).
19. A. S. McFarlane, *Nature (London)* **182**, 53 (1958).
20. P. J. Lachmann and H. J. Müller-Eberhard, *J. Immunol.* **100**, 691 (1968). Purified C3b inactivator was a gift of Drs. F. S. Rosen and P. J. Lachmann.
21. C. A. Alper, H. R. Colten, J. S. S. Gear, A. R. Rabson, F. S. Rosen, *J. Clin. Invest.* **57**, 222 (1976).
22. Supported by PHS grants AM-13855 and AM-16392. The possibility that CoF might be related to saurian C3 was first suggested by Dr. Michael J. Hobart before these studies were initiated.

9 September 1975; revised 5 January 1976

Laboratory Plate Tectonics: A New Experiment

Abstract. *A "continent" made of a layer of hexagonally packed black polyethylene spheres floating in clear silicon oil breaks into subcontinents when illuminated by an ordinary incandescent light bulb. This experiment may be a useful model of plate tectonics driven by horizontal temperature gradients. Measurements of the spreading rate are made to establish the feasibility of this model.*

Plate tectonics is now accepted by most earth scientists as a useful paradigm for understanding the evolution of the major features of geology (*1*). Attempts to understand the physics of the process have been limited by the extreme complexity of the problem (*2*). Only limited success has been achieved with analytic (*2*) and experimental (*3*) approaches.

A major outstanding problem is the