Certain mycoplasmas, including *M. arthritidis*, are able to convert arginine to ornithine by way of the three-enzyme arginine dihydrolase pathway (*7*). Kraemer (*8*) showed that some strains of *Mycoplasma* that are cytocidal to lymphoma cells in culture liberate a toxic substance into the medium. This substance may act by depleting the medium of arginine, since the cytotoxic effect can be prevented by the addition of arginine to the cultures. Whether the toxin is identical to one of the enzymes in the arginine dihydrolase pathway is not known. In our studies only nonviable mycoplasmas were used, eliminating the possibility that a toxin was produced during the culture period. Whether an inhibitory factor was released into the culture medium during the growth of the organism was not investigated. The present studies do not exclude the possibility that a heat-stable toxin is involved in the inhibition of PHA response, although the observation that lymphocytes exposed to the mycoplasmas for 48 hours were still capable of responding to PHA indicates the inhibitory factor was not cytocidal. The possibility remains, however, that the inhibition is produced by a heat-stable enzyme, which may act by depleting the medium of arginine. We have studied only one species of *Mycoplasma*, and since *Mycoplasma* belongs to an extremely heterogeneous family of organisms, our results cannot be assumed to apply to mycoplasmas in general.

The responsiveness of lymphocytes in vitro appears to be correlated with the immunologic competence of the host. A depressed response to PHA in lymphocytes in culture has been noted in diseases associated with a defect in immune response, such as Hodgkin's disease (*9*), chronic lymphocytic leukemia (*10*), sarcoidosis (*11*), agammaglobulinemia (*12*), and ataxia telangiectasia (*13*). The action of *M. arthritidis* on the immune system is not known, but it is possible that this organism produces an impairment of immune response in vivo comparable to the impairment of lymphocyte response demonstrated in vitro. Such a correlation has been observed in viral diseases. Montgomery *et al.* (*14*) demonstrated that the lymphocytes of patients with congenital rubella do not respond normally to PHA and found that the addition of rubella or Newcastle virus to cultures of normal lymphocytes inhibits their response to the mitogen. Smithwick and Berkovich (*15*) showed that measles virus inhibits the in vitro response to tuberculin purified protein derivative in lymphocytes from children having a positive reaction to this tuberculin, and they were able to correlate this effect with decreased reactions to tuberculin skin tests in tuberculous children during measles infection. Our studies were concerned only with the inhibition of PHA response; the effect of *M. arthritidis* on antigenic stimulation of sensitized lymphocytes awaits further investigation.

Mycoplasma infection has been postulated in the pathogenesis of a number of diseases of unknown etiology, including systemic lupus erythematosus, Reiter's syndrome, and rheumatoid arthritis (*16*). Although our data provide no information on the possible pathogenetic role of mycoplasmas, they do demonstrate that at least one species of *Mycoplasma* can profoundly affect lymphocyte function in vitro. Whether lymphocyte function is similarly affected in mycoplasma infections in man remains to be established.

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References and Notes

1. P. C. Nowell, *Cancer Res.* **20**, 462 (1960).
2. M. A. Bender and D. M. Prescott, *Blood* (abstr.) **20**, 103 (1962); E. H. Cooper, P. Barkhan, A. J. Hale, *Brit. J. Haematol.* **9**, 101 (1963).
3. R. W. Dutton and J. D. Eady, *Immunology* **7**, 40 (1964).
4. R. Copperman and H. E. Morton, *Proc. Soc. Exp. Biol. Med.* **123**, 790 (1966).
5. K. C. Cochrum, J. T. Okimoto, J. S. Najarian, *J. Appl. Physiol.* **24**, 247 (1968).
6. Packard Instrument Co.
7. R. T. Schimke and M. F. Barile, *J. Bacteriol.* **86**, 195 (1963); M. F. Barile, R. T. Schimke, D. B. Riggs, *ibid.* **91**, 189 (1966).
8. P. M. Kraemer, *Proc. Soc. Exp. Biol. Med.* **115**, 206 (1964).
9. E. M. Hersh and J. J. Oppenheim, *New Engl. J. Med.* **273**, 1006 (1965).
10. J. J. Oppenheim, J. Whang, E. Frei, III, *Blood* **26**, 121 (1965).
11. K. Hirschhorn *et al.*, *Lancet* **1964-II**, 842 (1964).
12. M. J. Cline and H. H. Fudenberg, *Science* **150**, 1311 (1965).
13. J. J. Oppenheim, M. Barlow, T. A. Waldmann, J. B. Block, *Brit. Med. J.* **2**, 330 (1966).
14. J. R. Montgomery, M. A. South, W. E. Rawls, J. L. Melnick, G. B. Olson, P. B. Dent, R. A. Good, *Science* **157**, 1068 (1967).
15. E. M. Smithwick and S. Berkovich, *Proc. Soc. Exp. Biol. Med.* **123**, 276 (1966).
16. L. E. Bartholomew, *Ann. N.Y. Acad. Sci.* **143**, 522 (1967); M. F. Barile, in *Methodological Approaches to the Study of Leukemias*, V. Defendi, Ed. (Wistar Inst. Press, Philadelphia, 1965), p. 171.
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Inherited C′2 Deficiency in Man: Lack of Immunochemically Detectable C′2 Protein in Serums from Deficient Individuals

Abstract. *Monospecific antiserum to highly purified second component of human complement (C′2) was used to show the absence of the protein from the serums of four persons homozygous for a hereditary deficiency of second-component activity. Serum from an individual heterozygous for the deficiency contained a reduced amount of this protein as compared to the concentration in normal serum. These observations indicate that genetic deficiency of this component is due to failure of synthesis of normal amounts of the protein rather than to synthesis of an antigenically related, hemolytically inactive analog of C′2.*

A deficiency of the second component (C′2) of human complement in a clinically healthy individual (*1*) was later shown (*2, 3*) to be an inherited characteristic. Three families with this deficiency have been described, and in each family the propositus was a homozygote for the defect (*2–4*). Several independent assays have established the presence of a small amount of second-component activity (usually less than 5 percent of normal) in the serums of the homozygous individuals. The serums from the heterozygotes showed approximately 50 percent of the normal activity (*1–5*).

Klemperer *et al.* (*3*) postulated that this deficiency was due to absence of the active protein rather than to the presence of an inhibitor of second-component activity. In our study, comparative immunochemical analyses were performed on both C′2-deficient and normal serums to determine directly whether the deficiency of the component in these serums was due to lack of the C′2 protein or to the presence of an inactive analog. It was presupposed that the potent, monospecific antiserum to normal C′2 used would detect a C′2 variant by immunochemical cross-reaction.

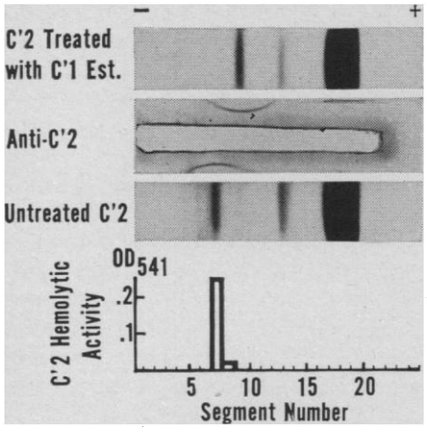

Fig. 1. Correlation between C'2 hemolytic activity, C'2 protein, and the antigen detected by antiserum to C'2 following electrophoresis on polyacrylamide gel. Protein bands in segments 13, 17, and 18 represent carrier protein, human serum albumin (*6*).

Antiserum to highly purified C'2 was produced (*6*), and its monospecificity was established in several ways. (i) The reaction of the antiserum with whole human serum and with purified C'2 in Ouchterlony plates produced a single precipitin line in both instances, and the two lines fused completely giving a reaction of identity. (ii) After polyacrylamide-gel electrophoresis of purified C'2 on three gels under identical conditions, one was stained for protein, the second was cut into 2-mm segments from which protein was eluted and tested for C'2 hemolytic activity (*6*), and the third was embedded in a plate of 2 percent agar, and the protein was allowed to diffuse into the agar and toward a trough filled with antiserum to C'2. An exact correspondence was obtained between the protein band in the first polyacrylamide gel, the C'2 hemolytic activity eluted from the second gel, and the immune precipitin arc formed in agar by the third gel (Fig. 1). (iii) The component was converted to C'2i, its hemolytically inactive and electrophoretically distinct product, by treatment with C'1 esterase for 30 minutes at 37°C (*6*). The product was then subjected to polyacrylamide-gel electrophoresis on three identical gels and analyzed in the same manner as the untreated component. Compared to untreated C'2, the protein band now assumed a more anodal position and so did the antigen detected by antiserum to C'2, the immune precipitin arc corresponding exactly to the position of the protein band (Fig. 1). As expected, no hemolytic activity could be eluted. (iv) The antiserum to human C'2 inhibited the hemolytic activity of C'2 in solution and in its cell-bound form. The inhibition data, not recorded here, are analogous to those reported by Mayer for antiserum to guinea pig C'2 (*7*).

In that the specificity of the antiserum to human component was now established, Ouchterlony analyses were performed on the C'2-deficient serums. Of the five serums tested, three were from individuals previously reported to be homozygous for the deficiency [P. and W. (*1–3, 8*); T.H. (*4, 8*)] and one has not as yet been described [K. (*8*)]. One serum (B.H.) was from the mother of the homozygous individual T.H., and this was heterozygous for the deficiency, possessing approximately 60 percent of normal C'2 hemolytic activity (*4, 8*). In addition, serums from two patients (M. and L.) with hereditary angioneurotic edema (*8, 9*) were also tested, both of which contained reduced C'2 activity.

As shown in Fig. 2, the antiserum failed to detect C'2 protein in the serums of homozygous C'2-deficient individuals, whereas it did detect C'2 protein in the serum from heterozygous individuals. Since normal human serum reacts with antiserum to C'2 only at dilutions less than 1 : 4, the results depicted in Fig. 2 indicate that the serums from all four of the homozygous C'2-deficient individuals tested contained less than one-fourth of the amount of C'2 protein present in normal serum. The serum of the heterozygous individual contained approximately 50 percent of the amount of C'2 present in normal serum, as judged by dilution tests and by the proximity of the precipitin line to the antigen well in Ouchterlony tests of undiluted serum (Fig. 2). Serums from both angioneurotic edema patients reacted with antiserum to C'2, showing two precipitin bands, both of which fused with the single band produced by antiserum to C'2 and C'2.

Thus, immunochemical analysis of genetically C'2-deficient serums has demonstrated a lack of C'2 protein in four homozygous individuals and a re-

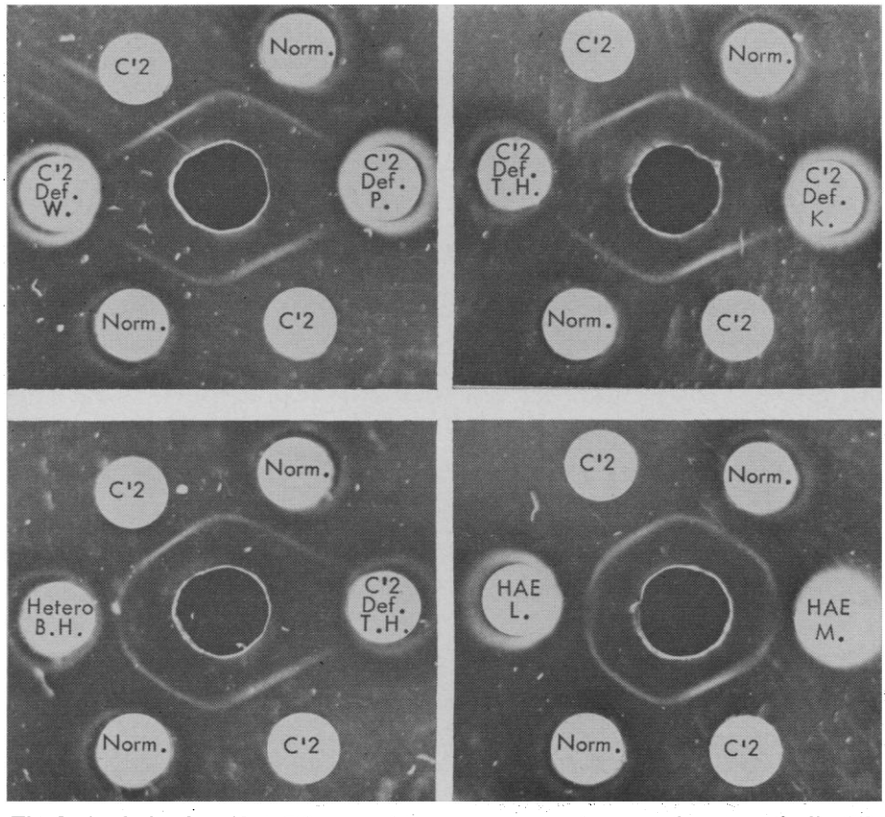

Fig. 2. Analysis of purified C'2, normal human serum, and serums from genetically C'2-deficient individuals by Ouchterlony test using a specific antiserum to human C'2. All serums were tested undiluted and 1*M* glycine was incorporated into the agar. *C'2*, purified C'2; *Norm.*, normal human serum; *C'2 Def.*, serum from homozygous, genetically C'2-deficient individuals; *Hetero.*, serum from a heterozygous individual; *HAE*, serum from patients with hereditary angioneurotic edema. Note that the precipitin lines of C'2 and its antibody extend into the wells containing serums from the homozygotes of the inherited C'2 deficiency.

duction to approximately half the normal concentration in a single heterozygote. The total lack of reaction between the homozygous serums and antiserum to C'2 precludes the presence of an antigenically related, hemolytically inactive analog of C'2 protein in these serums. Since all deficient individuals tested do possess residual C'2 activity (*1–5*), it must be concluded that the deficiency of C'2 protein is not absolute, and that there are small amounts of protein in the abnormal serums, which, however, are too small to be detected by the methods used in this study.

Note added in proof: Since this paper was submitted a similar observation was made by Dr. M. Klemperer and presented as an oral communication to the Complement Workshop, Boston.

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References and Notes

1. A. M. Silverstein, *Blood* **16**, 1338 (1960).
2. M. R. Klemperer, H. C. Woodworth, F. S. Rosen, K. F. Austen, *J. Lab. Clin. Med.* **66**, 886 (1965); *J. Clin. Invest.* **45**, 880 (1966).
3. M. R. Klemperer, K. F. Austen, F. S. Rosen, *J. Immunol.* **98**, 72 (1967).
4. N. R. Cooper, *Fed. Proc.* **27**, 314 (1968); ———, R. tenBensel, P. F. Kohler, *J. Immunol*, in press.
5. H. Gewurz, R. J. Pickering, L. H. Muschel, S. E. Mergenhagen, R. A. Good, *Lancet* **1966-II**, 356 (1966).
6. M. J. Polley and H. J. Müller-Eberhard, *J. Exp. Med.* **128**, 533 (1968).
7. M. M. Mayer and J. A. Miller, *Immunochemistry* **2**, 71 (1965).
8. Serums from homozygous C'2-deficient individuals P. and W. and from angioneurotic edema patient M. were supplied by Dr. K. Frank Austen; from homozygous C'2-deficient individual K. and angioneurotic edema patient L. by Dr. Richard Pickering; and homozygous C'2-deficient T.H. and heterozygous B.H. by Dr. Peter Kohler.
9. V. H. Donaldson and R. R. Evans, *Amer. J. Med.* **35**, 37 (1963); F. S. Rosen, P. Charache, J. Pensky, V. H. Donaldson, *Science* **148**, 957 (1965).
10. Supported by Dernham Senior Fellowship of the American Cancer Society, California Division No. D-133 and by PHS grant AI-07007. Publication No. 282 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California.

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Pyrethroid-Like Biological Activity of Compounds Lacking Cyclopropane and Ester Groupings

Abstract. *The following new insecticidal compounds respond to synergism by piperonyl butoxide and block nerve excitability in the same manner as the insecticide allethrin: 1-(4-allethronyl)-acetyl-2,2-dimethyl-3-isobutenylcyclopropane (the ketone analog of allethrin) and the esters of 5-benzyl-3 furylmethanol with 2,2,3,3-tetramethylcyclopropanecarboxylic acid, 2,2,3,3-tetramethylaziridinecarboxylic acid, and* N,N-*diisopropylcarbamic acid. Therefore pyrethroid-like activity is not restricted to esters of cyclopropanecarboxylic acids.*

Pyrethrum and a few of its many synthetic analogs play important roles in insect control because they are highly insecticidal (especially if used with a synergist), have low toxicity to mammals, and leave no hazardous or persistent residues on crops or food. Their use is limited by high cost, relative to that of most insecticides, resulting from either a limited botanical source (pyrethrum, for example) or, in the case of the synthetic analogs, costly preparation of compounds of such complex structures.

During the past 50 years, many workers have tried to expand the uses for pyrethroids and to elucidate the relations between their structures and activities; they have gained much information regarding the requirements for insecticidal activity by certain analogs of pyrethrin I, the most active natural pyrethroid, and of allethrin, the most important synthetic compound of this type, which contains an allyl group in place of the pentadienyl group of pyrethrin I (*1*). Insecticidal activity is improved when the respective alcohol component, pyrethrolone or allethrolone, is replaced by 5-benzyl-3-furylmethanol (*2*). Replacement of the acid component, chrysanthemumic acid, by 2,2,3,3-tetramethylcyclopropanecarboxylic acid changes insecticidal activity very little (*3*).

Although many compounds related to cyclopropanecarboxylic acid esters have been tested, no known highly insecticidal compounds of this type lack either the cyclopropane ring or the ester group. So far all studies indicate that the spatial configuration of the groupings in both the acid and alcohol moieties, and (in particular) a cyclopropane ring containing geminal dimethyl substituents, are important structural features.

Three compounds (Table 1) prepared by us and of special interest are related in structural topography to allethrin (A) but lack either the ester function or the cyclopropane ring. They include the ketone analog (B) of allethrin and the carbamates (D and E) related to compound C, all of which are insecticidal. The finding that the new compounds are insecticidal raises the question of whether or not their mode of action is the same as that of pyrethrin I or allethrin. Our present knowledge of the mode of action of pyrethroids allows this question to be answered only in terms of electrophysiological parameters. Allethrin acts on the giant axons of the cockroach (*Periplaneta americana* L.) to give slight depolarization, increased magnitude and prolongation of the negative afterpotential and eventual blockage of conduction (*4*). The increased negative afterpotential, which is sometimes accompanied by repetitive afterdischarges followed by blockage of conduction, is best explained in terms of sodium and potassium conductances across the nerve membrane (*5*).

Allethrin (A) is obtained by treating ±-*trans*-chrysanthemumoyl chloride with ±-allethrolone in the presence of pyridine (*1*). The ketone analog of allethrin (B) is prepared by a substitution reaction of ±-4-chloroallethrone (*6*) at the methyl group of the acetyl side chain of ±-*trans*-1-acetyl-2,2-dimethyl-3-isobutenylcyclopropane (*7*). This substitution is achieved by way of the ethoxycarbonyl derivative in the presence of sodium ethoxide (*8*). 5-Benzyl-3-furylmethanol (*2*) is converted to the various esters by reaction with the acid chloride of 2,2,3,3-tetramethylcyclopropanecarboxylic acid (*3*), in the presence of pyridine, to give compound C; or with phosgene to give the chloroformate, followed by either 2,2,3,3-tetramethylaziridine (*9*) to give compound D, or diisopropylamine to give compound E (*10*).

Toxicity studies involved treatment of susceptible adult female houseflies (*Musca domestica* L.) and of adult male and female milkweed bugs (*Oncopeltus fasciatus* Dallas) on the dorsum of the thorax and on the ventrum of the abdomen, respectively, with 1 μl of acetone containing each test compound in solution. In certain studies with houseflies, 5 μg of the synergist piperonyl butoxide was applied in 1 μl of acetone solution to the abdomen 0.5 to 1.0 hour before treatment with the insecticide. Mammalian toxicity studies involved intraperitoneal administration of the compounds in dimethylsulfoxide solution to male white mice. Symptoms of poisoning were recorded, but only-24 hour LD_{50} (lethal dose, 50