tremely rare. The major bronchi showed hemorrhage within the lumen, but the lining epithelium was not damaged. There was extensive alveolar hemorrhage both within alveolar walls and into the alveolar lumina. These changes were present within 24 hours after exposure, reached a maximum in 72 hours, and involved all lobes of the lungs. At about 48 hours after exposure, infiltration of alveoli by polymorphonuclear leukocytes and macrophages was prominent. This was best seen in the area of the alveolar ducts. The inflammatory cellular infiltrate had disappeared at the end of 7 days. At this time, the hemorrhage had receded markedly but still persisted in small foci as long as 42 days after exposure. Hemosiderin-laden macrophages appeared within alveoli at 48 hours, and their number reached a peak between 3 and 7 days. Some macrophages with hemosiderin were demonstrable throughout the experimental period. No apparent residual damage was present within the bronchi or alveoli of any of the animals 6 weeks after a single exposure to these enzymes.

The mechanical properties of the lungs need to be evaluated to assess lung damage after multiple enzyme exposures.

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Genetically Controlled Total Deficiency of the Fourth Component of Complement in the Guinea Pig

Abstract. *Guinea pigs with a total deficiency of the fourth component of complement (C4) have been discovered. There was no evidence for the presence of a C4 inhibitor in the serum of these animals. Mating studies indicate that C4 deficiency is transmitted as a simple autosomal recessive trait. A colony of these animals is being established at the National Institutes of Health. They will provide an opportunity to more precisely define the role of complement in immune phenomena and the defense against disease.*

Genetically controlled deficiencies of several components of complement have been described and have helped to elucidate the function of these components. Rabbits with a total deficiency of C6 have been observed in Germany (*1*) and Mexico (*2*) and have been extensively studied. Certain strains of mice lack C5 protein entirely (*3*). Partial C2 deficiency has been found in several human kindred and appears to be due to depressed synthesis of C2 protein (*4*); partial C3 deficiency in humans has also been described (*5*). Most recently, partial deficiency of C4 was detected in three individuals during the screening of 42,000 healthy Japanese. Since these individuals were found in certain isolated groups, it was suggested that there was a genetic basis for their low levels of C4 (*6*). Our purpose now is to report the discovery of guinea pigs with a genetically controlled, total deficiency of C4.

While trying to produce guinea pig immunoglobulin allotype antiserum, noninbred NIH multipurpose guinea pigs were immunized with immune aggregates consisting of heat-killed *Proteus* and strain 2 guinea pig antibodies to *Proteus* in complete Freund's adjuvant. The immune aggregate presumably fixed complement components from the strain 2 guinea pig serum. One month following immunization, one of the male NIH guinea pigs (henceforth referred to as C4 def A) was found to have precipitating antibodies that reacted by Ouchterlony anaylsis with the serum of 25 strain 2 guinea pigs, 25 strain 13 guinea pigs, 8 Abyssinian guinea pigs, and 125 Hartley guinea pigs. Immunoelectrophoresis indicated that this precipitating antibody reacted with a guinea pig serum component of fast gamma mobility. Further analysis revealed that this multipurpose guinea pig was totally deficient in C4 and was producing a specific antiserum to guinea pig C4.

This antiserum gave a single strong line on Ouchterlony analysis with partially purified guinea pig C4 (Cordis Corporation, Miami, Fla.). It was capable of agglutinating cells sensitized with hemolytic antibody and the first and fourth components of complement (EAC1,4) but not cells sensitized with hemolytic antibody alone (EA) or hemolytic antibody and the first component of complement (EAC1). Total hemolytic complement of this serum was zero (*7*), as was the titer of C4 (*8*), but other component titers (including C3 assayed as a complex) appeared to be normal. The antiserum from this C4-deficient animal was used to screen a large portion of the multipurpose breeding colony in the NIH Rodent Production Section. It was found that approximately 2 percent of 250 animals tested from the colony failed to give a precipitin line by Ouchterlony analysis. The serum from these guinea pigs, which included both males and females, also showed a total deficiency of C4 hemolytic activity (*8*). This was also true in an analysis in which rat complement was utilized as a source of the late-acting complement components. The latter method of analysis is capable of detecting the formation of one sensitized activated C1,4 site per cell (SAC1,4/cell) at a serum dilution of 1:470,000 of normal guinea pig serum. Both normal guinea pig serum and partially purified C4 restored the hemolytic activity of the serum of a non–antibody-producing, C4-deficient animal, and C4 could be titrated by adding a dilution of partially purified C4 to the serum of this guinea pig. Thus, there was no evidence of a C4 inhibitor in the serum. The absence of C4 hemolytic activity has been persistent over the 5-month period during which some of these guinea pigs have been observed.

Mating studies have shown that the

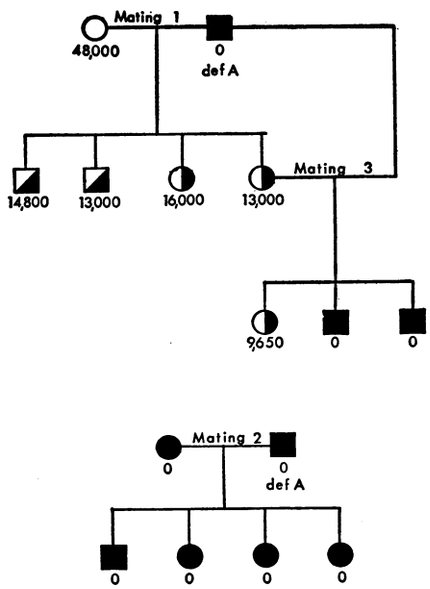

Fig. 1. Mating studies with C4-deficient animals: ■, C4-deficient male; ●, C4-deficient female; ◑, heterozygous female; ◪, heterozygous male; and ○, normal female. The serum C4 titer of each animal is indicated below the appropriate symbol.

observed deficiency of C4 is transmitted as a simple autosomal recessive trait. The animal designated C4 def A was mated with several normal multipurpose guinea pigs. Hybrid animals with intermediate levels of C4, as determined by hemolytic titration of C4, resulted in every case. The results of one such mating (mating 1) are illustrated in Fig. 1. It was also found that serum from these animals gave a distinctly weaker precipitin line in Ouchterlony analysis with the antiserum to C4. In another mating (mating 2) C4 def A was bred with a female multipurpose guinea pig lacking C4; none of the four offspring showed detectable C4 by either hemolytic assay or Ouchterlony analysis (Fig. 1). In a third mating, a heterozygous female, resulting from the mating of C4 def A and a normal multipurpose female, was mated with her father (C4 def A). Three offspring resulted, two of which had no detectable C4 and one of which had heterozygous levels of C4 by hemolytic analysis (Fig. 1) and a weak precipitin line by Ouchterlony analysis (Fig. 2). Not reported in detail are a series of matings between homozygous deficient parents which resulted in 11 progeny; all of these were totally deficient in C4, of which seven were male and four female. A series of matings between a homozygous deficient male and heterozygous females resulted in 14 progeny, seven of which were C4 deficient. Of these, four were male and three female. A single mating between two heterozygous parents resulted in four offspring, two of which were C4 deficient. Both of these animals were female.

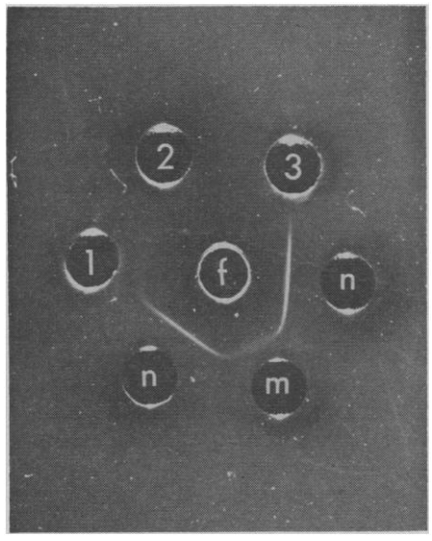

Fig. 2. Ouchterlony analysis of mating 3. Center wall contains antiserum to C4 obtained from def A, who is also the father of this mating (*f*); *m*, heterozygous mother; *1*, heterozygous offspring; *2*, homozygous deficient offspring; *3*, homozygous deficient offspring; *n*, multipurpose guinea pig with normal level of C4.

The evidence presented supports the discovery of guinea pigs with an inherited deficiency of C4. It is likely that homozygous deficient animals have a total absence of C4 hemolytic activity. The technique used for titration of C4 is sufficiently sensitive to detect levels below 0.01 of 1 percent of normal C4. Moreover, the ability of a C4-deficient guinea pig to produce antibodies against C4 is strong evidence in favor of a total absence of the serum protein or at least a portion of the molecule which normally is endowed with this activity. It is possible, however, that C4 is present in an immunochemically modified and inactive form.

This report represents the second description of complement deficiency in guinea pigs. In 1919, a strain of guinea pigs totally lacking hemolytic activity was discovered at the Vermont Agricultural Station (*9*). Although the lesion has been regarded as involving the "classical" third component of complement (now known to include C3, C5, C6, C7, C8, and C9) (*10*), the deficit cannot be defined in modern terms since the strain became extinct several decades ago. The NIH multipurpose guinea pig has been produced at NIH for two decades. The origin of the stock is not currently known.

The C4-deficient guinea pig is the first example of a total deficiency of one of the early components of complement. These animals offer a unique opportunity to study many in vivo and in vitro functions of complement in the laboratory animal. Currently a colony of these animals is being established at the NIH. Under ideal conditions these animals have remained healthy and appear to have normal fertility and infant survival.

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8. To EAC1 (1.5×10^8 cells per milliliter, with 100 effective C1 molecules per cell) in 0.5 ml of dextrose veronal buffer (DVB) containing gelatin, calcium, and magnesium was added 0.5 ml of an appropriate dilution of serum or partially purified C4 in DVB. After incubation of the mixture at 30°C for 30 minutes, 0.5 ml of partially purified C2 in DVB (100 effective molecules per cell) was added. After incubation for 10 minutes, 5.0 ml of normal guinea pig serum, diluted 1:25 in ice cold 0.01*M* ethylenediaminetetraacetate buffer (*p*H 7.4), was added. The mixture was incubated at 37°C for 1 hour. The DVB buffer was prepared according to the method of H. J. Rapp and T. Borsos [*J. Immunol.* **91**, 826 (1963)], except that an equimolar concentration of dextrose was substituted for sucrose.
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