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12. Coatings used on the columns were 5 percent DC-200, 5 percent QF-1, 5 percent mixed bed of DC-200 and QF-1, and 3 percent SE-30 with 6 percent QF-1 in a mixed bed. All coatings were made on DMCS Chromosorb W.
13. Silica gel G was used as the adsorbent. Chromatoplates were developed in *n*-heptane, compounds were identified by cochromatography with pure standards.
14. Values expressed as percent followed by standard error in percent: *p,p'*-DDT, 57.1 ± 12.9; *p,p'*-DDE, 18.0 ± 6.7; and *p,p'*-DDD, 24.9 ± 13.7.
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Pulmonary Hemorrhage in Hamsters after Exposure to Proteolytic Enzymes of Bacillus subtilis

Abstract. *Single exposures of Syrian hamsters by aerosol inhalation or intratracheal instillation to proteolytic enzymes of* Bacillus subtilis *produced massive pulmonary hemorrhage within the first week. Of 46 animals exposed, eight died as a result of extensive hemorrhage. The remainder made uneventful recovery with no apparent residual pulmonary disease 6 weeks after exposure.*

Recent reports in the literature indicate that some workers who handle proteolytic enzymes derived from *Bacillus subtilis* suffer pulmonary reactions. Asthmatic manifestations, as well as symptoms suggestive of a more peripheral pulmonary reaction, have been reported (*1*). Allergic reactions including nasal irritation, coughing, wheezing, and difficult breathing have also been documented (*2*). Severe respiratory symptoms in a number of workers handling enzyme preparations for washing apparently resulted from complex allergic reactions in bronchi and in peripheral lung tissue (*3*). The primary respiratory reactions included bronchospasm, chest pain, and hemoptysis. The Food and Drug Administration and the Federal Trade Commission have received notices of skin rash and other types of allergic reactions among housewives using stain-removing products (*4*). Contact dermatitis has also been reported (*5*). Respiratory disorders resembling asthma and influenza are considered serious problems among workers in detergent plants (*3*).

The use of enzymes, of both plant and bacterial origin, has increased in recent years and now involves large numbers of persons, both in the manufacture and in the application of such enzymes. More recently the use of bacterial enzymes has reached the level of the household user and they have thus been introduced into millions of homes. Their possible health hazards have not been adequately investigated (*6*).

Experimentally, enzymes of plant origin were employed as inhalants and also applied by intratracheal instillation to determine the pathological effects on the tracheobronchial tree and pulmonary parenchyma of laboratory animals. Papain, a plant protease, as well as ficin and bromelain, has been shown to produce emphysema in hamsters when it was inhaled (*7, 8*).

Table 1. Treatment of animals and time of killing after treatment. Abbreviations: A, aerosol; I, intratracheal. Results are given as the number of animals in each category.

Time of death after treatment	Experimental		Control	
	Alcalase (A)	Maxatase (I)	Saline (A)	Saline (I)
24 hours	4	4	2	1
48 hours	3	4	2	1
72 hours	1	4		1
7 days	4	2	2	1
10 days		3		
14 days	3	2	2	1
20 days		1		
28 days	4	3	2	
42 days	3	1	2	1

In the present studies, similar experiments with bacterial enzymes were undertaken to explore the possible pathological changes in the lungs of laboratory animals. The enzymes used were Alcalase (*9*) and Maxatase (*10*), two proteolytic enzyme products derived from *Bacillus subtilis,* which are utilized in the manufacture of a variety of household enzyme detergent agents. Alcalase is a finely divided powder containing approximately 60 percent sodium sulfate, 5 percent sodium chloride, and 35 percent organic material of which 5 to 10 percent is the enzyme (*2*). Maxatase is a fine powder, the major part of whose enzyme activity is proteolytic, but according to the manufacturer it also contains a small amount of α-amylase.

The animals used in the current experiments were Syrian golden hamsters of both sexes; age range was 37 to 165 days.

The solubility of the two enzymes in water determined the route of administration. Alcalase was administered as an aerosol and Maxatase by intratracheal instillation. Three percent solutions of the enzymes were administered by methods previously described (*7, 11*). A total of 46 animals were treated with the enzymes and an additional 18 animals served as controls; these received physiological saline. The disposition of the animals is indicated in Table 1.

A slight loss of weight was exhibited by a number of the experimental animals during the first few days after exposure. Some animals developed severe respiratory symptoms characterized by dyspnea, rales, and cough. A bloody nasal discharge was observed in a few. Eight of the 46 experimental animals died; six within the first 24 to 48 hours after treatment, one after 7 days, and one after 20 days. In each case, massive lung hemorrhage was observed. The remainder were killed at prescribed intervals of 24, 48, and 72 hours, as well as 7, 14, 28, and 42 days after exposure. Complete necropsies were performed on all animals, special attention being given to the respiratory organs. The method of fixation of the lungs and the histologic staining techniques employed have been described earlier (*12*).

The tracheas of the experimental animals failed to show significant pathologic alteration. Loss of cilia, usually the first evidence of damage, was ex-

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