# Enhanced Susceptibility of Pertussis Inoculated Mice to Pollen Extract.* (24785)

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It is now well known that mice inoculated with *Hemophilus pertussis* vaccine develop enhanced sensitivity to lethal effects of histamine, serotonin, endotoxin, peptone, and anaphylactic shock(1). The ensuing data will demonstrate that pertussis-inoculated mice can also be killed with doses of water soluble extract of pollen of rye grass (*Lolium perenne*†) which are not lethal to uninoculated animals. The effect of administration of mixtures of water soluble extracts of rye grass pollen (WSR) and rabbit antisera to WSR will also be discussed.

*Materials and methods.* Swiss-Webster male and female mice weighing 17 to 25 g were inoculated intraperitoneally with 0.1 ml of pertussis vaccine (Cutter) containing 50 billion organisms/ml. Five days later, pertussis-injected as well as uninoculated animals were challenged i.v. with either 0.4 ml or 0.1 ml of a solution of WSR containing 50 mg of extract/ml of saline. The lyophilized WSR had been prepared by the method of Sehon *et al.*(2) and contained 0.04 mg of N mg of extract. Antisera were obtained from rabbits injected with WSR in Freund's adjuvant for 2 months. Such sera had titers of 1:4000 to 1:8000 as measured by hemagglutination technic(3) employing WSR coupled to red cells (*via* bis diazotized benzidine) as the antigen. Sera from rabbits bled the previous day were used in preparation of the serum-pollen mixtures. Fifty mg of WSR was added to each ml of unheated serum and the combination incubated at 37°C for 30 minutes; no precipitation occurred. One-tenth ml of this mixture was then injected intravenously into mice inoculated with pertussis vaccine 5 days previously. Deaths were noted for 24 hours.

*Results.* The data in Table I indicate that 50% of pertussis-inoculated mice survived injection of 5 mg of WSR and only 18% survived 20 mg of this material. In contrast, normal, uninoculated animals suffered no fatalities from equivalent challenge doses of WSR. Additional experiments indicated that pertussis-injected pollen-sensitive mice could be protected from lethal effects of WSR with 0.5 mg of cortisone (Merck) administered 6 hours before challenge. The antihistamine pyranisamine maleate (Neoantergan) however, offered no protection.

The data in Table II indicate that only 29% of pertussis sensitized mice challenged with pollen + antiserum survived; in contrast 100% of pertussis inoculated mice challenged with antiserum alone or pollen alone remained alive. The difference between these percentages is statistically significant ($p = .01$). Uninoculated mice did not succumb to the pollen-antiserum mixture, while 8 of 9 pertussis-injected mice survived the combination of WSR in normal rabbit serum (NRS). The majority of deaths occurred within 2 hours after administration of serum-pollen mixtures and resembled anaphylactic shock. However, the convulsions preceding death seemed more severe than those observed in mouse anaphylaxis. The possibility that addition of WSR to its corresponding antiserum resulted in formation of soluble antigen-antibody complexes with anaphylaxis-inducing properties was considered. However, the fact that normal rab-

TABLE I. Enhanced Susceptibility of Pertussis Inoculated Mice to Pollen Extract.

| Mice sensitized with | Mice challenged with | Survivors /Total | % survivors |
|---|---|---|---|
| Pertussis vaccine | 5 mg WSR* | 7/14 | 50† |
| *Idem* | 20 | 3/17 | 18‡ |
| Nothing | 5 | 15/15 | 100† |
| " | 20 | 14/14 | 100‡ |

* Water soluble rye grass extract.
†‡ Difference between these percentages is statistically significant ($p = .01$).

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† Sharp and Sharp, Everett, Wash.

bit serum (NRS) plus WSR killed one pertussis-inoculated mouse (Table II) led to experiments with additional normal rabbit sera. The data in Table III indicate that normal rabbit serum also may potentiate the effect of WSR in pertussis inoculated mice. Of 27 mice challenged with NRS plus WSR, 30% survived; of 15 mice challenged with WSR alone, 86% survived The difference between these percentages is statistically significant ($p = .01$). It should also be mentioned that normal rabbit serum which enhanced toxicity of WSR (Table III) had a hemagglutination titer of zero when tested with WSR coupled to red cells.

*Discussion.* Subsequent experiments involving a number of different sera confirmed the above results; however, the occurrence of a significantly greater number of deaths in mice injected with pollen-serum mixtures is quite irregular. Rabbit serum alone did not kill any animals. Pollen alone did not kill more mice than pollen plus serum. With pollen-serum mixtures one could kill a significantly greater percentage of pertussis sensitized mice on a particular day; however one could never be sure of duplicating the result on succeeding day. The reason for this variability is unknown, but such irregularities with different serum-antigen mixtures have been reported (4). The fact that normal rabbit serum (titer of zero) and immune rabbit serum (titer of 1:8000) both caused a greater number of deaths when mixed with WSR suggested that the combination of rabbit serum and WSR lead to formation of anaphylatoxin. Rocha e Silva and Aronson(5) reported that agar incubated with guinea pig serum produced anaphylatoxin, whereas agar incubated with citrated plasma did not do so. In addition, heating of serum at 60°C for 60 minutes before activation with agar completely destroyed its capacity to be activated. We found that the mixture of WSR and citrated plasma is as active as the combination of WSR and serum. Experiments with heated serum, however, have thus far been inconclusive. Although the question of anaphylatoxin is unsettled, enhanced activity of mixtures of pollen and *normal* serum should be kept in mind. Recently, the increased biological activity of a mixture of pollen extract plus its corresponding antiserum was attributed to formation of soluble antigen-antibody complexes, although no control with normal serum was mentioned(6).

Finally, it is of interest that pertussis inoculated mice possess enhanced sensitivity to pollen extract when they are also histamine sensitive. The possibility must therefore be considered that pollen extracts have histamine releasing properties which may account for the notoriously high incidence of false positive skin tests with these materials.

TABLE II. Effect of Injecting Mice with a Mixture of Pollen Extract and Its Homologous Antiserum.

| Mice sensitized with | Mice challenged with | Survivors /Total | % survivors |
|---|---|---|---|
| Pertussis vaccine | WSR*+ antiserum† | 5/17 | 29§ |
| *Idem* | WSR | 21/21 | 100§ |
| " | Antiserum | 15/15 | 100 |
| " | WSR + NRS‡ | 8/ 9 | 89 |
| " | NRS | 15/15 | 100 |
| Nothing | WSR + antiserum | 15/15 | 100 |

* Water soluble rye grass extract.
† Titer 1:8000.
‡ Normal rabbit serum.
§ Difference between these percentages is statistically significant ($p = .01$).

TABLE III. Effect of Injecting Mice with a Mixture of Pollen Extract and Normal Rabbit Serum.

| Mice sensitized with | Mice challenged with | Survivors /Total | % survivors |
|---|---|---|---|
| Pertussis vaccine | WSR* + NRS† | 8/27 | 30‡ |
| *Idem* | WSR | 13/15 | 86‡ |
| " | NRS | 10/10 | 100 |

* Water soluble rye grass extract.
† Normal rabbit serum—titer of 0.
‡ Difference between these percentages is statistically significant ($p = .01$).

*Summary.* Pertussis inoculated mice have an enhanced susceptibility to lethal effects of water soluble extract of rye grass pollen. A mixture of this pollen extract with specific immune rabbit serum or normal rabbit serum will kill more pertussis inoculated mice than pollen extract alone.

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## Spontaneous Hereditary Diabetes Mellitus in Chinese Hamster (*Cricetulus griseus*). 1. Pathological Findings.*† (24786)

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Spontaneous diabetes mellitus has been reported in a number of nonrodent species, including cattle, horses, pigs, sheep, dogs and cats(1). Estimates of relative frequency of this type of diabetes in domesticated animals vary considerably, the generally accepted figure is approximately 1:1000(1). The reported incidence for dogs ranges from 1:260 to 1:800, and for cats approximately 1:1500. The disease occurs in all age groups of animals; however, in dogs there is a marked peak at upward of 8 years. The majority of diabetic dogs are females, especially neutered females, whereas in cats, diabetes is more common in males(2-4). Microscopic findings most frequently observed in animals are "hydropic degeneration" of the islets, hyaline and amyloid changes. Diminution in number and size of islet tissue, and even complete absence were also described. In dogs, diabetes mellitus is less frequently a disease of islet tissue proper than a probable sequel to acute pancreatitis common in obese animals(2). Reports of spontaneous diabetes in laboratory rodents appear to be lacking. The literature concerning experimental surgical and drug-induced diabetes, however, is extensive(4). The present paper describes clinico-pathological findings in spontaneous diabetes mellitus of Chinese hamsters (*Cricetulus griseus*) maintained at this institution(5). The evidence indicates that it is hereditary. The genetic and hereditary aspects will be the subject of a separate report. In addition, preliminary data on chemotherapy, screening of hypoglycemic agents and metabolic studies in these hamsters will be published elsewhere.

*Symptoms.* Abnormal signs are usually observed from 18 days of age and later develop into either mild or severe cases. Both sexes seemed affected with about equal frequency. Diabetic hamsters exude a peculiar pungent body odor; the fur is damp and the abdomen stained yellow and soiled with urine. Some hamsters excreted up to 50-70 ml of urine in 24 hours, reflecting severe polyuria. Metabolic studies of fluid intake, urine excretion and insulin assays are in progress. At onset of symptoms, the hamsters gain considerable weight, become sluggish and severely dehydrated. Blindness is noted occasionally, the pupils turning gray, or the cornea becoming opaque. The mortality in untreated animals is extremely high. Sudden death may result from the mildest stress, such as transfer of animals from one cage to another, or by slight changes in room temperature (5-8°F). *Laboratory findings.* Clinical biochemical investigations were originally restricted to basic diagnostic determinations such as blood and urine sugar, NPN, hematocrit, urine ketone bodies, and specific gravity. Blood sugar levels, for normal hamsters, ranged 103 to 116 mg %, averaging 110 ± 6 mg %. Diabetic hamsters had levels between 200 and 600 mg %. An isolated urine sugar value in one animal was over 828 mg %. Specific gravity of urine was

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