# REVERSE PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIGS USING EXTRACT OF TIMOTHY GRASS POLLEN

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UDC 612.79.017.32

Two variants of the method of reproduction of reverse passive cutaneous anaphylaxis in guinea pigs are described. A rabbit antiserum against timothy grass pollen and an extract of the pollen of this species are used.

The phenomenon of reverse passive cutaneous anaphylaxis (RPCA), by means of which different antigens can be compared in an experiment on the same animal, has been described by a number of workers [2, 4, 5]. By means of this method, antibodies undetectable by the passive cutaneous anaphylaxis (PCA) test can be recorded [4, 5]. However, according to the available data, this phenomenon can be difficult to reproduce. Even if antigens such as serum proteins are used, the RPCA is frequently unsuccessful in guinea pigs [3].

In the investigation described below the possibility of reproducing the RPCA in guineapigs by the use of rabbit antiserum against timothy grass pollen and the corresponding antigen was studied.

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Fig. 1. Reverse passive cutaneous anaphylaxis in a guinea pig after intradermal injection of extract of timothy grass pollen and intravenous injection of specific rabbit antiserum. 1-4) Extract of timothy grass pollen injected intradermally in various dilutions; 5) extracting fluid injected indradermally (control).

## EXPERIMENTAL RESULTS

Defatted timothy grass pollen was extracted with Evans' fluid [1] in the proportion of 1 g pollen to 10 ml liquid. The extract was sterilized by filtration and the sterility subsequently verified.

The antiserum was obtained from rabbits immunized with nondefatted timothy grass pollen together with Freund's adjuvant.

Noninbred guinea pigs weighing 250-300 g, with white fur, were used in the experiments. The hair from the sides of the trunk was shaved 18-20 h before the experiment.

The RPCA phenomenon was reproduced in two ways. In the first method the guinea pigs received an intradermal injection of 0.1 ml of pollen extract at each point (0.1 ml of Evans' fluid in the control), and 45-60 min later an intravenous injection of 1-1.5 ml antiserum with the dye Evans' blue (10 mg per animal).

In the second variant, to obtain a reaction each animal was given an intravenous injection of 1 ml antiserum, and 24 h later an intradermal injection of

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0.1 ml pollen extract or Evans' fluid (control). An intravenous injection of a 10% solution of Evans' blue was given 30-40 min later (1 ml per animal).

The result of the test in both cases was recorded 30-40 min after the injection of Evans' blue on the inner surface of the skin.

Altogether 6 experiments were performed on 26 animals.

## EXPERIMENTAL RESULTS

The experiments showed that the methods described above are suitable for obtaining a clearly defined RPCA by the use of extract of timothy grass pollen and the corresponding antiserum (Fig. 1).

The RPCA was reproduced more regularly by the second variant of the method. However, this reaction developed differently in the animals: in some guinea pigs it was weak in intensity (the diameter of the stain at the site of injection was less than 5 mm even when high concentrations of allergen were given).

The question of the genesis of the RPCA is debatable, but great importance is attached to the ability of the antigens to become fixed to the skin tissues [3, 4].

As a result of the present experiments two hypotheses can be put forward. In the first it is assumed that extracts of timothy grass pollen are firmly bound to the skin. It is also possible that success in the reproduction of the RPCA is much more dependent on the individual characteristics of the animals, for when the same antigen—antibody system was used appreciable differences in the intensity of the RPCA were observed in different animals.

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