MURINE PASSIVE CUTANEOUS ANAPHYLAXIS TEST (PCA) FOR THE "ALL OR NONE" DETERMINATION OF ALLERGENICITY OF BOVINE WHEY PROTEINS AND PEPTIDES

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1. INTRODUCTION
The study of allergenicity of proteins and peptides has attracted considerable research activities in the past two decades, utilizing several in vitro and in vivo techniques. The usefulness of the in vitro techniques, such as immuno-precipitation tests (1), radio-allergo-sorbent-test (2) and enzyme-linked-immuno-sorbent-test (3), is limited due to a poor correlation between results obtained by these tests and provocation tests in the patients. Consequently, the in vivo assays, passive cutaneous anaphylaxis test (PCA) (4-7) and anaphylactic shock models (3) using guinea pigs in both cases, are still widely employed and recommended when assessing the allergenicity of various compounds.

The present report describes the use of mice in PCA for the in vivo determination of the allergenicity of bovine whey proteins and peptides, and advocates for a more general replacement of guinea pigs by mice.

2. MATERIALS AND METHODS
2.1. Animals. The mice employed in the PCA test were as follows:

Group A: Inbred BALB/c Bom maintained at our laboratory for several generations on a diet without milk proteins (Ewos Brood stock feed-R-3, DK 8600, Silkeborg, Denmark)

Group B: Inbred BALB/c Bom maintained for several generations on pellets containing 15% skimmed bovine milk proteins (The Panum Institute, University of Copenhagen, Denmark).

Group C: BALB/c Bom maintained by random breeding in a closed colony at the Royal Veterinary and Agricultural University, Copenhagen. These mice were maintained on the milk containing diet.

Mice of both sexes aged 5-8 weeks were used and given water and feed ad libitum.

2.2 Antibody preparation. Murine antiserum against whey proteins was prepared as described previously (7), using low doses of protein for high IgE production as suggested by Jarrett and colleagues (8).

2.3. Antigen preparations. Concentrated whey proteins (CWP), hydrolyzed CWP and peptide fractions of partially hydrolyzed CWP (figure 1) were prepared as described previously. The pro-
Gelfiltration of hydrolyzed whey protein on Biogel P-30. Samples of hydrolyzed whey protein (5.0 ml) were separated and the eluted peptides pooled as indicated. Molecular weight markers were bovine serum albumin (BSA), lactoglobulin (LG), lactalbumin (LA), trypsin inhibitor (A), insulin chain B (B) and insulin chain A (C).

Protein content of these preparations was measured with the micro Kjeldahl method or by measuring the absorbance at 280 nm using bovine serum albumin (Sigma) as standard (7).

2.4. Evans Blue preparation. A solution of 10 mg/ml of Evans Blue (Sigma) in isotonic saline was prepared and stored at -20 °C in aliquots of 10 ml until use. Samples of proteins ranging from 0 to 4800 μg were mixed with 3.0 ml of the Evans Blue solution just prior to intravenous administration.

2.5. Passive cutaneous anaphylaxis test. The mice were anaesthetized by intraperitoneal injection of propanidid (Sombrevin) (500 mg/kg), and the fur on a 3 x 4 cm square on the backside was removed using an electric razor. Samples of 100 μl, containing antibody preparation mixed with Freund's incomplete adjuvant (1:1), were injected intradermally in the center of the shaved area using 26-gauge needles. The use of FIA resulted in well-defined local colour reactions, whereas antiserum mixed with saline (1:1) gave more diffuse, less intensive blue colouration of the entire backside.

Following a period of 3 hours after administration of antibody, samples of 250 μl of the antigen-Evans Blue solutions were injected intravenously in the tail vein. The blue skin reactions of the surface were recorded 30 min after the injection, at which time the mice were killed by dislocation of the neck and the skin removed to observe the colour reaction on the inside (corium) of the skin.

3. RESULTS
3.1. Sensitivity of the PCA method. Using an intravenous administration of 160 μg whey protein in 250 μl Evans Blue solution the employed murine antibody preparation could sensitize the mice even at a 128-fold dilution.

The sensitivity of the PCA method was studied using the murine antibody preparation at a 1:8 dilution. The sensitivity was studied in three different groups of mice. A dilution series ranging from 0.04 μg/ml to 400 μg/ml of whey protein in Evans Blue solution was prepared. At each dilution step three mice of each group passively sensitized with the mice antiserum were challenged intravenously with 250 μl antigen-Evans Blue Solution. The sensitivity results are presented in table 1.