

tal C.M.V. infection described by Rola-Pleszczynski et al.¹³ The maternal immune defect may impair her ability to contain viral spread and may facilitate transplacental transmission of C.M.V. 3 of the mothers who had the antigen-specific defect were studied during the early post-partum period, whereas the mother studied 4 yr post partum demonstrated a normal proliferative response to C.M.V. Thus it appears that depressed lymphocyte responsiveness to C.M.V. may be a reflection of the physiological immunosuppression of pregnancy and may eventually return to normal.

A maternal defect may facilitate transmission of the virus to the fetus but does not explain persistent excretion of virus by the infant. The cell-mediated C.M.V.-specific defect which we have found in the infants could account for persistent viral infection. This postulate can now be directly examined prospectively by following the children's in-vitro cell-mediated immunity to C.M.V. and their viruria. Correlation between recovery of cell-mediated immunity and cessation of viral excretion would be evidence for a cause/effect relationship.

Schauf et al. studied 3 children with C.M.V. infections and found that the percentage of T lymphocytes was depressed in all 3, and 2 also had depressed lymphocyte responses to mitogens.¹⁴ They postulated that these immunological abnormalities may explain the prolonged C.M.V. viruria. Unlike their patients, ours had normal numbers of T lymphocytes and normal generalised cell-mediated immunity. Our patients illustrate that prolonged C.M.V. infection with viruria need not be accompanied by a generalised defect in cell-mediated immunity but may be the result of a C.M.V.-specific cell-mediated defect.

If the C.M.V.-specific cell-mediated immune defect is responsible for failure to clear virus from infected organs, restoration of lymphocyte function with such agents as transfer factor may be beneficial. We are now exploring this possibility by administering transfer factor prepared from lymphocytes demonstrating C.M.V.-specific proliferation to children with congenital C.M.V. infections.

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RESPIRATORY ALLERGY TO URINE PROTEINS OF RATS AND MICE

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Summary Five atopic laboratory workers with asthma and other manifestations of allergy to rats and mice were sensitive to low-molecular weight urine proteins—an α_2 -globulin in the rat and prealbumin in the mouse. Positive skin prick and inhibition test reactions to the urine proteins were obtained and radioallergosorbent tests (R.A.S.T.) for specific IgG antibody were also positive. These findings show that proper control of the collection and disposal of the animals' urine is necessary to minimise the likelihood of sensitisation.

Introduction

ALLERGIC respiratory and skin symptoms are not common in animal handlers. Sensitivity to rats and mice has been reported in more than 10% of workers,¹ but may be unable to continue their work as a result. Identification of the relevant allergens and their source is necessary for control of this problem. In five asthmatic subjects sensitive to rats or mice, or both, the urine was found to be a major source of allergenic proteins.

Patients and Methods

Patients

Four patients had a history of hayfever and one of asthma. All five gave a positive reaction to one or more common allergens. All handled rats and four also handled mice.

Symptoms appeared within 1 year in four patients, and 4 years in the fifth patient. The initial symptoms were rhinitis of rapid onset and conjunctivitis on exposure to animals, and urticaria where the animals' feet made contact with urine on the skin. Asthma developed after a few weeks, at first several hours after exposure, and in one patient only after exercise. Within a year all five had asthma after a few minutes' exposure.

When the patients were examined in hospital after being separated from the animals for several days no abnormal results were found in pulmonary function tests or on radiographic examination of the chest.

Test Preparations

Hair. Shaved from the animals, was defatted in ether, extracted in Coca's solution, Seitz filtered, dialysed against distilled water, and freeze-dried.

Urine was collected and pooled from male and female Sprague-Dawley CFY rats two to three months old and from 6-month-old male and female CFLP mice. It was dialysed against distilled water, centrifuged, and freeze-dried.

Electrophoresis of Rat and Mouse Urines in Agarose with Protein Staining (fig. 1)

Mouse urine.—More than 90% of the mouse urine extract was a prealbumin containing two antigenically similar components. Traces of protein were also present in the α and γ globulin regions.

Rat urine.—This contained several proteins, most of which were in the serum albumin and α_2 -globulin regions.

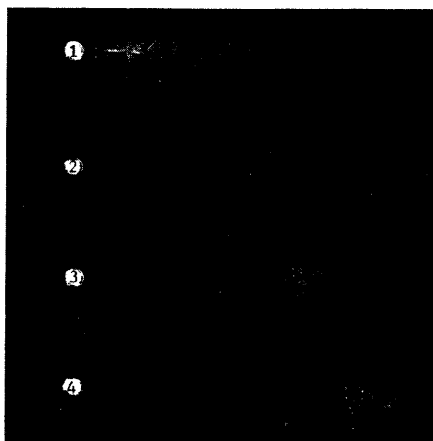


Fig. 1—Gel electrophoresis of (1) rat urine extract, (2) rat serum, (3) mouse serum, and (4) mouse urine extract.

The rat and mouse urinary α_2 -globulin proteins were antigenically identical, but there was no antigenic similarity between the major mouse prealbumin and the rat α_2 -globulin components.

Sephadex G100 Fractionation of Urinary Proteins

3 major fractions were obtained by gel filtration from both rat and mouse urines and were compared by two-dimensional immunoelectrophoresis against specific rabbit antisera.²

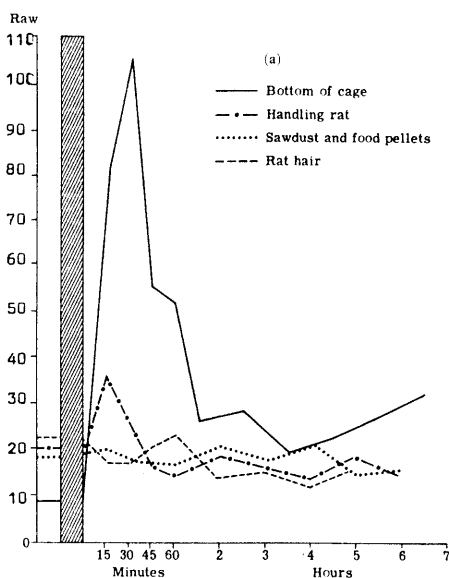


Fig. 2(a)—Immediate asthmatic reaction (increased airways resistance, Raw) to dust from the bottom of rat cage; weaker reaction to handling rats. No reactions to rat hair extract or foodstuffs. 2(b)—Immediate reaction to rat urine preparation and no reaction to rat serum.

Fraction 1 contained high-molecular-weight macroglobulins (cf. blue dextran). There was cross-reaction between the rat and mouse urinary α_2 -macroglobulins.

Fraction 2 contained proteins of intermediate molecular weight (cf. bovine serum albumin 68 000 daltons). The proteins which cross-reacted with serum proteins, especially the albumin and globulins in rat urine, were in this fraction.

Fraction 3 contained large amounts of low-molecular-weight urinary proteins (cf. cytochrome C, 12 400 daltons)—in the mouse a prealbumin and in the rat an α_2 -globulin.

The test materials were membrane-filtered and heated at 56°C for 1 hour before being used for skin and inhalation tests. They were put up for inhalation tests in Coca's fluid, with 50% glycerol added for skin tests.

Skin Prick Tests

Tests were made with:

(1) 23 common inhalant and food allergens and rat and mouse hair extracts (Bencard).

(2) Rat and mouse urine extracts in concentrations of 0.01, 0.1, and 1 mg/ml, and rat and mouse serum diluted 10 and 100 times.

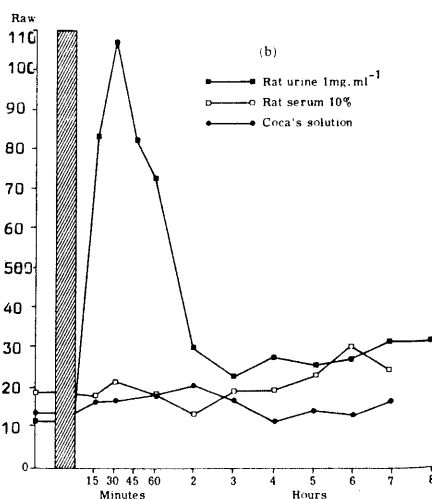
(3) Fractions 1, 2, and 3 from rat urine and fraction 3 from mouse urine in concentrations of 0.01, 0.1, and 1 mg/ml.

Inhalation Tests

These tests were made with solutions of the urine extracts in concentrations of 0.01, 0.1, 1, and 10 mg/ml, hair extracts in concentrations of 1 and 10 mg/ml, and serum diluted 10 and 100 times. Aerosols in air were used at a flow rate of 8 l/min through a Wright's nebuliser.¹ One test was made per day, starting with a control test with Coca's fluid. The initial test was made with a concentration ten times less than that giving a 3 mm weal on prick testing. If negative, a ten-fold greater concentration was used the next day. A fall in forced expiratory volume (F.E.V.₁) of 15% or more or an equivalent change in airways resistance (Raw) was taken as positive and no further tests were made with the particular allergen.

Precipitin Tests

These were made by agar-gel double diffusion* with the rat and mouse sera and urine extracts.



Radioallergosorbent Tests for Specific IgE Antibody

The test preparations were coupled to cyanogen-bromide-activated paper discs.³ Control tests on sera from all five patients were made with three cord-blood sera in the first set of tests and in tests on sera from four patients several months later. Five cord-blood and five blood-bank sera were used as controls.

Results**SKIN PRICK TESTS AND INHALATION TESTS****Rat Allergens**

(1) *Rat urine*.—Immediate skin and bronchial reactions were obtained with whole-urine extracts (1 mg/ml) in three patients; in the two other patients, combined immediate and non-immediate asthmatic reactions were obtained with a concentration of 0.01 mg/ml. Fig. 2(a) shows that there was a reaction to the dust from the bottom of the cage and to handling of the rat, but not to the extract of rat hair. Fig. 2(b) shows the reaction to the rat-urine extract.

With *urine protein fractions* the major skin allergen in all five patients was found in fraction 3, which contained the urine specific γ_2 -globulin but no serum-derived components. Reactions were obtained at 0.001 mg/ml compared with 0.01 and 0.1 mg/ml of the original material and the other fractions.

(2) *Rat serum*.—Three patients gave immediate prick test reactions; only one of them gave an immediate asthmatic reaction. Another who did not give immediate skin or bronchial reaction to the serum gave a non-immediate asthmatic reaction.

One patient gave comparable reactions to the inhalation test with rat serum and the whole urine extract; the others reacted only, or more vigorously, to the urine extract.

(3) *Rat-hair extract*.—Four of the five patients gave immediate prick test reactions to the rat-hair extract (Bencard). There was no asthmatic reaction to freeze-dried rat-hair extract at 10 mg/ml in two subjects giving positive reactions to the urine at 1 mg/ml.

Mouse Allergens

(1) *Mouse urine*.—The four patients handling mice gave immediate skin reactions to comparable concentrations of the whole-urine preparation and fraction 3 at 0.01 to 1 mg/ml. Inhalation tests gave immediate reactions in 3 subjects at 0.01 to 10 mg/ml, and one other gave a combined immediate and non-immediate asthmatic reaction to 0.01 mg/ml. The patient who was not exposed to mice gave neither skin nor bronchial reactions.

(2) *Mouse serum*.—Three of the four mouse handlers gave immediate skin reactions and bronchial reactions, one giving a non-immediate reaction as well. The non-exposed patient gave no reactions.

(3) *Mouse-hair extract*.—All four of the mouse handlers gave positive immediate skin prick test reactions. No asthmatic reaction was elicited in either of the skin-test-positive patients tested with freeze-dried extracts at 10 mg/ml.

With *urine protein fractions*, skin test reactions to fraction 3, the prealbumin of mouse urine, and the major bulk of the urinary protein were equivalent to those of the whole urine extract. The patient who did not handle mice did not react.

RADIOALLERGOSORBENT TESTS

R.A.S.T.s for specific IgE antibodies showed higher values and were all positive for the relevant urine extract. There were 2 positive tests to rat serum and 3 positive to mouse serum. Tests repeated with sera taken from the patients some months later showed similar results with slightly lower values.

PRECIPITIN TESTS

Tests with unconcentrated sera against the rat mouse urinary and serum extracts were negative.

Discussion

These results show that the urine of rats and mice is an important source of allergenic protein. Sensitivity to such heterologous proteins can follow inhalation of airborne dust contaminated with urine, and a case of extrinsic alveolitis caused by allergy to rat serum proteins has been reported. In the rat urine, the bulk of the urine proteins were serum proteins (in addition to specific urine α_2 -globulin)⁶ whereas in the mouse urine more than 90% was low-molecular-weight urine albumin. These low-molecular-weight proteins were demonstrable in the animals' sera by crossed immunoelectrophoresis. The mouse prealbumin has, however, been reported⁷ in the serum in amounts 200 to 400 times less than in the urine. The quantity of urinary protein is greater in males, increasing considerably with age.

All five patients were atopic, and it seems likely, in mind the findings in workers manufacturing enzymes of *Bacillus subtilis* for biological detergent that such subjects would be sensitised more readily. Positive and stronger skin and bronchial reactions elicited by the whole-urine extracts than by the animal sera extracts. Similarly, higher values for specific antibodies were obtained with whole-urine extract skin prick tests than the low-molecular-weight urinary proteins provoked the strongest reactions. Low-molecular-weight proteins (10 000–25 000 daltons) in extracts of rat and mouse pelts have also been reported as being most potent in eliciting skin test reactions in sensitive subjects.⁹

Reduction of sensitisation in workers handling laboratory animals will require stricter control of the collection and disposal of the animals' urine, a source of potential allergenic heterologous proteins.

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