

# Experimental intestinal hypersensitivity. Effect of four anti-allergic drugs on protein uptake, permeability to sugars and mucosal mast-cell activation

M. W. TURNER, P. BOULTON\* and S. STROBEL

*Molecular Immunology Unit, Institute of Child Health, London, UK*

## Summary

The ability of four drugs with anti-allergic action to modulate the uptake of bystander protein, lactulose/rhamnose permeability ratios and mast cell activation was studied in rats presensitized with egg albumin in alum and challenged intraduodenally with the same antigen. Beclomethasone dipropionate (BDP) and nedocromil both significantly reduced the uptake of the bystander protein, bovine serum albumin ( $P < 0.002$  and  $P > 0.02$  respectively). BDP also significantly reduced sugar permeability ( $P < 0.01$ ). In animals with elevated lactulose/rhamnose permeability ratios we confirmed our earlier observation of a significant correlation between levels of the specific mucosal mast cell protease Rat Chymase II (RChyII-previously known as RMCPII) and the sugar ratios. None of the drugs had any influence on the levels of mast cell protease II released following challenge and there was no correlation between the histological light microscopic appearance of the mast cells and the experimental treatment administered. Our results suggest that in the gut the pharmacological effect of anti-allergic drugs may be complex. Some, such as nedocromil, appear to act only on the mechanisms underlying increased protein uptake whereas others, such as BDP, appear to abrogate both increased protein uptake and increased sugar permeability.

*Clinical and Experimental Allergy*, Vol. 25, pp. 448–455. Submitted 23 May 1994; revised 15 July 1994; accepted 25 October 1994.

## Introduction

Direct systemic and/or local effects of intestinal hypersensitivity reactions are difficult to investigate experimentally in man and there has been a need for a satisfactory *in vivo* animal model which would permit investigations into the underlying mechanisms. One such model is that of Bloch and Walker [1] in which rats are rendered hypersensitive to one protein antigen (e.g. ovalbumin [OA]) and then when challenged locally in the gut absorb increased amounts of an unrelated 'bystander' protein (e.g. BSA) which can be detected in the serum. We were able to confirm this observation

whilst simultaneously measuring changes in gut permeation to low molecular weight sugars in rats with unequivocal evidence of mucosal mast cell activation [2]. The model appeared to be potentially useful for investigations on the efficacy of anti-allergic drugs in mucosal hypersensitivity reactions and a small pilot study of protein uptake in rats receiving disodium cromoglycate and beclomethasone dipropionate before challenge suggested that the model could indeed be adapted [3].

Using the above model we have now investigated the modulatory action of four different anti-allergic drugs by performing simultaneous evaluations of the uptake of a 'bystander' protein, sugar permeability and mucosal mast cell activation as indicated by release into the circulation of Rat Chymase II (RChyII) in presensitized rats challenged intraduodenally with antigen. Histological evidence of mast cell degranulation was also sought in

Correspondence: Professor M. W. Turner, Molecular Immunology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.

\*Present address: Glaxo Group Research, Greenford, Middlesex, UB6 0HE, UK.

**Table 1.** Experimental protocol used to investigate modulatory action of various anti-allergic drugs (modified after Bloch and Walker [1] and Turner *et al.* [2])

| Stage                                                                                            | Procedure                                                                                                                                                   |
|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Immunize                                                                                         | 100 µg OA (i.p.) with alum<br>14 days                                                                                                                       |
| Oral feed                                                                                        | Administered in 2 ml<br>0.15 M NaHCO <sub>3</sub> - 0.15 M NaCl:<br>Either 1 g BSA alone or 1 g<br>BSA + drug<br>1 h                                        |
| Laparotomy                                                                                       | Instil into lumen of small intestine<br>a mixture of:<br>100 mg OA<br>140 mg lactulose<br>20 mg rhamnose<br>in 0.1 M K <sub>2</sub> HPO <sub>4</sub> pH 7.0 |
| Blood collection*                                                                                | 1, 3, 6 h                                                                                                                                                   |
| Urine collection **                                                                              | 0-6 h                                                                                                                                                       |
| *Blood samples were assayed for maximum BSA levels (radio-immunoassay) and RChyII levels (ELISA) |                                                                                                                                                             |
| **Urine samples were analysed for lactulose/rhamnose ratios by thin layer chromatography.        |                                                                                                                                                             |
| Sham-immunization group:                                                                         | Identical protocol except that animals were immunized with alum alone.                                                                                      |
| Sham-challenge group:                                                                            | Identical protocol except that OA was omitted at laparotomy stage.                                                                                          |

tissue sections obtained from the jejunum of selected animals.

## Materials and methods

### Experimental protocol

The basic protocol used was based on that described by Bloch and Walker [1] and Turner *et al.* [2] and is illustrated in Table 1.

Adult Sprague-Dawley rats were obtained from Charles River UK (Margate, UK). They were maintained throughout on an egg- and milk-free diet. After a 6 week equilibration period they were sensitized with a single i.p. injection of 100 µg OA (Sigma Grade V, Poole, UK) in 100 mg alum (total volume 1 ml). After 14 days 1 g BSA (Sigma Fraction V dissolved in 2 ml of 0.15 M NaHCO<sub>3</sub> - 0.15 M NaCl) was introduced into the stomach using a plastic-coated metal cannula. One hour later a small midline abdominal incision was

made under combined Sagatal-Hypnorm anaesthesia. The pylorus was occluded and 2 ml of a solution of OA (100 mg), lactulose (140 mg) and rhamnose (20 mg) in 0.1 M K<sub>2</sub>HPO<sub>4</sub>, pH 7.0 were introduced into the duodenum using a 25 gauge needle and the incision closed. Blood was obtained by tail-vein bleeding immediately before the BSA gavage stage, and further samples were obtained by cardiac puncture 1, 3 and 6 h after intraduodenal challenge, supplementing the anaesthesia with Hypnorm as necessary. Urine samples were obtained over 6 h following antigen challenge as previously described [2]. Sixteen sham challenge control animals underwent an identical protocol except that OA was omitted at the laparotomy stage. An additional sham immunization control group comprised eight animals which were not sensitized but received the BSA feed and OA challenge exactly as did the positive control antigen challenge group.

The modulatory action of four anti-allergic drugs was investigated in this system by the simultaneous administration of the drug with the BSA. The four drugs used were sodium cromoglycate (a chloride channel blocker), beclomethasone dipropionate (adrenocorticoid steroid), nedocromil (chloride channel blocker) and doxantrazole (mast cell stabilizing agent). The details were as follows: Sodium cromoglycate (SCG) was obtained from Fisons plc (Loughborough UK) and administered at a dose of 20 mg/100 g body weight (group: *n* = 14); beclomethasone dipropionate (BDP) was obtained from Glaxo Group Research (Greenford, UK) and used at a dose of 1 mg/100 g body weight (group: *n* = 17); nedocromil was obtained from Fisons plc and used at 20 mg/100 g body weight (group: *n* = 16); doxantrazole was obtained from Wellcome (Beckenham, UK) and used at 1 mg/100 g body weight (group: *n* = 13). The drug doses were chosen on the advice of each manufacturer following extensive experience obtained in experimental animals.

### Histochemical studies of rat tissues

Following the removal of blood at the 6 h sampling time the anaesthetized animals were killed with an overdose of pentobarbitone sodium. Samples of both jejunal and ileal tissue were removed immediately after death and transferred to 10% formol saline fixatives (40% formaldehyde [10 ml], distilled water (90 ml) and sodium chloride (0.85 g). After fixation for 5-6 h the tissues were transferred to 70% ethanol and were then successively passed through absolute ethanol (2 × 1 h), and ethanol:xylene (1:1) mixture (30 min), xylene (2 × 1 h) and finally into Ralwax II (BDH Ltd) (2 × 1 h under vacuum). After hardening, paraffin blocks were stored at room temperature and then, after coding, transferred to

the Histopathology Department at Fisons, Loughborough (Dr B. Greenwood) for subsequent processing. Sections of the paraffin blocks were prepared and stained with 1% alcian blue (CI 74240), safranin, 1% toluidine blue (CI 52040) or haematoxylin-eosin. Two sections of each block were stained for examination under light microscopy and the process repeated again before summarizing the observed morphology. These evaluations were decoded at the Institute of Child Health in London.

### Assays

**Radioimmunoassay for immunoreactive BSA** Immuno-reactive BSA in rat serum samples was measured using a competitive radioimmunoassay (RA) as described previously [2]. Briefly, the following were mixed in a plastic tube: 10  $\mu$ l of serum or BSA standard; 100  $\mu$ l of rabbit anti-BSA diluted 1/15,000; 100  $\mu$ l of normal rabbit serum (diluted 1/100); 100  $\mu$ l of  $^{125}$ I-BSA diluted to 10,000 cpm; 100  $\mu$ l of PBS containing 0.1% Tween-20, 1% PVP and 0.05%  $\text{NaN}_3$ . After overnight incubation at room temperature, 100  $\mu$ l of donkey anti-rabbit precipitating serum (diluted 1/25) were added. After 4 h at room temperature the mixture was centrifuged at 1500 *g* for 45 min, the supernatant decanted and the radioactivity in the precipitate counted in an LKB gamma counter. The BSA content of each unknown was calculated from the standard curve.

**Determination of lactulose/rhamnose ratios in urine samples** Rhamnose (mol. wt. 164) and lactulose (mol. wt. 342) probes are believed to cross the intestinal epithelium essentially by transcellular and paracellular routes respectively and changes in the absorption ratio reflect pathology or experimental stress. The levels of lactulose and rhamnose in samples of rat urine were determined by thin layer chromatography as previously described [2].

Briefly, urine samples were desalted using duolite ion exchange resin and then applied in multiple 5  $\mu$ l volumes to a thin layer chromatography plate. The chromatograms were subjected to two initial developments using methanol — butanol-1-ol water followed by three successive runs in the following solvent mixtures: (i) butanol-1-ol-ethanol-glacial acetic acid-water; (ii) butanol-1-ol-ethyl acetate-pyridine-glacial acetic acid-water; (iii) butanol-1-ol-ethanol-glacial acetic acid water. Sugars were located on the plate using 4-aminobenzoic acid dissolved in methanol and orthophosphoric acid. After heat treatment at 120–125° for 10 min the plates were scanned and the concentrations of the sugars in the urines determined from the peak heights obtained.

**Assay of rat chymase II (RChyII) in rat serum samples** Serum levels of rat Chymase II were determined by an ELISA procedure using specific anti-RChyII reagents kindly provided by Dr H.R. Miller. The assay has previously been described in detail [2].

Briefly, microtitre plates were coated with affinity purified sheep anti-RChyII diluted to 1  $\mu$ g/ml in carbonate-bicarbonate buffer pH9.6, 100  $\mu$ l of antibody solution were incubated in each well overnight at 4°. This solution was then removed and residual binding sites blocked with 4% normal sheep serum, diluted in coating buffer. The plates were then washed six times with PBS and used immediately.

Some 100  $\mu$ l volumes of diluted samples and standards were incubated in duplicate in the plate for 90 min at 37°C. After washing, a sheep anti-RChyII horseradish peroxidase conjugate (150  $\mu$ l/well, diluted 1/2000) was added and the plate incubated at 37°C for 90 min. Colour was developed using OPD substrate and absorbance values read at 492 nm using a Titertek Multiscan plate reader. Unknowns were interpolated from the standard curve.

### Statistical analyses

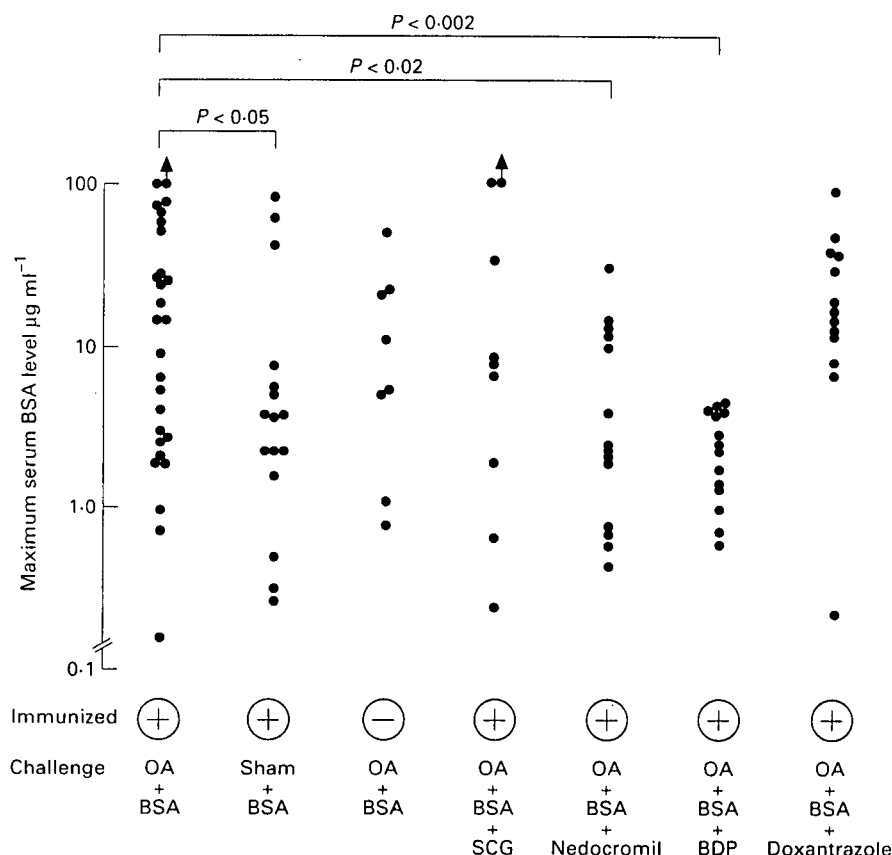
Non-parametric Wilcoxon rank sum or Mann-Whitney *U*-tests were used to analyse differences in BSA uptake, sugar excretion ratios or RChyII levels in the various experimental groups. One tailed tests were used throughout, since the direction of change could be predicted from previous experiments (decreased uptake of BSA, lower sugar excretion ratios and lower RChyII levels in animals receiving drugs relative to corresponding values in the antigen challenge control group). Kendall's rank correlation method was used to determine rank correlation coefficients.

### Results

#### *Uptake of bystander protein (BSA) across the gut*

The highest overall level of immuno-reactive BSA detected in each animal in samples obtained 1, 3 and 6 h post challenge is plotted in Fig. 1. As previously noted [2,3] there was a large overlap between the values observed in the positive challenge group and those of the sham challenge group. Nevertheless, there was a significantly increased uptake of BSA in the positive challenge animals ( $P < 0.05$ ; Wilcoxon rank sum test). Of the animals receiving a drug together with the ovalbumin challenge, only those treated with nedocromil and BDP showed significant reductions in BSA uptake relative to

**Fig. 1.** Groups of rats were either presensitized to ovalbumin (+) or were sham immunized (−), 14 days later they received an intraduodenal challenge of ovalbumin or buffer alone (sham challenge group) and were bled 1, 3 and 6 h later. All animals received BSA by gavage 1 h before the challenge. The data plotted for each individual animal is the maximum BSA level detected in the three serum samples. Drug modulation of BSA uptake was attempted using sodium cromoglycate (SCG), nedocromil, beclomethasone dipropionate (BDP) and doxantrazole.



the positive challenge group ( $P < 0.02$  and  $P < 0.002$  respectively; Wilcoxon rank sum test).

#### Sugar permeability results

The lactulose/rhamnose clearance ratios of the animals are plotted in Fig. 2. The ratios of the positive challenge group of rats were significantly higher than the ratios of both the sham challenge group and the sham immunised animals ( $P < 0.0001$  and  $P < 0.005$  respectively; Mann–Whitney  $U$ -tests). The sugar ratios of the animals receiving a drug with the ovalbumin challenge were also elevated except for the group receiving BDP. For these animals the distribution of sugar ratios was significantly reduced with respect to the positive challenge group ( $P < 0.01$ ; Mann–Whitney  $U$ -test).

#### Serum RChyII levels

Levels of the rat mast cell chymase RChyII were measured in the serum samples obtained 1 h after challenge with ovalbumin and the results are plotted in Fig. 3. The levels of RChyII in the positive challenge animals

were significantly higher than both of the negative control groups ( $P < 0.00003$ ; Mann–Whitney  $U$ -test) but none of the drugs used appeared to have had any influence on the levels of serine protease released at challenge.

#### Correlations between protein uptake, sugar permeability and RChyII release

We have previously reported a statistically significant correlation between lactulose/rhamnose excretion ratios and the levels of RChyII released in 14 rats experiencing a local challenge [2]. Interestingly, however, there was no correlation between protein uptake and RChyII release or between protein uptake and the lactulose/rhamnose excretion ratio. The present study permitted similar analyses on a much larger number of animals. Using the Kendall method for calculating rank correlations between protein uptake, sugar permeability and RChyII release of each group of animals, statistically significant associations were found between the RChyII levels and lactulose/rhamnose ratios of the positive challenge group ( $n = 24$ ,  $\tau = 0.34$ ,  $P = 0.01$ ), the group receiving SCG ( $n = 14$ ,  $\tau = 0.43$ ,  $P = 0.03$ ), the group

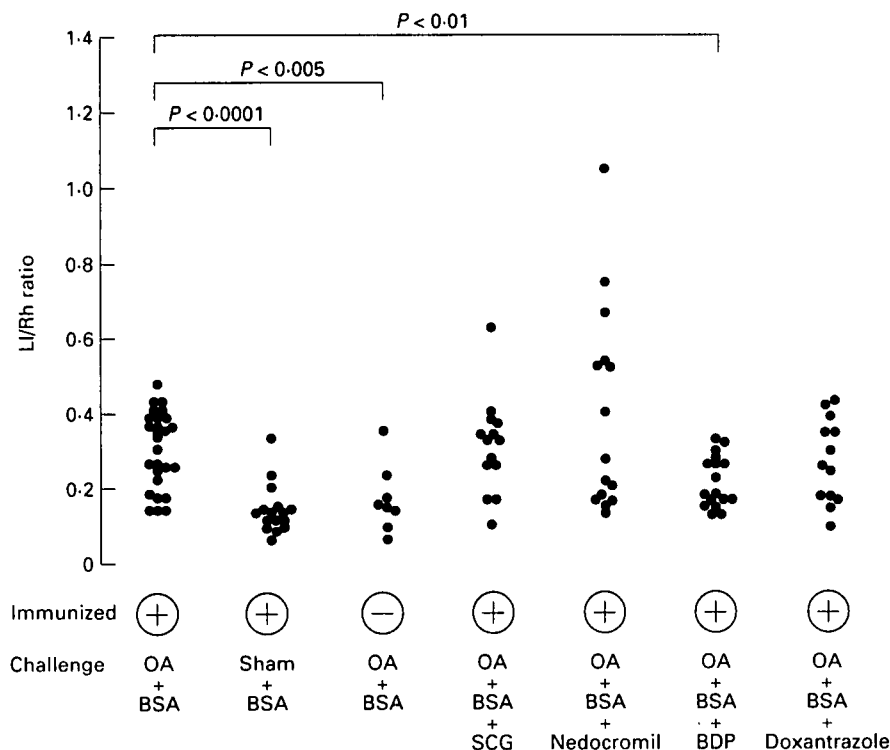


Fig. 2. Lactulose/rhamnose permeability ratios (L/Rh) in the groups of rats studied. See legend to Fig. 1 for details.

receiving nedocromil ( $n = 15$ ,  $\tau = 0.49$ ,  $P = 0.011$ ) and the group receiving doxantrazole ( $n = 13$ ,  $\tau = 0.53$ ,  $P = 0.01$ ). There was no significant correlation for the group receiving BDP ( $n = 17$ ,  $\tau = 0.066$ ,  $P = 0.7$ ) presumably because the lactulose/rhamnose ratios of animals receiving this drug were not elevated. None of the BSA uptake-RChyII or BSA uptake-sugar permeability comparisons approached significance.

#### *Histological evaluation of rat intestinal tissue in relation to antigen challenge*

Histological evidence of mast cell degranulation was sought in samples of rat jejunum obtained from animals undergoing: (i) OA challenge without drugs, (ii) sham challenge, (iii) OA challenge in the presence of SCG and (iv) OA challenge in the presence of nedocromil. In each case tissues obtained from four different animals, coded to disguise the group of origin, were evaluated in duplicate. There was no correlation between the histological light microscopic appearance of the mast cells and the experimental treatment group. The jejunal tissues of four animals with the highest levels of serum RChyII were histologically graded as normal and tissue from two of the sham challenge animals with minimal release of RChyII were scored as showing evidence of some degranulation.

#### **Discussion**

The dissection of the mechanisms underlying food allergy continue to present a formidable challenge to experimental allergists. We have previously exploited the animal model of Bloch and Walker [1] for basic studies of intestinal permeability and mediator release following intraduodenal antigen challenge [2]. The induction of intestinal hypersensitivity was confirmed by measuring serum levels of the rat mast cell protease II, RChyII. This mediator is released by mucosal mast cells but not by connective tissue mast cells [4,5] and we have also used it as a sensitive indicator of mast cell activation in the gut following repeated feeds of an antigen (OA) to rats previously sensitized with this protein [6].

Since RChyII has been shown to cleave type IV collagen of basement membrane [7] it has been suggested that release of this mediator results in shedding of epithelial cells from the intestinal mucosa and directly contributes to the increased mucosal permeability associated with anaphylactic reactions in the gut [8]. In partial support of this hypothesis we have observed significant positive correlations between serum levels of RChyII and sugar permeation (as measured by lactulose/rhamnose ratios) in both our earlier study [2] and, in the present investigation, in all of the challenged groups of animals except those receiving BDP ( $n = 66$ ). In con-

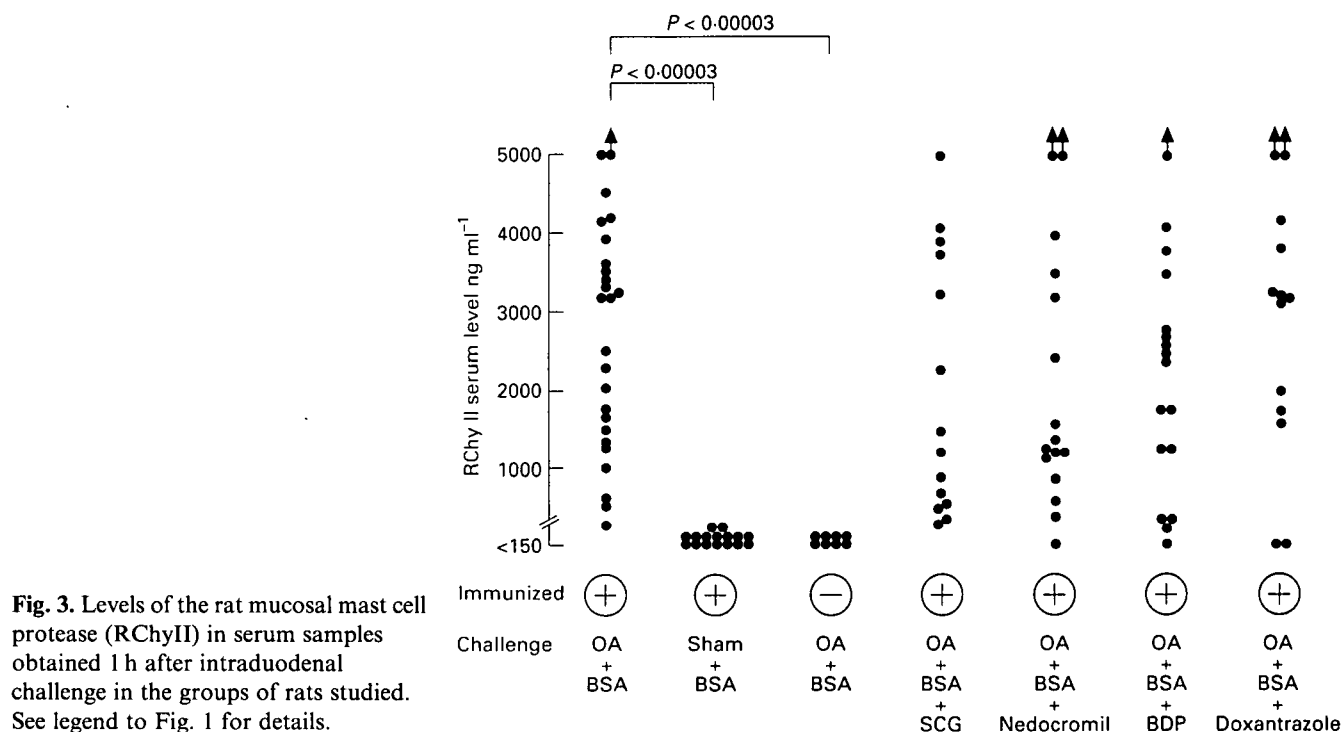


**Fig. 3.** Levels of the rat mucosal mast cell protease (RChyII) in serum samples obtained 1 h after intraduodenal challenge in the groups of rats studied. See legend to Fig. 1 for details.

trast, in both of these studies the uptake of bystander protein was not correlated with either sugar permeation or RChyII levels. Similarly, using the modified protocol with repeated feeding to challenge the gut of sensitized rats we were again unable to detect any simple correlation between uptake of bystander protein and release of RChyII [6]. Since each one of these three studies was based on the same experimental animal model it would be premature to conclude that there is no correlation between the level of mediator release and permeability to protein. One of the inherent difficulties of the experimental design used in this model is that it cannot take account of the large amount of individual variation that exists between different animals with respect to (i) the kinetics of RChyII release (ii) rates of transfer of RChyII from local sites to the serum and (iii) the kinetics of protein uptake from the gut. Samples were obtained at 1, 3 and 6 h post challenge and the maximum value of BSA observed was selected for the purposes of analysis. The true maximum, achieved at some intermediate point, might be very different. Frequent sampling through an indwelling canula would be possible for a short time span but is not feasible (or permissible) over 6 h in the numbers of animals required in each group.

The decision to use a single dosage of each drug, based on information provided by the manufacturers, was, we believe, justified in order to minimize the usage of

animals. As an approximation the levels of SCG, BDP and nedocromil used were 5, 100 and 20 times the maximal human dosage respectively. Doxantrazole never entered clinical use and no comparable figure is available.

The choice of the drugs used in the present study was, in part, based on the work of Pearce *et al.* studying the effects of various anti-allergic compounds on histamine secretion by isolated intestinal mast cells [9, 10]. This suggested that SCG was inactive whereas a structurally related flavonoid (quercetin) and doxantrazole were effective inhibitors [9, 10].

More recently, Perdue and Gall have established an *in vivo* model of intestinal anaphylaxis using Hooded Lister rats sensitized with ovalbumin. Abnormalities in the absorption of water and electrolytes were associated with mast cell degranulation and could be prevented by doxantrazole but not by sodium cromoglycate [11, 12]. In an alternative model Sprague-Dawley rats were immunized with *Nippostrongylus brasiliensis* and subsequently challenged with whole worm antigen after previous administration of either SCG or the corticosteroid betamethasone [13]. Gastrointestinal permeability was evaluated using [<sup>51</sup>Cr] EDTA and the gut was examined for morphological evidence of intestinal anaphylaxis. The authors found that low doses of betamethasone (0.2 mg/kg) were unable to prevent mucosal damage or

modulate [ $^{51}\text{Cr}$ ] EDTA uptake whereas high dose beta-methasone (2 mg/kg) completely prevented intestinal anaphylaxis.

In our earlier pilot study [3] both BDP and SCG significantly reduced protein uptake in Sprague Dawley rats. Our present findings have confirmed the effect on bystander protein uptake for BDP and extended this positive association to sugar permeability. However, whereas we were unable to confirm the observations with SCG we did obtain evidence of reduced uptake of bystander protein using the related compound nedocromil which seems to have an increased activity compared with SCG on a weight for weight basis. These variable laboratory findings appear to parallel the mixed early clinical experience when using cromoglycate for the treatment of presumed intestinal hypersensitivities. Some groups reported clinical benefit with SCG [14,15], others described equivocal results [16] and yet others found no benefit [17].

More recently there have been reports from two groups in which sugar permeability was evaluated in parallel with antigen challenge and sodium cromoglycate administration in patients with food allergy. André *et al.* [18] used mannitol and lactulose to monitor intestinal permeability in 60 patients with food allergy and found evidence of benefit in those receiving sodium cromoglycate. Similarly, Fälth-Magnusson *et al.* [19] studied gastrointestinal permeability in a small number of children using different sized polyethylene glycols and concluded that permeability returned to normal after treatment with cromoglycate. In a later study of 16 children with immediate hypersensitivity to milk the same group concluded that peroral treatment with SCG moderated the immediate hypersensitivity reactions with respect to both tolerated antigen dose and intestinal permeability properties [20]. Nevertheless it is important to caution against simplistic extrapolation of pharmacological studies from species to species. The present study may illuminate basic mechanisms in the rat but may not mirror events in the human gut.

Under light microscopy there was no obvious histological difference between jejunal tissue obtained from animals with high serum levels of RChyII and tissue obtained from sham challenge animals with low serum levels of the protease. This confirms previous studies such as that of Freier *et al.* [21] in which intestinal tissue was removed 45 min after and OA gavage challenge of rats presensitized to this antigen. Similarly, under light microscopy Patrick *et al.* [22] observed little distortion of the mucosal architecture after intraluminal challenge of sensitized rats but did find evidence of disruption to the basement membrane and collagenous matrix using electron microscopy. Nevertheless, it should be noted that

Perdue *et al.* [23], using a direct perfusion challenge protocol, were able to demonstrate decreased numbers of darkly stained granulated mast cells in the lamina propria of the intestinal mucosa of sensitized rats following exposure to antigen.

A major unresolved issue arising from the present studies concerns the significance of serum RChyII levels. Figure 3 suggests that the protease is a most effective indicator of gut mast cells responding to an antigen specific challenge since there is essentially no overlap between protease levels in the positive and negative challenge groups. Furthermore, our data support the hypothesis that the protease damages the intestinal mucosa and increases its permeability to low molecular weight sugars. However, we were unable to establish a similar link with the increased protein permeability which occurs concomitantly and the processes underlying the changes in permeability to sugars and proteins may well be quite distinct. Further experimental work is needed to establish more precisely the role of RChyII in the induction of intestinal damage. In particular it may be necessary to quantify mucosal mast cell protease levels in gastrointestinal secretions in order to observe correlations with changes in sugar and protein permeability and delineate some of the mechanisms underlying food induced gastrointestinal disease.

In conclusion, the *in vivo* model of intestinal anaphylaxis used in these investigations suggests that pharmacologically mediated changes in protein and/or sugar permeability can be observed in the absence of changes in the release of RChyII from mucosal mast cells. There was no direct evidence of morphological changes in mucosal mast cell architecture in this study but this may be a reflection of the indirect (gavage) challenge procedure used. It is increasingly clear that this is a very complex area. There are numerous other factors such as eosinophils and mast cell derived cytokines which may influence permeability and recent work suggests that non-IgE mediated pathways may also need to be considered [24].

#### Acknowledgements

We gratefully acknowledge essential financial support from Action research, Nestlé Nutrition, Glaxo Laboratories Ltd and Fison plc. We thank Dr H. R. P. Miller for the provision of reagents for the RChyII assays, Dr Brian Greenwood for undertaking the histological examinations and Miss Gabrielle Barnett for assistance with some of the analyses and Miss Amanda Crane for the preparation of the manuscript.

## References

- 1 Bloch KJ, Walker WA. Effect of locally induced anaphylaxis on the uptake of bystander antigen. *J Allergy Clin Immunol* 1981; 67:312-6.
- 2 Turner MW, Boulton P, Shields JG et al. Intestinal hypersensitivity reactions in the rat. I. Uptake of intact protein, permeability to sugars and their correlation with mucosal mast-cell activation. *Immunology* 1988; 63:119-24.
- 3 Boulton P, Shields JG, Strobel S, Levinsky RJ, Turner MW. Modulation of intestinal hypersensitivity reactions in the rat by the anti-allergic drugs beclomethasone dipropionate and disodium cromoglycate. *Med Sci Res* 1988; 16:811-2.
- 4 Miller HRP, Woodbury RG, Huntley JF, Newlands G. Systemic release of mucosal mast-cell protease in primed rats challenged with *Nippostrongylus brasiliensis*. *Immunology* 1983; 49:471-9.
- 5 Miller HRP, Huntley JF, Newlands GFJ, Irvine J. Granule chymases and the characterization of mast cell phenotype and function in rat and mouse. In: Schwartz LB (ed) *Neutral Proteases of Mast Cells*. Monogr Allergy. Basel: Karger, 1990; 27:1-30.
- 6 Turner MW, Barnett GE, Strobel S. Mucosal mast cell activation patterns in the rat following repeated feeding of antigen. *Clin Exp Allergy* 1990; 20:421-7.
- 7 Sage H, Woodbury RG, Bornstein P. Structural studies of human type IV collagen. *J Biol Chem* 1979; 254:9893-9000.
- 8 King SJ, Miller HRP. Anaphylactic release of mast cell protease and its relationship to gut permeability in *Nippostrongylus* primed rats. *Immunology* 1984; 51:633-60.
- 9 Pearce FL, Befus AD, Gauldie J, Bienenstock J. Mucosal Mast cells. II. Effects of anti-allergic compounds on histamine secretion by isolated intestinal mast cells. *J Immunol* 1982; 128:2481-6.
- 10 Pearce FL, Befus AD, Bienenstock J. Mucosal mast cells. III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. *J Allergy Clin Immunol* 1984; 73:819-23.
- 11 Perdue M, Gall DG. Transport abnormalities during intestinal anaphylaxis in the rat: effect of anti-allergic agents. *J Allergy Clin Immunol* 1985; 76:498-503.
- 12 Perdue M, Gall DG. Intestinal anaphylaxis in the rat: jejunal response to in vitro antigen exposure. *Am J Physiology* 1986; 250:G427-G431.
- 13 D'Inca R, Hunt RH, Perdue MH. Mucosal damage during intestinal anaphylaxis in the rat. Effect of betamethasone and disodium cromoglycate. *Diges Dis Sci* 1992; 37:1704-8.
- 14 Businco L, Cantini A, Benincori N et al. Effectiveness of oral sodium cromoglycate (SCG) in preventing food allergy in children. *Ann Allergy* 1983; 51:47-50.
- 15 Papageorgiou N, Lee TH, Nagakura T et al. Neutrophil chemotactic activity in milk-induced asthma. *J Allergy Clin Immunol* 1983; 72:75-82.
- 16 Vaz GA, Tan LK-T, Gerrard JW. Oral cromoglycate in treatment of adverse reactions to foods. *Lancet* 1978; i:1066-8.
- 17 Freier S. A therapeutic trial of disodium cromoglycate in gastrointestinal allergy. *Acta Allergol* 1977; 13(suppl):109-12.
- 18 André C, André F, Colin L, Cavagna S. Measurement of intestinal permeability to mannitol and lactulose as a means of diagnosing food allergy and evaluating therapeutic effectiveness of disodium cromoglycate. *Ann Allergy* 1987; 59:127-30.
- 19 Fälth-Magnusson K, Kjellman NI, Magnusson KE, Sundqvist T. Intestinal permeability in healthy and allergic children before and after sodium cromoglycate treatment assessed with different sized polyethyleneglycols (PEG400 and PEG1000). *Clin Allergy* 1984; 14:277-86.
- 20 Fälth-Magnusson K, Kjellman NI, Odelram H, Sundqvist T, Magnusson K. Gastrointestinal permeability in children with cow's milk allergy: effect of milk challenge and sodium cromoglycate as assessed with polyethyleneglycols (PEG400 and PEG1000). *Clin Allergy* 1986; 16:543-51.
- 21 Freier S, Eran M, Goldstein R. The effect of immediate-type gastrointestinal allergic reactions on brush border enzymes and gut morphology in the rat. *Pediatr Res* 1985; 19:456-9.
- 22 Patrick MK, Dunn IJ, Burt A et al. Mast cell protease release and mucosal ultrastructure during intestinal anaphylaxis in the rat. *Gastroenterology* 1988; 94:1-9.
- 23 Perdue MH, Chung M, Gall DG. Effect of intestinal anaphylaxis on gut function in the rat. *Gastroenterology* 1984; 86:391-7.
- 24 Oettgen HC, Martin TR, Wynshaw BA et al. Active anaphylaxis in IgE-deficient mice. *Nature* 1994; 370:367-70.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.