Br. J. Pharmac. (1985), **85**, 787–795

Immediate hypersensitivity reactions in epithelia from rats infected with *Nippostrongylus brasiliensis*

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1 Colonic epithelia from rats infected with the nematode *Nippostrongylus brasiliensis* have been studied under short circuit conditions and in response to challenge with worm antigen.

2 Challenge from the serosal but not the mucosal side with antigen caused a transient increase in inwardly directed short circuit current. No effects were observed in comparable tissues from non-infected animals.

3 Simultaneous measurements of short circuit current and of the fluxes of sodium or chloride ions showed there was an increase in electrogenic chloride secretion and an inhibition of electroneutral sodium chloride absorption, associated with antigen challenge. This result, together with the inhibitory effects of piretanide on the response to antigen challenge, indicate that chloride ions are a major carrier of the short circuit current response. However, the equivalence of the biophysical response to ion fluxes was not established, there being an excess of chloride secretion.

4 The mast cell stabilizing agent, FPL 52694, significantly inhibited the current responses to antigen, while cromoglycate and doxantrazole were ineffective. Mepyramine, an H_1-receptor antagonist, and indomethacin, an inhibitor of fatty acid cyclo-oxygenase, were without effect on the responses to antigen challenge.

5 Anti-rat IgE produced qualitatively similar responses to antigen in both normal and sensitized colonic epithelia. However, the responses were significantly greater in tissues derived from infected animals. Maximally effective antigen concentrations prevented subsequent responses to anti-rat IgE in sensitized tissues, while anti-rat IgE only attenuated the responses to antigen.

6 The ways in which antigen challenge modifies epithelial function is discussed, particularly in relation to its possible role in promoting rejection of the nematodes during secondary infection.

Introduction

The recognition and rejection of nematode parasites by mammalian hosts appears to require the integrated activity of most major components of the immune system including antibodies, lymphocytes and bone marrow derived cells (Ogilvie & Love, 1974). The expulsion of a primary nematode infection of laboratory animals takes several days (Ogilvie, 1967; Wakelin, 1978). A secondary infection, however, will be rejected by the host within 4–5 h (Miller *et al.*, 1981). This is believed to be due to an immediate (Type I) hypersensitivity reaction since (a) the response to re-infection is very rapid, (b) increased serum IgE is associated with developing immunity (Ogilvie, 1964; Orr & Blair, 1969) and (c) there is an hyperplasia of intestinal mast cells (Taliaferro & Sarles, 1939; Miller, 1980) which are discharged during helminth expulsion by the rat (Miller, 1971).

There are many ways in which increased numbers of mast cells and parasite specific IgE antibodies may be mobilised to rapidly reject a re-infestation. Antibody itself may produce a damaging effect on the parasite (Ogilvie *et al.*, 1973), as may substances released from specifically primed mast cells or basophils (Rothwell *et al.*, 1974; Kelly & Dineen, 1976). The release of pharmacologically active substances in nematode infections has been reviewed by Boreham & Wright (1976). Potential mediators of parasite expulsion from the intestine of rat include histamine and 5-hydroxytryptamine (5-HT) (Urquhart *et al.*, 1965; Sharp & Jarrett, 1968), prostaglandins (Dineen *et al.*, 1974; Dineen & Kelly, 1976), SRS-A (Murray, 1972) and kinins (Fal, 1974). In addition nematodes can release agents which affect the functioning of the epithelium, for example cholinesterase (Ogilvie *et al.*, 1973), and in

turn these activities may be modified by the immune system.

The local immune responsiveness of the intestine has been examined using antigens other than those to parasitic nematodes. These include ovalbumin in sensitized rats (Byars & Ferraresi, 1976; Perdue *et al.*, 1983) and β-lactoglobulin in cow milk sensitized guinea-pigs (Cuthbert *et al.*, 1983). In the latter study it was suggested that electrogenic chloride secretion, in response to antigen challenge, was the basis of fluid secretion responsible for diarrhoea associated with food intolerance. The purpose of this study was to investigate ion transporting mechanisms across colonic mucosa from rats infected with *Nippostrongylus brasiliensis* and during challenge *in vitro* with appropriate antigens. Isolated colonic epithelium was studied under conditions of zero transepithelial electrical potential obtained by the passage of a short circuit current (SCC), the magnitude of which is a direct measure of net electrogenic ion transport.

Methods

Sensitization of rats

Male Wistar rats (200–250g) were infected with 3000 third stage larvae (L_3) of the nematode *Nippostrongylus brasiliensis* by subcutaneous injection into the thigh. Twenty-five to 40 days later the rats were killed, serum collected and 4 cm of distal colon removed for *in vitro* study.

Preparation of secretory antigen

Adult worms obtained from the duodenum and jejunum of rats previously infected with *N. brasiliensis* were incubated in ten times their volume of sterile saline (0.9%) at 37°C for 5–6 h. The supernatant was then removed, divided into aliquots and frozen at −20°C until used. PAGE gels showed that the supernatant contained at least 9 distinct proteins which were released spontaneously from the parasites. There was a series of proteins of about 43,000 Daltons (pI 4.2–5.0), two or three proteins (pI ~ 5.3) and a single protein of about 100,000 Daltons (pI ~ 6.1). A 'worm-equivalent' (WE) of antigen was as previously defined by Ogilvie (1967).

Measurement of short circuit current (SCC)

Isolated epithelium was dissected from the descending colon and mounted in Ussing chambers (window area 0.6 cm^2) for voltage clamping. Each surface of the tissue was bathed in Krebs-Henseleit solution maintained at 37°C and gassed with 95% O_2:5% CO_2. SCCs were recorded using a W.P.I. Dual Voltage Clamp and displayed on pen recorders. Transepithelial conductance was measured by clamping transiently at −2 mV rather than zero. The resulting current change caused by this procedure was recorded and the conductance calculated using the Ohmic relationship.

Challenge was made by the addition of antigen to either the serosal (nutrient) or the mucosal (luminal) side of the preparation. Transient changes in SCC were measured as the total amount of charge transferred (current × time) and converted to uEq using the Faraday relationship. To do this the area under the SCC response curves was integrated by use of a planimeter (Allbrit).

In separate sets of experiments the net fluxes of sodium and of chloride were measured. Paired preparations (two adjacent pieces of epithelium) were used and trace amounts (2–3 μCi) of either ^{22}Na or ^{36}Cl were added to the mucosal bath of one tissue and the serosal bath of the paired preparation. One hour was allowed for isotopic equilibration after which four samples (P1–P4) were taken at 10 min intervals from the side opposite to that to which the isotope was added. Antigen challenge was made immediately after the second sample was taken. Small samples were also taken from the side to which the isotope was added at convenient times during the experiment. These samples were used to calculate specific activity, which remained constant throughout a given experiment. The radioactivity in each sample was measured as β-emission using liquid scintillation counting (Packard TriCarb 300) with a counting efficiency of 93%. The net flux of each ion was calculated and compared with the SCC responses measured simultaneously.

The Krebs-Henseleit solution had the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2 and glucose 11.1. This solution had a pH of 7.4 when gassed with 95% O_2 and 5% CO_2.

Throughout the text all values are given as means ± s.e. mean for the number of observations (n) noted.

Histamine acid phosphate (B.D.H.), mepyramine maleate (May & Baker Ltd.) and the lyophilised sodium salt of indomethacin (Merck Sharp and Dohme) were dissolved in water before use. We gratefully acknowledge gifts of disodium cromoglycate (1,3-*bis*[carboxychromone-5-yloxy]-2-hydroxypropane, disodium salt) and FPL 52694 (5-[2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) from Dr A.M. Edwards, Fisons plc (UK) and doxantrazole (3-[5-tetrazolyl]-thioxanthone-10,10,-dioxide) from Dr G. Perkins, Burroughs Wellcome Co. (U.S.A.). All these were dissolved in water before use. Piretanide was a gift from Hoechst Pharmaceuticals which was dissolved in sodium hydroxide solution and diluted as required. Anti-rat IgE serum was purchased from Miles Research Products

Division, Indiana, as a lyophilised powder (lot no. 27). It was reconstituted by addition of 2 ml distilled water. The potency of this batch was characterized by the suppliers as showing a precipitin band with up to 1:4 dilution of antiserum in an immunodiffusion assay against Rat Tumour IR 162 Ascites Fluid.

Results

The stable SCC across the descending colon of *N. brasiliensis* infected rats was $20.5 \pm 1.3\,\mu A\,(0.6\,cm^2)^{-1}$ ($n = 37$). This was an inward current representing net secretion of anions, net absorption of cations or a mixture of the two. The magnitude of this current was similar to that found in tissues from control, uninfected rats. In an unassociated series of experiments, carried out at the same time as those with infected rats, basal SCC was $19.5 \pm 1.5\,\mu A\,(0.6\,cm^2)^{-1}$ ($n = 24$).

Sidedness and repeatability of responses to worm antigen in isolated colon of worm-infected rats

Epithelium from descending colon, when challenged on the serosal but not on the mucosal side, showed a transient increase in inward current on the first but not subsequent challenge (Figure 1). The response, therefore, exhibits sidedness and is one which shows desensitization. Not unexpectedly there was some variation in the magnitude and duration of the responses in preparations from different animals, which may relate to the severity of the infection and of the immunological response to it. Figure 2 shows pooled values of SCC responses from preparations, all challenged with $10\,WE\,ml^{-1}$. Coincident with the

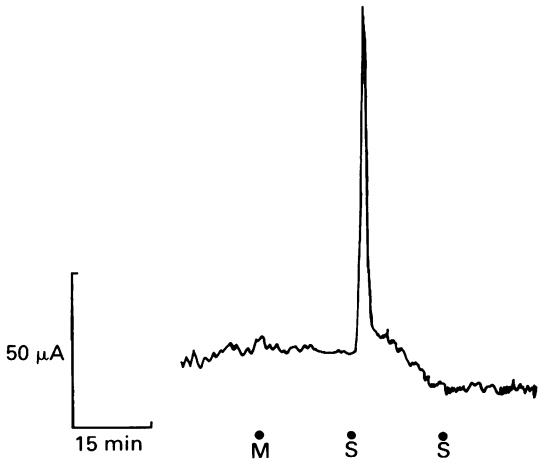

Figure 1 SCC recording from colonic epithelium obtained from a worm-infected rat. Antigen challenge ($10\,WE\,ml^{-1}$) to the serosal (S) but not the mucosal (M) side produced a transient inward current. The absence of a response to a second serosal side challenge showed that desensitization had occurred. Time calibration also indicates position of zero SCC.

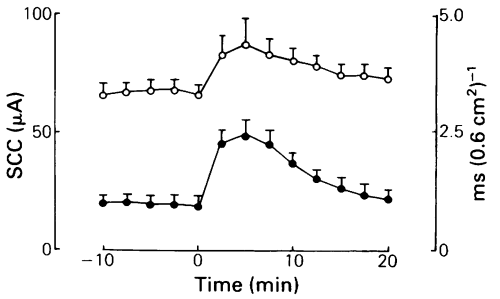

Figure 2 Pooled results of values for SCC (●) and transepithelial conductance (○) from isolated colonic epithelia from worm-infected rats following challenge with worm antigen ($10\,WE\,ml^{-1}$) applied to the serosal side bathing solution at time 0. Each value shows the mean for eight separate observations; vertical lines show s.e.means.

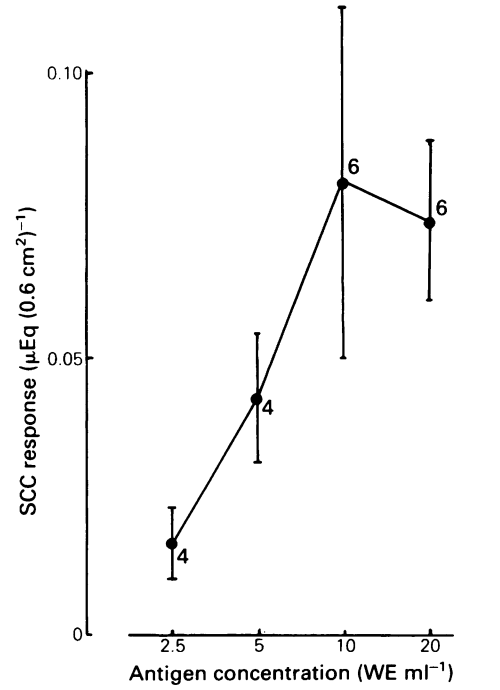

Figure 3 The relationship between antigen concentration and the response (net charge transfer) in isolated colonic epithelium from worm-infected rats. Each result was obtained from a separate preparation, which was challenged only once from the serosal side. The number of observations at each concentration is indicated.

Table 1 Chloride fluxes during antigen challenge

	SCC (μEq)	*J Cl (m→s)* (μEq)	*J Cl (s→m)* (μEq)	*J Cl (net)* (μEq)
P1	0.10 ± 0.02	1.56 ± 0.33	0.88 ± 0.20	0.68 ± 0.46
P2	0.10 ± 0.02	1.82 ± 0.33	1.07 ± 0.23	0.75 ± 0.50
P3	0.22 ± 0.04	1.93 ± 0.35	1.90 ± 0.46	0.03 ± 0.46
P4	0.12 ± 0.03	1.70 ± 0.39	1.26 ± 0.11	0.44 ± 0.46

SCC and unidirectional chloride fluxes (J Cl) were measured in five pairs of colon preparations during four consecutive 10 min periods (P1–P4). Antigen challenge (10 WE ml^{-1}) was made to the serosal side at the beginning of P3. Values obtained for each parameter during P2 were compared with those of P3 by paired Student's *t* test.

During antigen challenge the SCC increased transiently ($P < 0.02$). This was accompanied by a transient and significant increase in J Cl (s→m) ($P < 0.05$). There was no significant change in J Cl (m→s). The values of SCC were converted to μEq by integrating the area under the trace (see Methods). Mean values of SCC for pairs of preparations were used to calculate the values given in column 1. Each value gives mean ± s.e.mean for five measurements.

changes in SCC following challenge were changes of total transepithelial conductance, which showed approximately the same time course. Colonic epithelia from non-infected rats showed no response to addition of worm antigen.

The concentration-response relationship between worm antigen and SCC in descending colon taken from infected rats

As a single exposure to antigen modifies the response to subsequent exposures, or even abolishes the response when the antigen concentration is maximally effective, it was necessary to measure each response in a separate piece of tissue. A range of antigen concentrations between 2.5 and 20 WE ml^{-1} was used. Threshold concentrations were around 2.5 WE ml^{-1} and maximal effects were obtained at 10 WE ml^{-1}. Figure 3 shows the composite concentration-response relationship obtained in separate preparations.

Some idea of the extreme sensitivity of tissues to antigen can be obtained from the protein content of the antigen solution and taking the mean size of the proteins shown on PAGE gels (~43,000 daltons). Consideration of these values indicates that threshold responses are obtained with antigen concentrations in the pM range.

Identification of the charge carrying species

The results from measurements of chloride fluxes, using ^{36}Cl, are given in Table 1. When the values obtained during P3 are compared with those obtained in P2 it is shown that there was an increase in $J Cl_{ms}$ of 0.11 ± 0.19 μEq 0.6 cm^{-2} and in $J Cl_{sm}$ of 0.83 ± 0.24 μEq 0.6 cm^{-2}. These represent an increase in net chloride movement in the serosal to mucosal direction, that is a secretion, of 0.72 ± 0.41 μEq following antigen challenge. The change in $J Cl_{sm}$ was statistically significant ($P < 0.05$) using a paired *t* test.

Several things are apparent from these data. First the basal SCC cannot be accounted for by chloride secretion as net chloride movement is both too great and in the wrong direction. Second, antigen challenge

Table 2 Sodium fluxes during antigen challenge

	SCC (μEq)	*J Na (m→s)* (μEq)	*J Na (s→m)* (μEq)	*J Na (net)* (μEq)
P1	0.10 ± 0.02	0.57 ± 0.11	0.42 ± 0.16	0.15 ± 0.23
P2	0.10 ± 0.02	0.67 ± 0.14	0.38 ± 0.05	0.29 ± 0.15
P3	0.27 ± 0.04	0.36 ± 0.23	0.27 ± 0.09	0.09 ± 0.21
P4	0.18 ± 0.04	0.70 ± 0.21	0.54 ± 0.12	0.16 ± 0.30

SCC and unidirectional sodium fluxes (J Na) were measured in five pairs of colon preparations during four consecutive 10 min periods (P1–P4). Antigen challenge (10 WE ml^{-1}) was made to the serosal side at the beginning of P3. Values obtained for each parameter during P2 were compared with those of P3 by paired Student's *t* test.

During antigen challenge the SCC transiently increased ($P < 0.05$). Both J Na (m→s) and J Na (s→m) were reduced during P3 when compared with the P2 values but neither of these changes was significant statistically. Mean values of SCC (integrated over time) for pairs of preparations were used to calculate the values given in column 1. Each value gives mean ± s.e.mean for five measurements.

caused a significant ($P < 0.02$) increase in SCC. The changes in chloride movement during antigen challenge are in the correct direction for the SCC to be carried by chloride ions, but the net change in chloride movement ($0.72 \pm 0.41\,\mu$Eq) is some six times greater than the SCC response ($0.12 \pm 0.05\,\mu$Eq). Turning to sodium flux measurements, the data from which are given in Table 2, some clarification of the inequality seen between chloride fluxes and SCC responses becomes obvious. Again comparing the fluxes during period P3 with those during P2 it can be seen that $J\,Na_{ms}$ was reduced by $0.31 \pm 0.23\,\mu$Eq and $J\,Na_{sm}$ was reduced by $0.10 \pm 011\,\mu$Eq, neither of these values reaching statistical significance. It can also be seen that the net flux of sodium was always in the mucosal to serosal direction and that there was an apparent reduction in net sodium absorption of $0.21 \pm 0.28\,\mu$Eq. As will be discussed, the rat colon shows electroneutral NaCl absorption and this appears to be depressed by antigen challenge. This process would effectively reduce the amount of chloride which could carry current across the epithelium and account for the SCC. From these studies the chloride available to carry current is 0.51 $(0.72-0.21)\,\mu$Eq, which is still some 3 to 4 times the recorded values of SCC.

If chloride is the main current carrier for the SCC response then other electroneutral processes must account for the discrepancy between flux and current measurements and other types of evidence are required to identify the current carrying species. This has been obtained using an inhibitor of transepithelial chloride active transport, piretanide. Piretanide ($100\,\mu$M) was added to the serosal side bathing solution of one of each pair of colonic epithelial sheets. Five minutes later both tissues were challenged serosally with antigen ($10\,WE\,ml^{-1}$) and the transient changes in SCC measured. Piretanide alone was without effect on SCC but reduced the response to antigen to $0.04 \pm 0.02\,\mu$Eq ($n = 6$) which was significantly different from the control group, in which the response was $0.18 \pm 0.04\,\mu$Eq ($P < 0.05$; Student's t test). This is strong evidence that a major fraction of the current is carried by chloride ions, a possibility supported by the measurements of chloride flux.

Effects of mast cell stabilizing agents on the SCC response to antigen challenge

Three different mast cell stabilizing agents were used in separate series of experiments: they were disodium cromoglycate, doxantrazole and FPL 52694. Each set of experiments was carried out with a single concentration of the mast cell stabiliser (10^{-5}M) applied to solutions bathing both sides of the tissue. Ten minutes were allowed for equilibration before antigen challenge. The SCC responses obtained in response to $10\,WE\,ml^{-1}$ were compared with those obtained in tissues obtained from adjacent segments of colon but not pretreated with the stabilizing agents. None of the agents under test altered resting SCC. The responses to antigen in the presence of disodium cromoglycate ($0.20 \pm 0.06\,\mu$Eq $(0.6\,cm^2)^{-1}$, $n = 6$) were not significantly different from the corresponding control values ($0.27 \pm 0.10\,\mu$Eq $(0.6\,cm^2)^{-1}$). Doxantrazole treatment reduced the response to antigen ($0.21 \pm 0.05\,\mu$Eq $(0.6\,cm^2)^{-1}$ ($n = 6$)) compared to controls ($0.37 \pm 0.06\,\mu$Eq $(0.6\,cm^2)^{-1}$) to a level approaching significance ($P < 0.1$). The response to antigen was also reduced by FPL 52694 ($0.05 \pm 0.01\,\mu$Eq $(0.6\,cm^2)^{-1}$) compared to controls ($0.09 \pm 0.02\,\mu$Eq $(0.6\,cm^2)^{-1}$), this difference being significant by Student's t test at $P < 0.05$. These three sets of experiments were done on separate batches of animals preinfected with separate batches of worms. The control responses illustrate the variation in the sensitivity to antigen, referred to earlier, which presumably reflects varying degrees of sensitization to the parasites.

Potential mediators of the SCC response

The possible involvement of histamine in the response was investigated by use of the histamine H_1-receptor antagonist, mepyramine. Mepyramine (10^{-5}M) was applied to the solutions bathing each surface of short circuited colonic epithelia 5 min before serosal side challenge with antigen ($10\,WE\,ml^{-1}$). Mepyramine had no effect on SCC but increased the response to antigen ($0.21 \pm 0.06\,\mu$Eq $(0.6\,cm^2)^{-1}$) compared with control values ($0.17 \pm 0.06\,\mu$Eq $(0.6\,cm^2)^{-1}$, $n = 6$). This increase was not statistically significant. Following the response to antigen challenge two control preparations were treated with histamine (10^{-5}M) which caused a sustained increase in colonic SCC. The mean response was $0.09\,\mu$Eq $(0.6\,cm^2)^{-1}$ $(15\,min)^{-1}$ which was 27% of the response to worm antigen. This action of histamine was completely prevented in paired preparations treated with mepyramine.

Metabolites of arachidonic acid are likely candidates as mediators of anaphylaxis. To test this hypothesis the results of antigen challenge in isolated colons were compared using adjacent segments of epithelium, one preincubated for 1 h with the cyclooxygenase inhibitor indomethacin ($5\,\mu$M). Indomethacin pretreatment did not significantly alter the response to worm antigen ($10\,WE\,ml^{-1}$) which was $0.37 \pm 0.07\,\mu$Eq $(0.6\,cm^2)^{-1}$ ($n = 6$) when compared with the control group ($0.43 \pm 0.06\,\mu$Eq $(0.6\,cm^2)^{-1}$).

Responses to anti-rat IgE

It is known that rats infected with *Nippostrongylus brasiliensis* have increased serum IgE levels (Ogilvie,

Table 3 Effects of anti-rat IgE on short-circuited colonic epithelium

SCC responses to serosal challenge with anti-rat IgE μEq $(0.6\,cm^2)^{-1}$			
Worm-infected tissues ($n = 6$)		*Control tissues* ($n = 5$)	
1/1000	1/100	1/1000	1/100
0.045 ± 0.016	0.064 ± 0.008	0.020 ± 0.010	0.035 ± 0.009

Preparations of colonic epithelium from worm-infected and control rats were challenged serosally with one dilution of anti-rat IgE. The magnitude of the response to 1/100 dilution of anti-rat IgE was significantly greater in worm-infected than in control rat colon ($P < 0.05$).

1964; Orr & Blair, 1969). However,not all the circulating IgE antibodies will be to antigens derived from the parasites so that it might be expected that tissues from both normal and infected animals would respond to anti-rat IgE. Leung & Pearce (1984) have suggested there is a degree of sensitization to environmental antigens in laboratory rats. Furthermore, because of the mast cell hyperplasia shown in infected animals (Miller, 1980), it might be expected that tissues from infected animals would show an exaggerated response to anti-rat IgE. These hypotheses have been tested using a sheep anti-serum to rat IgE.

Both control tissues and those from infected animals responded to anti-rat IgE, applied to the serosal side of the tissue, with an inwardly directed, transient SCC. Using a dilution of 1/100 only a single response was obtained on the first exposure to antiserum, exactly the same situation that obtains when worm antigen (10 WE ml^{-1}) is applied to epithelia from infected animals. Lower concentrations of antiserum (1/1000 dilution) produced submaximal responses.

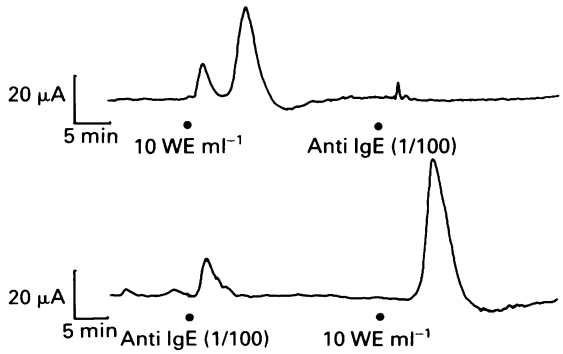

Figure 4 Paired preparations of colon from a worm-infected animal. Each preparation was exposed to antigen (10 WE ml^{-1}) and to anti-rat IgE (1/100 dilution) with a 30 min interval between. The order of application was reversed in the two preparations. Pre-exposure to antigen prevented a response to the antiserum but not *vice versa*. The time calibration also indicates the position of zero SCC.

Table 3 summarizes the responses obtained in tissues from control and infected rats to anti-rat IgE. Graded responses to the two dilutions used were seen with both types of tissue. The response to the higher concentration is presumably maximal since no further response could be elicited with further addition of antiserum. The responses were larger in tissues from infected animals, significantly so for the higher concentration of antiserum.

Cross-desensitization between worm antigen and anti-rat IgE was investigated. In each of four experiments, primary challenge with antigen followed 30 min later by anti-rat IgE showed that desensitization to the antiserum had occurred. This is illustrated in Figure 4. In this particular case the antigen produced a biphasic response while subsequent exposure to the antiserum was without effect. In the companion tissues where the order of challenge was reversed, desensitization was not so clearly demonstrable. This is also shown in Figure 4. Here antiserum gave a monophasic response, comparable in magnitude and latency to the first part of the biphasic response in the paired tissue. Thirty minutes later the worm antigen produced now a monophasic, rather than a biphasic response, with a latency corresponding to the second phase of the response in the paired tissue. In other responses, clear separation of the two components with worm antigen was not seen. However, as in the experiment demonstrated, exposure to worm antigen desensitized the tissue to antiserum, while exposure to antiserum attenuated the response to subsequent exposure to antigen.

Responses in other parts of the alimentary tract

In a few experiments the effect of antigen challenge on SCC was examined on epithelia prepared from parts of

the alimentary tract, other than the colon, taken from rats infected with *N. brasiliensis*. These were terminal ileum, jejunum, duodenum and stomach. All but the stomach preparations showed transient, inwardly directed, SCC responses on challenge with antigen from the serosal side. The stomach preparation showed no response to antigen.

Discussion

The significance of the results presented can be approached from two distinct viewpoints. First, what are the consequences for transepithelial ion transport of a type I hypersensitivity reaction occurring in cells of the lamina propria of the colon? To this can be added a consideration of the mediators and mechanisms involved. Secondly, it can be asked if the effects on epithelial function are related in any way to the mechanisms by which animals rid themselves of intestinal parasitic infections.

There can be little doubt that the response to antigen challenge is mediated by an immune mechanism. The response to antigen is obtained only in tissues from animals infected with the parasite, otherwise the tissues behave entirely normally with respect to their basal transporting characteristics. Also antiserum directed against rat IgE causes qualitatively similar responses and, furthermore, exposure to a maximally effective concentration of antigen desensitizes the tissues to challenge with antiserum.

We have not shown the locus of action of the worm antigen but the evidence favours an interaction, either directly or indirectly, with mast cells embedded in the lamina propria. First, challenge is effective only when made from the basolateral side of the tissue. Furthermore, the response to anti-rat IgE is enhanced in conditions where mast cell hyperplasia is known to occur (Befus & Bienenstock, 1979). Additionally the mast cell stabilizing agent, FPL 52694 attenuates the response to antigen, although the better known agent disodium cromoglycate was without effect. However, this is in keeping with direct studies on the release of histamine from free rat mast cells. In particular, cromoglycate shows a high degree of tissue and species specificity in its action (for a review see Pearce, 1983) and is maximally active against connective tissue mast cells of the rat but totally ineffective against isolated mucosal mast cells from the lamina propria of this species (Pearce *et al.*, 1982). FPL 52694 is more potent than cromoglycate in the inhibition of mediator release from rat serosal mast cells (Leung *et al.*, 1984) but its effect on isolated intestinal cells has yet to be reported.

It is known that anti-rat IgE will liberate histamine from peritoneal and mucosal mast cells of the rat and that this stimulus is less effective than worm antigen with mast cells from infected animals (Befus *et al.*, 1982). These findings are in accord with our observations upon SCC in isolated epithelia. From the results of our cross-desensitization experiments (Figure 4), it is possible that mast cell bound IgE may account for only part of the biophysical response. Both IgE and IgG antibodies may sensitize mast cells (Moodley & Mongar, 1981).

Although we have not identified the mediator(s) involved in the SCC response we can feel confident that neither histamine nor prostanoids are the sole mediators of the SCC. Other possible candidates such as leukotrienes, kinins and 5-HT, which are also released from mast cells (Foreman, 1981) have not been investigated in this study, although the failure of antigen challenge to be modified by indomethacin militates against a major involvement of kinins (Cuthbert *et al.*, 1984). Our results with indomethacin were surprising since in guinea-pig colon sensitized to milk proteins, the SCC responses to challenge with β-lactoglobulin were entirely abolished by inhibition of fatty acid cyclo-oxygenase (Cuthbert *et al.*, 1983).

The sensitivity of the antigen responses to the loop diuretic, piretanide, (Zeuthen *et al.*, 1978) is a good indication that the major part of the current response is due to stimulation of active, electrogenic chloride secretion. Flux measurements support this notion but the lack of equivalence between the SCC values and the flux measurements leaves the doubt that other ions might be involved to a minor extent as current carriers in the response. Indeed, the increase in net movement of chloride toward the mucosal bath, even allowing for changes in electroneutral NaCl transport (Bridges *et al.*, 1983), are in excess of the amount required by the current responses. This extra chloride must be moved across the epithelium by an electrically silent process, maybe in exchange for bicarbonate.

It is far more difficult to deal with the possible involvement of the secretory process we have described in the expulsion of the parasite from the host. The antigen we used is secreted spontaneously from the parasite, but it is also clear that it would need to be released on or penetrate to the serosal side of the epithelium in order to generate the secretion. Immunocytes of the lamina propria are strategically located to influence other cells of the gut, which is a primary shock organ of the rat (West, 1959). The immune system will certainly affect the activity of intestinal smooth muscle (Coulson, 1957). Castro *et al.* (1976) have shown gut transit times to be decreased in *Trichinella spiralis* infected rats although inhibitors of intestinal motility did not prevent the characteristic rapid expulsion of these parasites from infected animals (Bell *et al.*, 1982). Gut lymphoid tissue may also affect the functioning of epithelial cells (Castro, 1982). Intestinal anaphylaxis in the rat is accompanied by enhanced mucosal permeability (Barth *et al.*, 1966;

Murray *et al.*, 1971), by the release of mucus from goblet cells (Lake *et al.*, 1980; Miller *et al.*, 1981) and also by a decrease in the net absorption of fluid, occurring within a few minutes of worm antigen contacting the gut mucosa (Castro *et al.*, 1979). These features may combine to create an unfavourable environment within the lumen of the gut, thus contributing to nematode expulsion. In the alimentary tract chloride secretion takes place in the crypts and compensatory cation drag plus osmotic water movement leads to formation of visible secretion at the mouth of the crypts (Welsh *et al.*, 1982). We cannot know from our work whether formation of secretion flushes parasites away from the mucosa or provides an environment which is unfavourable to the parasites. It has long been held that an immune response involving mucosal mast cells is responsible for expulsion of the parasites, yet the subject remains controversial (for discussion see Askenase, 1980; Befus & Bienenstock, 1982). Nevertheless, our results describe for the first time a way in which a parasitic infection may affect the transporting activity of the colonic epithelium. As an *in vitro* system in which an IgE mediated immune response can be monitored and quantified it is a valuable addition to the variety of techniques which can be used to examine anti-allergic drugs directed against mast cells in the gastrointestinal tract.

A reprint similar to this one, in which the nematode Trichinella spiralis was used, has appeared recently (Russell & Castro, 1985).

A.W.B. is grateful to Fisons plc for financial support.

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(Received November 12, 1984.
Revised February 25, 1985.
Accepted March 26, 1985.)