*Immunology* 1983 **50** 547

# Immunological responses to fed protein antigens in mice

## IV. EFFECTS OF STIMULATING THE RETICULOENDOTHELIAL SYSTEM ON ORAL TOLERANCE AND INTESTINAL IMMUNITY TO OVALBUMIN

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*Accepted for publication 15 June 1983*

**Summary.** We have studied the role of the reticuloendothelial system (RES) in intestinal and systemic immunity in mice immunized orally with ovalbumin (OVA). Stimulation of the RES by oestradiol completely prevented the induction of systemic tolerance normally found in mice fed 25 mg OVA and this applied both to humoral immunity and delayed-type hypersensitivity (DTH). In addition, an active DTH response could be detected in the mucosa and mesenteric lymph nodes (MLN) of oestradiol-treated, OVA-fed mice on oral challenge with OVA. Oestradiol had no direct effect on lymphocyte function and we propose that RES activation may be one mechanism which predisposes to small intestinal disease associated with food hypersensitivity.

## INTRODUCTION

Intestinal delayed-type hypersensitivity (DTH) to dietary proteins plays an important part in small intestinal diseases which are associated with enteropathy and malabsorption (Ferguson & Mowat, 1980). However, such conditions are uncommon, probably because feeding soluble proteins to normal animals results in immunological tolerance rather than active hypersensitivity (Tomasi, 1980). Recently we showed that cyclophosphamide (CY) abrogated the state of tolerance normally found in mice fed ovalbumin (OVA) and allowed active DTH to develop in the intestinal mucosa and gut-associated lymphoid tissues (Mowat & Ferguson, 1981a, 1982a; Mowat *et al.*, 1982). Suppressor T cells ($T_s$) have been shown in the tolerance of systemic DTH which occurs after feeding OVA (Miller & Hanson, 1979; Challacombe & Tomasi, 1980; Titus & Chiller, 1981a) and our studies indicated that the effect of CY on intestinal immune responses to OVA was due to interference with a suppressor cell system (Strobel *et al.*, 1983). Therefore we have proposed that a deficiency of $T_s$ could predispose to potentially harmful intestinal DTH to food proteins and may be one mechanism for the development of childhood enteropathies.

Primary defects in $T_s$ activity have not yet been described in humans with food-sensitive enteropathy, but it is possible that such defects could arise secondary to a disturbance in another part of the lymphoid system. In mice, the induction of tolerance by intravenous injection of sheep red blood cells (SRBC) is inhibited by stimulating the reticuloendothelial system (RES) with oestradiol and this is related to deficient generation of suppressor cells (Yoshikai *et al.*, 1981).

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In this paper we have investigated the possibility that the function of the RES may also influence immune responses to orally administered antigens and have followed the induction of intestinal and systemic immunity in mice fed OVA after stimulating the RES with oestradiol. Our results are consistent with the hypothesis that stimulation of the RES may play a role in the development of enteropathy associated with food hypersensitivity.

## MATERIALS AND METHODS

*Mice*
Male BALB/c mice were first used at 6–8 weeks of age.

*Oestrogen*
Mice were injected subcutaneously with 2 mg oestradiol benzoate (Benztrone; Paynes and Byrne).

*Ovalbumin*
Ovalbumin (Sigma fraction V) was dissolved in distilled water for oral administration. Heat-aggregated OVA was prepared by the method of Titus & Chiller (1981b) and stored at −20° before use. For parenteral immunization of mice, OVA was incorporated in Freund's complete adjuvant (FCA; H37Ra Difco Ltd) and 100 μg injected intradermally into one footpad.

*Induction of oral tolerance*
Mice were fed 25 mg OVA by intragastric tube 3 days after receiving oestradiol and 14 days later were immunized with OVA in FCA (Group 4). Control mice either received saline before feeding on OVA (Group 2), were injected with oestradiol and fed water (Group 3) or injected with saline and fed water (Group 1). All mice were tested for systemic immunity 21 days after immunization.

*Measurement of systemic immunity*
Blood was obtained from the retroorbital plexus under light ether anaesthesia and serum antibodies measured by passive haemagglutination of OVA-coated SRBC as described previously (Mowat & Ferguson, 1981a). Systemic DTH was assessed by measuring the OVA-specific increment in footpad thickness 24 hrs after an intradermal injection of 100 μg heat-aggregated OVA in saline (Titus & Chiller, 1981b).

*Induction of intestinal DTH*
Three days after receiving oestradiol, mice were fed 2 mg OVA and 28 days later were challenged with 0·1 mg/ml OVA in the drinking water for a period of 10 days. Control mice received oestradiol alone or were fed and challenged with OVA alone. Intestinal DTH responses were assessed in the mesenteric lymph node (MLN) and jejunal mucosa on completion of the 10-day challenge.

*Migration inhibition of MLN lymphocytes*
MLN cells were assayed for sensitization to OVA as described previously (Mowat & Ferguson, 1982a, b). Briefly, $10^8$ MLN cells/ml of RPMI-1640 (Gibco Ltd) were centrifuged in 10-μl glass capillary tubes (Drummond Microcaps), the tubes cut at the cell interface and placed in the wells of leucocyte migration inhibition plates (Sterilin Ltd). The wells were filled with 0·45 ml medium or with medium containing 0·1 mg/ml OVA and the plates incubated for 18 hr at 37°. The migration areas were drawn using a drawing tube attached to a dissecting microscope and measured by planimetry.

Migration Indices (MI) were calculated as follows:

$$MI = \frac{\text{Area of migration in presence of OVA}}{\text{Area of migration in medium alone}}$$

In each assay, MLN cells were pooled from 3 to 4 mice and the results shown are the means of eight replicate wells.

*Measurement of mucosal architecture*
Villous and crypt lengths and crypt cell production rate (CCPR) were measured by a stathmokinetic technique employing colchicine as described previously (Mowat & Ferguson, 1981a, b). Mice were injected with 7·5 mg/kg colchicine (BDH Ltd) intraperitoneally and killed at intervals of 20–100 min thereafter. Pieces of jejunum were taken 10 cm from the pylorus, fixed in 75% ethanol/25% acetic acid and stained in bulk by the modified Feulgen reaction (Schiff-Difco Ltd). Villous and crypt lengths were measured by an eyepiece micrometer using a dissecting microscope and the CCPR calculated from the net accumulation of metaphases/crypt/hr.

*Intraepithelial lymphocyte counts*
Pieces of jejunum were fixed in formyl alcohol, stained with haematoxylin and eosin (H & E) and intraepithelial lymphocytes (IEL) counted by the method of Ferguson & Murray (1971). Numbers of IEL were expressed as IEL/100 epithelial cells.

*Carbon clearance tests*

Mice were injected with 160 mg/kg colloidal carbon (Pelikan Ink, Gunther Wagner, Hanover) in saline. After 1, 3, 6, 9 and 15 min 50 $\mu$l blood was taken from the retroorbital plexus, diluted in 4 ml distilled water and the optical density at 700 nm measured. The carbon clearance ($K_{16}$) was calculated from the slope of the linear regression function of $OD_{700}$ against time.

*Lymphocyte transformation tests*

Single cell suspensions of spleen cells were prepared in RPMI-1640 supplemented with 10% newborn calf serum (Gibco Biocult), 100 u/ml Penicillin G (Gibco) and 100 $\mu$g/ml streptomycin (Gibco) and washed three times before use. Two million cells in 100 $\mu$l were cultured in U-bottomed microtitre plates (Titertek Ltd) for 72 hr in 5% $CO_2$ in air at 37° in the presence of purified PHA (Wellcome).

Four hours before harvesting, cells were pulsed with 12·5 nCi/well [$^{14}$C]-thymidine. Cell-bound radioactivity was harvested using a Skatron Cell Harvester (Titertek Ltd) and counted on a Packard Liquid Scintillation Counter. Results are expressed as counts per minute (c.p.m.) per $10^7$ cells.

*Statistics*

Results expressed as means $\pm$ 1 standard deviation were compared by Student's *t* test, while crypt cell production rates were compared by covariance analysis.

## RESULTS

### General

Oestradiol had no harmful effects on the mice used in the study and there was no evidence of debility or weight loss.

### Reticuloendothelial system activity in oestradiol-treated mice

The carbon clearance tests performed 3 days after administration of 2 mg oestradiol showed a significant increase in RES activity in oestradiol-treated mice compared with controls (Fig. 1).

### Effect of oestradiol on induction of oral tolerance after feeding ovalbumin

Systemic DTH responses were assessed by intradermal footpad testing, 21 days after immunization with OVA in FCA (Fig. 2a). Mice fed 25 mg OVA had markedly suppressed DTH responses compared with untreated controls (Group 2 *vs* Group 1 80% suppression), while mice given oestradiol before feeding OVA (Group 4) had DTH responses which were greater than control values. Mice receiving oestradiol alone (Group 3) also had normal DTH responses, indicating that there was no residual effect of oestradiol on the immune response at the time of immunization with OVA in CFA.

A similar pattern was seen when haemagglutinating antibody levels were examined 21 days after immuni-

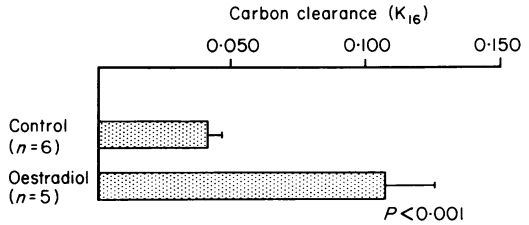


**Figure 1.** Carbon clearance activity ($K_{16}$) in BALB/c mice 3 days after 2 mg oestradiol and in controls. Mice were injected intravenously with 160 mg/kg colloidal carbon and carbon levels measured in blood at intervals thereafter by spectrophotometry. Results are the mean $\pm$ 1 SD for five or six mice.

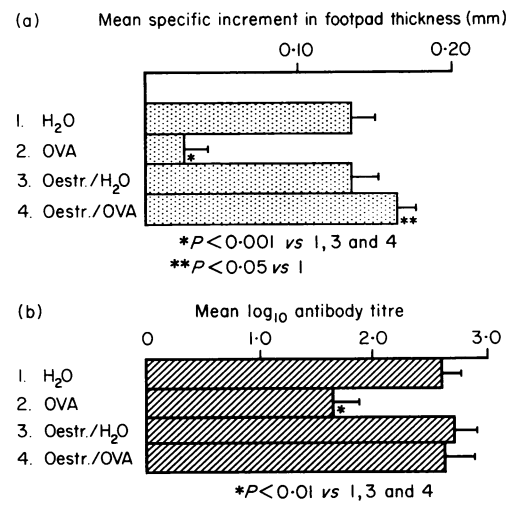


**Figure 2.** Effect of oestradiol on oral tolerance to OVA. Mice were immunized with 100 $\mu$g OVA in CFA 2 weeks after feeding 25 mg OVA and systemic DTH (a) and total serum antibody levels (b) measured 3 weeks later. Results are the mean specific increment in footpad thickness 24 hr after intradermal OVA (a) and the mean $\log_{10}$ haemagglutinating antibody titre (b) +1 SD for six to eight mice per group.

zation (Fig. 2b). Once again, mice fed OVA had significantly reduced antibody levels compared with controls (Group 2 *vs* Group 1 89% suppression) and the tolerance was abrogated in mice given oestradiol before feeding OVA (Group 4). The antibody response of mice receiving oestradiol alone was the same as control levels (Group 3).

### Intestinal DTH responses in oestradiol-treated OVA fed mice

Since oestradiol abrogated oral tolerance to OVA, we investigated whether an immune response could be induced in the gut-associated lymphoid tissues (GALT) and mucosa of oestradiol treated mice after oral sensitisation and challenge with OVA. In previous studies we have found migration inhibition of MLN lymphocytes to be a reliable assay for local DTH in the GALT of OVA fed mice (Mowat & Ferguson, 1981a, 1982a), while increases in IEL count and in crypt cell production rate measured the DTH response in the mucosa itself (Mowat & Ferguson, 1981a, b). These indices were therefore used to study intestinal DTH in oestradiol-treated, OVA-fed mice.

### Active DTH in the MLN of mice fed OVA after oestradiol treatment

Mice were fed 2 mg OVA and 28 days later challenged with OVA in the drinking water for 10 days. DTH to OVA in the GALT was then assessed by measuring the migration inhibition of MLN lymphocytes in the presence of 0·1 mg/ml OVA. Figure 3 shows that mice receiving oestradiol alone or fed and challenged with OVA alone had no evidence of MLN sensitization, with Migration Indices of $0{\cdot}98 \pm 0{\cdot}12$ and $1{\cdot}0 \pm 0{\cdot}07$ respectively. However, mice pretreated with oestradiol and fed OVA had significant levels of DTH in their MLN after oral challenge with OVA ($MI = 0{\cdot}72 \pm 0{\cdot}15$).

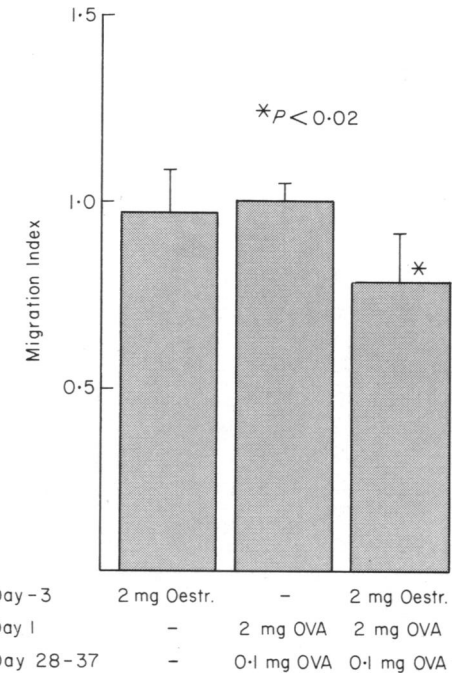


**Figure 3.** DTH to OVA in the MLN of oestradiol-treated mice. Mice were fed 2 mg OVA and 28 days later challenged orally with 0·1 mg/day OVA for 10 days, and MLN sensitization assessed by migration of lymphocytes in the presence of 0·1 mg/ml OVA. Results are mean Migration Index + 1 SD for three experiments using MLN pooled from three or four mice.

### Mucosal DTH in oestradiol-pretreated mice fed OVA

Mucosal DTH after OVA challenge was assessed by measurements of mucosal architecture and counts of IEL in the jejunum.

In mice receiving oestradiol alone or fed and challenged with OVA alone, there were no differences in villus length, crypt length ($122{\cdot}8 \pm 5{\cdot}4$ $\mu$m and $119 \pm 5{\cdot}0$ $\mu$m) or crypt cell production rates (7·6 and 8·3) (Fig. 4). However, mice pretreated with oestradiol before oral sensitization and challenge with OVA had significant increases in both crypt length ($138{\cdot}7 \pm 4{\cdot}1$ $\mu$m) and CCPR (12·9) compared with both the control groups. They also had significantly greater villus length than the group fed and challenged alone.

Similarly, oestradiol-pretreated mice had significantly increased numbers of IEL after oral sensitization and challenge with OVA (Fig. 5; $21{\cdot}7 \pm 2{\cdot}2$ IEL/100 epithelial cells) compared with oestradiol-treated or OVA-fed control mice ($13{\cdot}4 \pm 2{\cdot}2$ and $13{\cdot}2 \pm 2{\cdot}1$ respectively).

### Lymphocyte function in oestradiol-treated mice

Although the effect of oestradiol on the immune responses to fed OVA was considered to be due to stimulation of the RES, it was necessary to exclude a

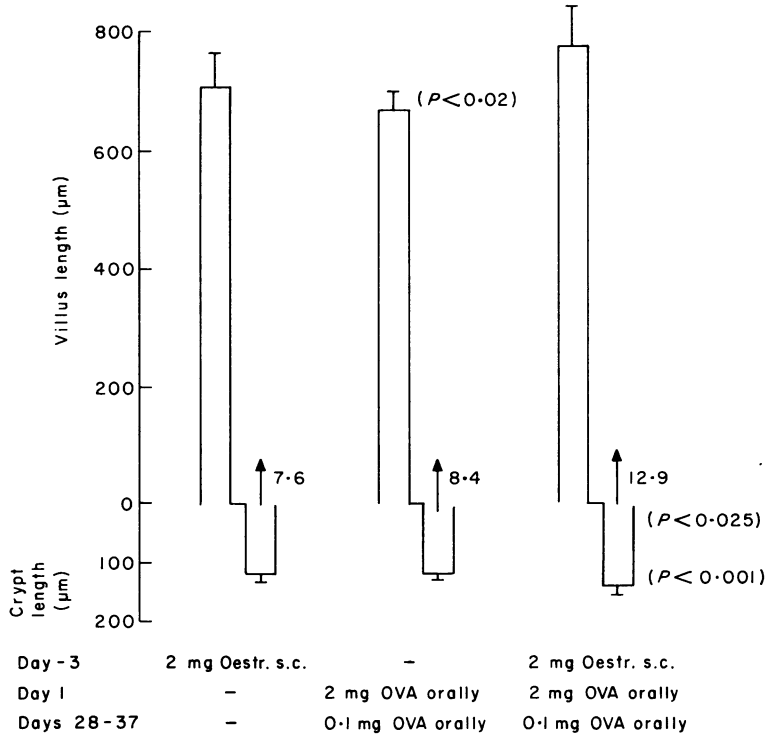


**Figure 4.** Mucosal DTH in oestradiol-treated, OVA-fed mice. Mice were fed 2 mg OVA and 28 days later challenged with 0·1 mg/day OVA for 10 days, and mucosal immunity assessed by alterations in villous and crypt length and in CCPR. *Bars* represent mean villous/crypt length + 1 SD and *arrows* the CCPR in six mice per group.

direct action on lymphocyte function. We therefore examined the responses of spleen cells from mice given oestradiol 3 days before to PHA *in vitro*. Table 1 shows that spleen cells from oestradiol-treated mice responded normally to the mitogen.

## DISCUSSION

These results show clearly that stimulation of RES activity by oestradiol prevents the induction of tolerance normally found in mice fed OVA and allows active local immunity to develop in the intestinal mucosa and GALT. The studies extend our previous findings on the induction of systemic and intestinal immunity to dietary antigens and contribute to understanding the mechanisms which regulate such responses in man.

The experiments described were based on the ability of oestradiol to stimulate the RES (Sljivic, Clark & Warr, 1975) and carbon clearance tests confirmed this effect in our mice. In addition, oestradiol-treated mice had normal responses to parenteral OVA and their spleen cells responded normally to mitogens *in vitro*. Thus, although oestrogens may have direct effects on the function of lymphocytes (Sljivic *et al.*, 1975; Cohn, 1979; Kittas & Henry, 1980), we do not consider that such an action can explain our results. Our demonstration that the RES is important in regulating immune responses to dietary proteins is supported by the fact that strain differences in the induction of oral tolerance to protein are determined by strain differences in RES function (Swarbrick, 1979). As oral tolerance is a complex phenomenon, involving several regulatory mechanisms which include $T_s$ (Ngan & Kind, 1978; Miller & Hanson, 1979; Challacombe & Tomasi, 1980; Titus & Chiller, 1981a), helper T-cell deficiency (Vives, Parks & Weigle, 1980; Titus & Chiller, 1981a) and tolerance of B cells (Vives *et al.*, 1980), activation of the RES could influence oral tolerance in several possible ways.

It has been suggested that the induction of oral

tolerance merely reflects the amount of antigen which the immune system encounters via the intestine (Asherson, Perera & Thomas, 1977), and so enhanced processing activity by the oestradiol-stimulated RES may alter the amount of available antigen, thus modifying the tolerant state. Such a mechanism has been proposed to explain the enhanced immune response and absence of tolerance in oestradiol-treated mice receiving high doses of SRBC intravenously, with normally tolerogenic doses becoming immunogenic due to increased clearance by the RES (Yoshikai *et al.*, 1979, 1981; Ezaki *et al.*, 1982). However, we consider it unlikely that this can explain our results as clearance of soluble antigens such as OVA by the RES is less easily modulated than that of particulate antigens like SRBC (Di Luzio & Morrow, 1971). In addition the induction of tolerance by feeding proteins is not due simply to the dose of antigen reaching the immune system but depends on the nature of the protein molecules which are absorbed (Strobel *et al.*, 1983). Finally, if altered protein clearance were the explanation for our findings, the effect of oestradiol on oral tolerance should be prevented by feeding larger doses of OVA. This is unlikely as systemic DTH responses are tolerized by as little as 2 mg fed OVA (Mowat *et al.*, 1982) but oestradiol completely abrogated tolerance of DTH even after feeding as much as 25 mg OVA.

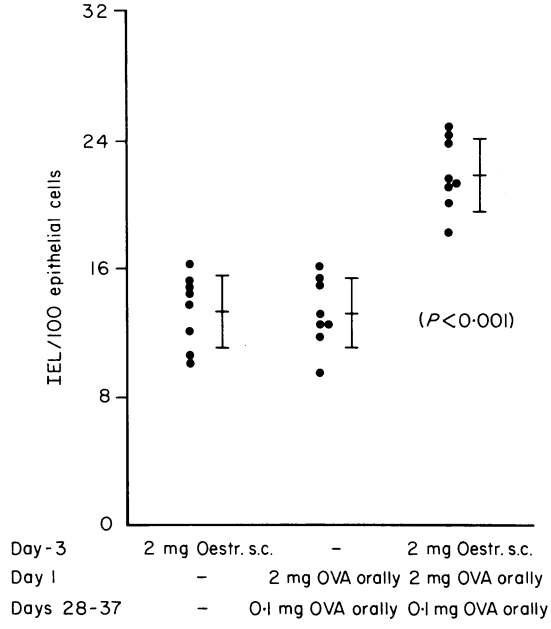


**Figure 5.** Intraepithelial lymphocyte counts in oestradiol-treated, OVA-fed mice after challenge with oral OVA. Bars represent mean IEL count/100 epithelial cells ± 1 SD for six mice per group.

**Table 1.** Effect of oestradiol on mitogen response of spleen cells

| | Spleen-cell source | |
|---|---|---|
| PHA | Control | Oestradiol |
| — | 878 ± 92 | 912 ± 121 |
| 0·5 μg | 11,406 ± 1210 | 10,850 ± 1112 |
| 5 μg | 11,730 ± 976 | 14,202 ± 1531 |
| 50 μg | 919 ± 100 | 620 ± 95 |

Spleen cells from three mice were cultured in presence of PHA for 72 hr and results shown are the mean c.p.m./$10^7$ cells ± 1 SD of four replicate wells. Oestradiol-treated mice received 2 mg oestradiol 3 days before being killed.

Alternative explanations for our results are based on the possibility that oestradiol stimulates the activity of antigen-presenting cells (APC) in OVA-fed mice. Stimulation of the RES has been shown to interfere with systemically induced tolerance to protein antigens and this reflects an enhanced ability of the RES to prime the immune response (Lukic, Cowing & Leskowitz, 1975). We would propose that this activation of APC inhibits the generation of $T_s$ in OVA-fed mice and that $T_s$ deficiency accounts for our present results. The ability of oestradiol to prevent the induction of tolerance in mice receiving SRBC intravenously is associated with deficient activation of $T_s$ (Yoshikai *et al.*, 1981) and we have already shown that elimination of $T_s$ by CY abrogates oral tolerance to OVA (Mowat *et al.*, 1982; Strobel *et al.*, 1983). Stimulation of APC activity by oestradiol may ensure that a greater proportion of fed OVA reaches the immune system in association with APC, bypassing $T_s$ which recognize antigen in the absence of APC (Feldmann & Kontiainen, 1976). In addition to quantitative changes in APC activity oestradiol may also modify $T_s$ function by altering the way in which APC process different molecular forms of the fed protein antigen. Tolerance after parenteral administration of protein antigens reflects differential handling of immunogenic and tolerogenic moieties by the RES (Lukic *et al.*, 1975) and oral tolerance also depends on the nature of the protein molecules reaching the immune system (Vives *et al.*, 1980; D. G. Hanson,

personal communication). In OVA-fed mice, a CY-sensitive suppressor mechanism is activated by tolerogenic moieties of OVA (Strobel *et al.*, 1983) and it is conceivable that oestradiol alters the processing of intestinally derived OVA in a manner which favours the presentation of immunogenic forms and the induction of $T_s$ is inhibited. Direct examination of APC function and T-cell populations in OVA-fed, RES-stimulated mice are planned to investigate these possibilities.

The most striking feature of our study was that oestradiol-treated, OVA-fed mice developed increases in CCPR and in IEL count in the jejunal mucosa and their MLN lymphocytes showed inhibition of migration in the presence of OVA. These changes have been shown previously to be associated with local, intestinal DTH responses (Mowat & Ferguson, 1981a, b, 1982a) and indicate that active DTH has developed in the intestine and GALT of the mice. These findings are identical to those found in CY-treated, OVA-fed mice and support our idea that abrogation of oral tolerance can lead to the induction of potentially harmful immunity to dietary proteins in the intestinal mucosa (Mowat & Ferguson, 1981a). We proposed that local DTH is responsible for the development of enteropathies associated with food hypersensitivity in infancy, and suggested that this occurs due to defects in gut-associated $T_s$ activity. Our present results indicate that stimulation of RES activity may be one way in which intestinal immunity to dietary antigens can occur and may be a cause of secondary $T_s$ deficiency. In childhood there are several possible factors which could produce RES activation including products released by endogenous or exogenous bacterial infections (Morrison & Ryan, 1979). In addition, it has been shown that feeding one protein antigen to mice results in enhanced RES activity and increased immunity to unrelated antigens (Newby, Stokes & Bourne, 1980) and so it is possible that one dietary antigen could influence immune responses to another. We propose that the RES has a critical role in determining the immunological consequences of feeding proteins and that stimulation of RES activity presents one mechanism which predisposes to food-sensitive enteropathy in infancy.

## ACKNOWLEDGMENTS

The authors would like to thank Mrs S. MacKenzie for skilled technical assistance.

Supported by MRC Grant No. 8011059.

## REFERENCES

ASHERSON G.L., PERERA M.A.C.C. & THOMAS W.R. (1979) Contact sensitivity and the DNA response in mice to high and low doses of oxazolone: low dose unresponsiveness following painting and feeding and its prevention by pretreatment with cyclophosphamide. *Immunology*, **36,** 449.

CHALLACOMBE S.J. & TOMASI T.B. (1980) Systemic tolerance and secretory immunity after oral immunisation. *J. exp. Med.* **152,** 1459.

COHN D.A. (1979) High sensitivity to androgen as a contributing factor in sex differences in the immune responses. *Arthr. Rheum.* **22,** 1218.

DI LUZIO N.R. & MORROW H.S. (1971) Comparative behaviour of soluble and particulate antigens and inert colloids in reticuloendothelial-stimulated or depressed mice. *J. Reticuloendothel. Soc.* **9,** 273.

EZAKI T., NAWA Y., HAYAMA T., YAMAGUCHI K. & KOTANI M. (1982) Modulation of the immune responses against SRBC after oestriol treatment in mice. *Clin. exp. Immunol.* **48,** 239.

FELDMANN M. & KONTIAINEN S. (1976) Suppressor cell induction *in vitro*. II. Cellular requirements of suppressor cell induction. *Eur. J. Immunol.* **6,** 302.

FERGUSON A. & MOWAT A.MCI. (1980) Immunological mechanisms in the small intestine. In: *Recent Advances in Gastrointestinal Pathology*. (ed. R. Wright), p. 93. W. B. Saunders Ltd.

FERGUSON A. & MURRAY D. (1971) Quantitation of intraepithelial lymphocytes in human jejunum. *Gut*, **12,** 988.

KITTAS C. & HENRY L. (1980) Effect of sex hormones on the response of mice to infection with *Toxoplasma gondii*. *Br. J. exp. Path.* **61,** 590.

LUKIC M.L., COWING C. & LESKOWITZ S. (1975) Strain differences in ease of tolerance induction to bovine-γ-globulin: dependence on macrophage function. *J. Immunol.* **114,** 503.

MILLER S.D. & HANSON D.G. (1979) Inhibition of specific immune responses by feeding protein antigens. IV. Evidence for tolerance and specific active suppression of cell mediated immune responses to ovalbumin. *J. Immunol.* **123,** 2344.

MORRISON D.C. & RYAN J.L. (1979) Bacterial endotoxins and host immune responses. *Adv. Immunol.* **28,** 293.

MOWAT A.MCI. & FERGUSON A. (1981a) Hypersensitivity in the small intestinal mucosa. V. Induction of cell mediated immunity to a dietary antigen. *Clin. exp. Immunol.* **43,** 574.

MOWAT A.MCI. & FERGUSON A. (1981b) Hypersensitivity reactions in the small intestine. 6. Pathogenesis of the graft-versus-host reaction in the small intestinal mucosa of the mouse. *Transplantation*, **32,** 328.

MOWAT A.MCI. & FERGUSON A. (1982a) Migration inhibition of lymph node lymphocytes as an assay for regional-cell mediated immunity in the intestinal lymphoid tissues of mice immunized orally with ovalbumin. *Immunology*, **47,** 365.

MOWAT A.MCI. & FERGUSON A. (1982b) Migration inhibition of lymph node lymphocytes as an *in vitro* assay for cell-mediated immunity in the draining lymph nodes of parenterally immunized mice. *Immunology*, **47,** 357.

MOWAT A.McI., STROBEL S., DRUMMOND H.E. & FERGUSON A. (1982) Immunological responses to fed protein antigens in mice. I. Reversal of oral tolerance to ovalbumin by cyclophosphamide. *Immunology*, **45,** 105.

NEWBY T.J., STOKES C.R. & BOURNE F.J. (1980) Altered polyvinylpyrrolidone clearance and immune responsiveness caused by small dietary changes. *Clin. exp. Immunol.* **39,** 349.

NGAN J. & KIND L.S. (1978) Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by the oral administration of ovalbumin. *J. Immunol.* **120,** 861.

SLJIVIC V.S., CLARK D.W. & WARR G.W. (1975) Effects of oestrogens and pregnancy on the distribution of sheep erythrocytes and the antibody response in mice. *Clin. exp. Immunol.* **20,** 179.

STROBEL S., MOWAT A.McI., DRUMMOND H.E., PICKERING M.G. & FERGUSON A. (1983) Immunological responses to fed protein antigens in mice. II. Oral tolerance for CMI is due to activation of cyclophosphamide-sensitive cells by gut-processed antigen. *Immunology*, **49,** 451.

SWARBRICK E.T. (1979) Antigen processing by the intestine. *M.D. Thesis*, University of London.

TITUS R.G. & CHILLER J.M. (1981a) Orally induced tolerance: Definition at the cellular level. *Int. Archs Allergy appl. Immun.* **65,** 323.

TITUS R..G. & CHILLER J.M. (1981b) A simple and effective method to assess murine delayed type hypersensitivity to proteins. *J. immunol. Meth.* **45,** 65.

TOMASI T.B. (1980) Oral tolerance. *Transplantation*, **29,** 353.

VIVES J., PARKS D.E. & WEIGLE W.O. (1980) Immunological unresponsiveness after gastric administration of human γ-globulin: antigen requirements and cellular parameters. *J. Immunol.* **125,** 1811.

YOSHIKAI Y., MAIKE S., MATSUMOTO T., NOMOTO K. & TAKEYA K. (1979) Effect of stimulation and blockade of mononuclear phagocyte system on the delayed footpad reaction to SRBC in mice. *Immunology*, **38,** 577.

YOSHIKAI Y., MAIKE S., MATSUMOTO T., NOMOTO K. & TAKEYA K. (1981) Effect of stimulation and blockade of mononuclear phagocyte system on the induction of suppressor T cells of delayed footpad reaction to SRBC in mice. *Immunology*, **43,** 241.