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THE ROLE OF DIETARY ANTIGEN IN THE AETIOLOGY OF POST WEANING DIARRHOEA

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Abstract

Experiments were undertaken to investigate whether a hypersensitivity response to dietary antigen might be a predisposing factor in the aetiology of post weaning diarrhoea. The results indicated that: 1. very small amounts of food given to baby pigs before weaning significantly increased the severity and accelerated the onset of the disease; 2. post weaning diarrhoea was dependent upon the presence within the diet of antigenic material; 3. weaning diets containing little antigenic material had less effect upon sucrose levels in the brush borders than did diets rich in antigens. These results are discussed in relation to the hypothesis that immune mediated intestinal damage may predispose to post weaning diarrhoea.

Post-weaning diarrhoea is a complex disease. Although enterotoxigenic strains of *E. coli* are clearly responsible for the severe diarrhoea seen in this condition, they are not on their own capable of initiating the disease. In order to reproduce the condition experimentally some additional stress must be applied to the small intestine. This may take the form of weaning (Porter *et al.*, 1974) or of rotaviral infection (Lecce *et al.*, 1982). Kenworthy and Allen (1966) studied the changes that occurred in the small intestine immediately after weaning and which preceded bacterial infection. They concluded that the initial lesions in post-weaning diarrhoea were villous atrophy associated with malabsorption, and other workers have also reported falls in disaccharidase levels in the intestinal brush borders (Gay *et al.*, 1976). It appears that these changes (which also occur following enteric viral infection (Snodgrass *et al.*, 1979; Kerzner *et al.*, 1977) may predispose the intestinal tract to bacterial infection. It is still not clear, however, what causes these changes in intestinal structure and function at weaning.

In this report we investigate the possibility that

the villous atrophy lesions and falls in enzyme levels and absorption capacity are the result of a transient hypersensitivity response to some of the antigens in the weaning diet. The reasons for advancing this suggestion are as follows:

Firstly, villous atrophy and malabsorption can be produced experimentally by hypersensitivity responses in the intestine of the mouse (Mowatt and Ferguson, 1981) and in the pig (Stokes *et al.*, 1981). Secondly, very similar intestinal lesions are seen in the human infant suffering from cow's milk allergy (Walker Smith, 1982) and, thirdly, the pig would be uniquely susceptible to such a response at weaning since it is suddenly introduced to large amounts of highly antigenic food for the first time at this period.

Such a hypothesis produces a number of very specific predictions which can easily be tested experimentally in order to see whether this explanation is correct, and in this paper we describe experiments that investigate two such predictions. The first is that by feeding very small amounts of the weaning diet to the pigs while still being suckled by the sow, the immune response of the intes-

tinal tract to these dietary antigens should be primed, so that following weaning the hypersensitivity response and hence the diarrhoea should be made more severe. Secondly, by weaning pigs onto diets that contain very little antigenic material, the post-weaning changes should be minimised.

Materials and Methods

Experimental Animals and Experimental Design

For each experiment two sows were selected which gave birth to litters within 24 h of each other. Pigs from the litters were placed randomly into the two experimental groups in order to remove any genetic environmental or parental effects from the experiment. The litters were housed in adjacent pens.

In the first experiment one group of baby pigs was allowed to eat the weaning diet for 3 h per day from days 7-10 inclusive. After day 10 they were not given any more solid food and were removed from the sow when she was fed in order to prevent them from eating the sow's food. The other experimental group was not allowed any solid food before weaning. After weaning, at three weeks of age, both groups were fed the weaning diet *ad libitum*. Faecal samples were obtained daily and examined for haemolytic *E. coli* and the water content was measured.

In the second experiment two litters born within 24 h of each other were again randomly ascribed to two groups. Both groups were fed bovine casein (in the form of cow's milk) from day 7-10 and then weaned at three weeks onto diets containing casein. One group received the diet containing native casein while the other was given the diet containing enzymatically hydrolysed casein. Bacteriological examinations and faecal water content investigation were again performed daily.

In the third experiment animals were subjected to the same experimental protocol as in the second experiment but they were killed ten days after weaning and the sucrase levels in the small intestine were measured by the method of Kidder and Manners (1975) and expressed as international units per gram of protein.

Faecal Examination

The animals were swabbed daily *per rectum* and the swabs were plated out on blood agar. *E. coli* were identified visually and haemolytic strains were tested for enterotoxin production using an ELISA. Five grammes of faeces were obtained manually from the rectum daily, weighed and then dried at 90 °C. The dry weight was measured and the faecal water content calculated. Water content greater than 80 % was considered to be clinical diarrhoea.

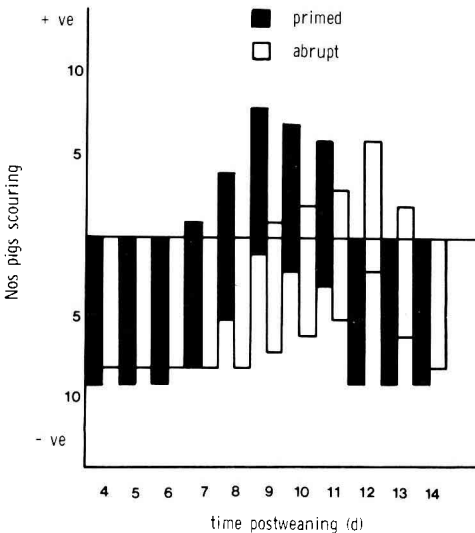


Fig. 1.— Incidence of diarrhoea post-weaning in pigs fed small amounts of weaning diet before weaning (closed bars) and pigs fed no weaning diet before weaning (open bars).

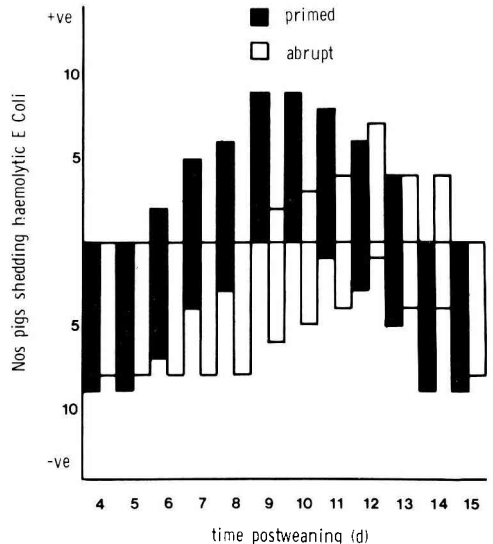


Fig. 2.— Incidence of haemolytic *E. coli* shedding post-weaning in pigs fed small amounts of weaning diet before weaning (closed bars) and pigs fed no weaning diet before weaning (open bars).

Table 1. — Haemolytic *E. coli* in faeces.

	Fed before weaning	Not fed before weaning	
Onset time (in days) (mean \pm S.D.)	7.6 \pm 1.2	10.8 \pm 1.6	P < 0.001
Duration days (mean)	5.4	3.0	P < 0.05
<i>Clinical diarrhoea</i> (> 80 % faecal water content)			
Onset time (in days) (mean \pm S.D.)	8.7 \pm 1.1	10.6 \pm 1.3	P < 0.01
Duration in days (mean)	2.8	1.8	P < 0.05

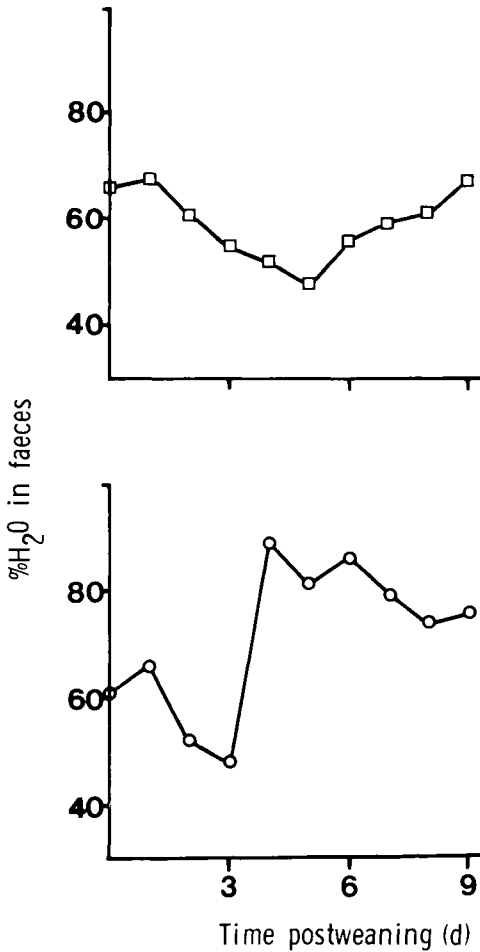


Fig. 3.— Percentage of faecal water content post-weaning in pigs primed before weaning with casein and fed the hypoantigenic casein diet (open squares) or the antigenic casein diet (open circles) after weaning.

Weaning Diets

In experiment 1 a conventional weaning diet was used containing 21 % protein and no antibiotics (Vitamealo; Beecham Animal Health Ltd., Brentford, U.K.).

In experiments 2 and 3, two diets were used containing 21 % protein. The protein source was casein and was incorporated either as normal casein or in its hydrolysed form (Sigma, Poole, Dorset, U.K.). The antigenicity of the two diets was compared by injecting equal amounts emulsified in Freund's complete adjuvant into laboratory mice. The anticasein antibodies were assayed by ELISA and the native casein-containing diet was found to produce an eightfold higher response than the hydrolysed casein diet. The diet containing hydrolysed casein was therefore considered to be hypoantigenic.

Statistics

Differences in sucrase levels and speed of onset of diarrhoea, and *E. coli* shedding, were compared using Student's *t*-test. Differences between groups in duration of diarrhoea, and *E. coli* shedding, were compared by an eight by two Chi-squared contingency test using single pig days of scouring as the individual events.

Results

Experiment 1

The effect, of small amounts of food ingested before weaning on post-weaning diarrhoea are shown in figures 1 and 2 and table 1. It can be seen that the time of onset of the shedding of haemolytic *E. coli* is significantly earlier in the group fed before weaning ($P < 0.001$) and that the period of shedding is significantly extended ($P < 0.05$) when compared to the group given no solid food before weaning. Similarly, the onset of diarrhoea was more rapid in this group ($P < 0.01$) and

lasted longer ($P < 0.05$) than in the group not primed by prior exposure to the weaning diet.

Experiment 2

In figure 3 the mean faecal water content of groups of pigs fed cow's milk for three days and then weaned onto antigenic or hypoantigenic diets is shown. From day 4 after weaning, mild diarrhoea was seen in the group fed the antigenic diet while those fed the hypoantigenic diet showed no signs of diarrhoea throughout the experiment. There was no apparent proliferation of haemolytic *E. coli* after weaning in either group.

Experiment 3

The sucrose levels at various points in the small intestine of pigs weaned onto antigenic and hypoantigenic diets is shown in figure 3. There were no differences in sucrose levels between groups in the upper small intestine. However, at a point 60% along the small intestine sucrose levels in pigs fed the antigenic diet were reduced to 0.6 of the levels seen in the other group ($P < 0.01$); at

80% along the small intestine they were reduced by a similar amount ($P < 0.05$) while 95% along the small intestine the group eating the antigenic diet had sucrose levels only half that of the group on the hypoantigenic diet ($P < 0.001$).

Discussion

The experiments described in this paper were designed in order to test two major predictions made from the assumption that an allergic response to food antigens could be a major predisposing factor to post-weaning diarrhoea. Both of these predictions were fulfilled by the results presented here. Exposure to small amounts of diet before weaning significantly increased the duration of the subsequent diarrhoea and significantly accelerated its appearance after weaning and had similar effects upon the shedding of enteropathogenic *E. coli*. Pre-weaning food intake in the primed group was very small and nutritionally insignificant but produced very important changes in the severity of the disease occurring two weeks later. An immunological explanation of this effect is satisfactory since the differences in response are identical to those seen between a primary and secondary immune response, but it is difficult to evoke a more conventional explanation for this effect. This finding is a reproducible one and we have already reported similar results (Miller *et al.*, 1982). The second and third experiments indicated that two diets, identical in nutritional terms and differing only in their degree of antigenicity, also produced very different effects in terms of post-weaning disease. Pigs fed the antigenic diet post-weaning showed diarrhoea but those fed the hypoantigenic diet did not. Furthermore, these pigs had significantly higher sucrose levels in the lower small intestine which, since sucrose levels normally fall rapidly in post-weaned pigs, suggests that such changes were less marked in pigs fed the hypoantigenic diet.

A mechanism by which hypersensitivity reactions in the intestine can produce the changes reported in post-weaning diarrhoea has been demonstrated by Mowatt and Ferguson (1981) in mice and in pigs by Stokes *et al.* (1981). They have found that type IV hypersensitivity responses to dietary antigens can bring about increases in mitotic rate in the enterocytes. This has the effect of increasing the speed at which the enterocytes migrate up the villi and are shed from its tip, with a proportionate decrease in the number of mature enterocytes. Since these are the cells that are absorptive in function and which have high levels of sucrose in their brush borders, a reduction in the number of mature cells will produce falls in both these parameters.

Diarrhoea may result from this increased cell

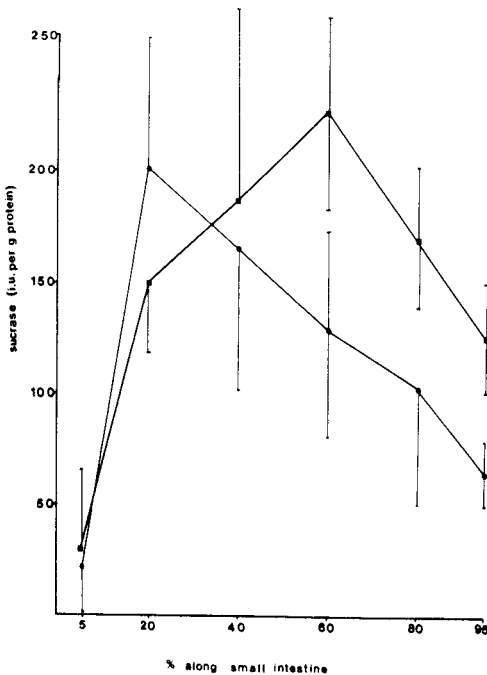


Fig. 4.— Brush border sucrose levels along the small intestine days after weaning in pigs fed the hypoantigenic diet (closed squares) and pigs fed the antigenic diet (closed circles). (mean \pm SD of six pigs per group).

turnover rate in two ways. A mild diarrhoea may occur due to the malabsorption syndrome alone reducing water absorption in the small intestine. This may explain why mild diarrhoea sometimes occurs post-weaning in the absence of bacterial proliferation. In addition these changes may produce a greater susceptibility to *E. coli* enterotoxin. Evidence exists that this is the case. Stevens *et al.* (1972) found the piglet small intestine showed greatly increased susceptibility to *E. coli* enterotoxin immediately after weaning and Ljungstrom *et al.* (1980) showed a similar increase in susceptibility to the related *Vibrio cholera* enterotoxin in experimentally induced villous atrophy in mice.

These results suggest that post-weaning diarrhoea may be produced by a combination of several factors during the immediate post-weaning period. For severe diarrhoea to occur enterotoxigenic bacteria must be present within the small intestine. It is probable that these bacteria are normally present within the intestine but that immediately after weaning they increase in numbers, perhaps as a result of the change in diet or the withdrawal of milk protection. The bacteria, however, cannot produce disease unless some additional changes occur in the intestine. Circumstantial evidence suggests that the important changes are villous atrophy and malabsorption, which together increase the susceptibility of the intestine to *E. coli*.

The evidence presented here indicates clearly that an immunological reaction to dietary antigens

may be the predisposing factor to the disease and this is supported by field evidence that poor feeding methods can be responsible for the disease and in particular that an inadequate intake of solid food prior to weaning may precipitate the condition (English *et al.*, 1980). These workers found that a pre-weaning intake of at least 400 g of high protein food was necessary to prevent post-weaning diarrhoea and in experiments reported elsewhere (Miller *et al.*, 1982) we have shown that while very small amounts of food prime the post-weaning hypersensitivity response, such large intakes pre-weaning can tolerate the immune system and so suppress the post-weaning reaction. These experiments suggest, therefore, that by offering young pigs solid food in the appropriate manner, post-weaning diarrhoea can be reduced in severity or prevented altogether.

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Question

From Dr Larvor to Dr Miller

Have you studied the effects of classical pharmacological antagonists of allergy mediators weaning diarrhoea?

Answer

No, but it would be an interesting approach which we are actively considering.

Question

From Dr Pahud to Dr Miller

Did you observe any cases of persisting hypersensitivity in your pigs which would be comparable to atopic infants, such as in cases of allergy to milk proteins?

Answer

No. In atopic infants only a small number of individuals are affected and it is almost certainly associated with failure of normal immunoregulatory mechanisms what we observe in the newly weaned pig is a transient immune response which is controlled within a few days by the normal immunoregulatory process. The immune response is probably quite normal, it is the sudden presentation of food antigens which is the problem.