Increased mucosal damage during parasite infection in mice fed an elemental diet

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SUMMARY We have examined the effects of parasite infection on the mucosal architecture of mice maintained on an elemental diet (Vivonex). Techniques used were conventional histology, microdissection and measurement of individual villi and crypts, and measurement of crypt cell proliferation rate by a metaphase accumulation technique. In normal, non-parasitised mice the elemental diet caused no change in villus height, crypt depth, or crypt cell proliferation. Likewise, the only effects of chronic protozoal infection or *Nippostrongylus brasiliensis* infection on the intestine of mice fed a normal diet have been a slight crypt hypertrophy and an increase in crypt cell proliferation rate without villous atrophy. However, the combination of elemental diet and parasite infection resulted in increased mucosal damage when compared with infected mice on a normal diet. Elemental diet mice infected with the nematode *Nippostrongylus brasiliensis* had significantly reduced villus height and correspondingly raised crypt length and metaphase accumulation rate. Elemental diet mice infected with the protozoan *Giardia muris* did not have villous atrophy but there was a significant increase in crypt length and metaphase accumulation when compared with infected normal diet mice. These experiments show that in two animal models of enteric infection, elemental diet has altered the host parasite relationship to the detriment of the host.

Elemental diets are widely used in gastrointestinal practice. However, although their nutritional properties have been extensively evaluated, there is limited information available on the effects of this radical alteration in diet on intestinal physiology, microbiology, and immunology. We have reported that when mice were reared on an elemental diet and infected at the age of 3 months with the parasite *Nippostrongylus brasiliensis*, they became lethargic, wasted, and some died (Ferguson, 1976; Ferguson *et al.*, 1978). Infection of rats with this nematode parasite results in changes in intestinal histopathology, with short villi, long crypts, and acceleration of epithelial cell turnover (Symons, 1965; Loehry and Creamer, 1969; Ferguson and Jarrett, 1975). The adverse clinical effects of elemental diet in infected mice would be in keeping with the presence of a more severe degree of malabsorption and enteropathy than usual. We have, therefore, examined further the effects of combined elemental diet and parasite infection in mice, by using a range of techniques to study mucosal damage—morphometry of villi and crypts, together with studies on crypt cell prolifera-

tion. Two parasites have been examined, the nematode *Nippostrongylus brasiliensis* and the protozoan *Giardia muris*.

Methods

ANIMALS

Two strains of mice were used—CBA (healthy and free of parasites) and Sha Sha (a strain known to be chronically infected with the protozoa *Giardia muris* and *Hexamita muris*) (MacDonald and Ferguson, 1978). At the time these experiments were conducted the principal organism in the Sha Shas was *Giardia*.

Nippostrongylus brasiliensis INFECTION

CBA mice were infected by a subcutaneous injection of 1000 *N. brasiliensis* larvae (from a culture maintained at the Wellcome Laboratories of Experimental Parasitology, Glasgow). Culture of larvae and intestinal worm counts were performed by standard techniques (Jennings *et al.*, 1963; Jarrett and Urquhart, 1968).

DIETS

The animals on a normal diet had free access to a standard mouse pellet feed, FFG(M), which is based

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on wheat, barley, maize, soya bean meal, white fish meal, and molasses.

Elemental diet animals had free access to Vivonex (Eaton Laboratories) rendered solid by incorporation in agar as follows. The contents of one packet, 80 g, were dissolved in 100 ml tepid water and mixed with 100 ml 5% agar which had previously been dissolved by boiling and then cooled to around 60°C. The two were mixed together using an egg whisk and allowed to solidify. Thereafter the solidified Vivonex was stored at 4°C. The mice were weaned directly onto the elemental diet after separation from the mother 16 days after birth.

POST-MORTEM EXAMINATION AND CONVENTIONAL HISTOPATHOLOGY

Animals were anaesthetised, the abdomen opened and small intestine taken from around 10 cm from the pylorus, for histopathological examination. This level of intestine was selected because it is the site of maximum concentration both of *Nippostrongylus brasiliensis* worms and of *Giardia muris* protozoa. Specimens were wax embedded and routinely cut and stained with haematoxylin and eosin.

For the study of liver weights and histology, individual mice were weighed, anaesthetised, the abdomen opened, the liver dissected out and immediately weighed. A piece of liver was then fixed in formol saline, wax embedded, routinely processed and stained with haematoxylin and eosin. Another piece of liver was snap frozen, and frozen sections were stained with Sudan IV.

MEASUREMENTS OF INTESTINAL ARCHITECTURE AND OF CRYPT CELL PRODUCTION

Groups of experimental animals were given intraperitoneal colchicine at a dose of 5 mg/kg body weight, and were killed at measured time intervals from one to two hours later. From each animal a segment of jejunum 10 cm from the pylorus was taken and fixed in 75% absolute alcohol, 25% acetic acid mixture. The tissue was then stained in bulk by the Feulgen reaction.

Fig. 1 Liver histology in a CBA mouse aged 10 weeks reared (a) on a normal diet, (b) on an elemental diet. H and E $\times 320$ (original magnification).

With a dissecting microscope, and by using fine forceps and a cataract knife, single villi with associated crypts were cut free from the edge of the specimen, placed on a slide in a drop of 45% acetic acid, and a coverslip was applied gently. By using an eyepiece graticule and a light microscope the lengths of 10 villi and 10 crypts per specimen were measured. The preparation was then gently squashed by light pressure on the coverslip. This liberates the crypts from the lamina propria matrix and flattens them out. The number of metaphases were then counted

Table 1 Effects of elemental diet (Vivonex) on mouse liver

Experimental group	Number of animals	Weight of mouse (g)			Weight of liver (g)			Liver histology
		Mean	SE	P*	Mean	SE	P*	
Normal diet	6	19.4	0.3		1.07	0.03		3 animals normal 3 animals occasional fatty change (< 25% of parenchyma)
Elemental diet	7	18.2	1.6	> 0.05	1.21	0.02	< 0.001	All 7 animals had marked fatty change (> 75% of parenchyma)

*Significance of difference from normal diet group.

in each of 10 crypts per specimen, and the mean value obtained.

Experiments and results

As in previous experiments the elemental diet mice grew satisfactorily and appeared normal and healthy, although the body weight was 5–10% less than that of normal diet animals. They were strikingly placid in behaviour and at post-mortem examination the livers appeared large and pale, and the caecum was small and did not contain gas. Faecal pellets were tiny and few in number.

In order to confirm that the livers of elemental diet animals were indeed abnormal, measurements of body weight, liver weight, and examination of liver histology were carried out in a group of six normal diet and six elemental diet mice aged 10 weeks. The results, summarised in Table 1 and illustrated in Figs. 1a and b, confirm the reduced body weight, increased liver weight, and the presence of fatty liver in elemental diet animals.

The Sha Sha mice used for the experiments were confirmed to have heavy *Giardia* infection of the jejunum at post-mortem examination.

EFFECTS OF ELEMENTAL DIET ON SMALL INTESTINE OF NORMAL MICE

Six normal diet mice and six elemental diet mice were killed at age 3 months and intestines examined by conventional histology and by the microdissection technique. Macroscopically, the elemental diet mice had narrow calibre intestine with clear contents. Conventional histology revealed no difference when normal diet and elemental diet specimens were compared. Studies of epithelial cell kinetics confirmed similar values for villus length, crypt length,

and accumulated metaphases per crypt per hour for normal diet and elemental diet animals of the CBA strain (Fig. 2).

EFFECTS OF ELEMENTAL DIET ON SMALL INTESTINE OF *Giardia* INFECTED MICE

In animals taking a normal diet, chronic *Giardia* infection is associated with longer crypts and a significantly raised metaphase accumulation rate when compared with the uninfected animals of the CBA strain (MacDonald and Ferguson, 1978). When mice of the Sha Sha strain were given an elemental diet, and studied at age 3 months, it was found that the elemental diet animals had significantly longer crypts and further increment in metaphase accumulation rate to 18.7 metaphases per crypt per hour (Fig. 2). Thus the known effect of giardiasis in accelerating cell production and cell transit rate up the sides of the villi are further enhanced by elemental diet treatment of the animals.

EFFECTS OF ELEMENTAL DIET ON COURSE OF *Nippostrongylus brasiliensis* INFECTION

The effects of *Nippostrongylus brasiliensis* infection on the animals' weight was examined in six normal diet and six elemental diet mice. Each was weighed immediately before the subcutaneous injection of larvae, and daily thereafter for 12 days. The results, shown in Fig. 3, confirm that the group of elemental diet mice weighed significantly less than the normal diet animals before infection. In both groups there was a drop in weight on day 2 post-infection. From the seventh day after infection, the elemental diet animals remained consistently and significantly lighter than the normal diet group and, whereas from day 2 there was a daily increment in normal diet

Fig. 2 Intestinal mucosal architecture in normal diet and elemental diet mice of the CBA strain (uninfected) and Sha Sha strain (chronically infected with *Giardia muris*). At least six mice per group. Values given are mean $\pm$ 1 standard error. The arrow indicates the number of accumulated metaphases per crypt per hour in each experimental group. *P < 0.01.

Fig. 3 Weights (mean $\pm$ standard error) of normal diet and elemental diet CBA mice, before and for 12 days after infection with 1000 *Nippostrongylus brasiliensis* larvae.

animals' weight, the weight of the group of elemental diet animals dropped between day 6 and day 9.

The influence of diet on the time course of infection was examined in 17 normal diet and 19 elemental diet mice, which were given 1000 larvae subcutaneously. Groups of animals were killed on days 6, 8, 9, and 15 after infection. The results of worm counts are illustrated in Fig. 4. The variation in the number of worms found is consistent with our previous experience of this infection in mice. There was a sharp drop in the number of worms in the intestine between the eighth and ninth day in the normal diet animals, similar to the 'self cure' phenomenon which terminates this infection in the rat (Jarrett *et al.*, 1968). In the elemental diet animals, although worm counts dropped after day 8, there were still substantial numbers of worms in all the animals examined up to day 15.

EFFECTS OF ELEMENTAL DIET ON SMALL INTESTINE HISTOLOGY AND ARCHITECTURE IN *Nippostrongylus brasiliensis* INFECTION

In a preliminary experiment 12 normal diet and 15 elemental diet mice were infected with 1000 larvae and killed at intervals from six to 15 days. Specimens were taken at 10 cm from the pylorus for conventional histology. In the normal diet animals changes were minor and comprised slightly increased lymphoid cell infiltrate of the lamina propria and villus shortening in a proportion of the animals (Fig. 5a). All four specimens studied from animals killed on day 15 were normal. In the elemental diet mice, the range of severity of pathology was greater. Several animals had very short or absent villi and long crypts and others had less severe abnormalities (Figs. 5b, 5c). Results of subjective grading of the histology of the specimens is shown in Table 1.

Fig. 4 Worm counts at intervals after *Nippostrongylus brasiliensis* infection, in mice fed a normal diet and elemental diet.

Fig. 5 Histology of the jejunum in CBA mice (a) fed a normal diet, day 9 after infection with 1000 *Nippostrongylus brasiliensis* larvae, (b) and (c) fed an elemental diet, day 9 after infection with 1000 *Nippostrongylus brasiliensis* larvae. H and E $\times 90$ (original magnification).

In order to carry out a more detailed investigation at a single time point, of the inter-relating effects of diet and infection on intestinal architecture, the seventh day post-infection was chosen as a suitable

time when both normal diet and elemental diet animals were likely to have similar numbers of worms established in the intestine. Thus measurements of villus and crypt length and of metaphase accumulation rate were carried out in six normal diet infected animals, six elemental diet infected animals, and six normal diet uninfected mice, all of the CBA strain. Results are given in Fig. 6. They show that in normal diet infected animals, the slightly increased crypt length and metaphase accumulation rate were not significantly higher than in controls. However in elemental diet animals there was villus shortening, significantly increased lengthening of crypts, and the metaphase accumulation rate was 16.6 metaphases/crypt/hour, as compared with 8.9 for the infected, normal diet group.

Fig. 6 Intestinal mucosal architecture in normal diet, uninfected mice of the CBA strain, and in normal diet and elemental diet CBA mice on the seventh day after infection with 1000 larvae of *Nippostrongylus brasiliensis*. Six mice per group. The arrow indicates the number of accumulated metaphases per crypt per hour in each experimental group. * $P < 0.01$.

Discussion

Elemental, chemically defined diets such as Vivonex are used in the management of a number of gastrointestinal diseases, for they have no residue, are not immunogenic, and can be absorbed across the small intestinal mucosa in the absence of pancreatic and other digestive secretions (Russell, 1975). Although designed for human use, Vivonex appears to be nutritionally adequate for laboratory rodents. We have found that Vivonex reared animals tend to be about 10% lighter than animals reared on a normal diet, and they have fatty liver but the diet is not

Table 2 Subjective assessment of small intestinal histology of 12 normal diet and 15 elemental diet mice, at various times after infection with 1000 *Nippostrongylus brasiliensis* larvae

Experimental group	Days post-infection	Number of animals with histological grading of:		
		Normal or virtually normal	Partial villous atrophy	Subtotal villous atrophy (flat surface)
Normal diet	6	3	—	—
	8	—	2	—
	9	2	1	—
	15	4	—	—
Elemental diet	6	1	2	—
	8	1	2	1
	9	1	2	—
	15	—	3	1

associated with any significant mortality and we have reared one group of animals on Vivonex for a year without ill effect.

The nature of the luminal contents is one of several factors which control the relative sizes of villi and crypts and the rate of epithelial cell renewal in the small intestine (Eastwood, 1977). The effects of the elemental diet Vivonex on the structure of the gut mucosa have been studied in rats, in whom Vivonex fed animals have longer villi and shorter crypts than controls (Nelson *et al.*, 1978), suggesting a slower cell proliferation rate; and such animals also have colonic mucosal atrophy (Janne *et al.*, 1977). In these respects, the effects of elemental diet on intestinal epithelial cell kinetics are similar to those of bypass operations, or total parenteral nutrition (Eastwood, 1977; Williamson, 1978). The present series of experiments have been carried out in mice, and results in normal animals indicate that there may be a species difference in this effect of elemental diet on cell turnover. By using a sensitive microdissection technique in addition to conventional histology, we have found that an elemental diet has no effect on the intestinal architecture of normal conventionally reared, parasite free mice.

Although on theoretical grounds the presence of easily absorbed nutrients at the luminal surface of the small intestine might be expected to reduce the amount of damage associated with extrinsic agents, such as parasites, we found that *Nippostrongylus brasiliensis* infection of elemental diet fed mice produced a more severe clinical illness than the same magnitude of infection in an animal fed normal diet (Ferguson, 1976; Ferguson *et al.*, 1978). We have now shown that these clinical effects are paralleled by increased severity of mucosal abnormality in the small intestine. These aspects have been examined most fully in relation to *Nippostrongylus* infection, in which the parasite load at different times can be measured. Not only do the animals fed an elemental diet lose weight after infection, but they also have an impaired ability to eliminate the complete parasite load, as illustrated by the presence of worms in the

small intestine on the 15th day post-infection. Furthermore, the usual effects of *Nippostrongylus* infection in mouse intestine—namely, of accelerated epithelial cell turnover—were enhanced by the elemental diet and a further aspect of pathology, villus shortening early in the course of infection, was produced. Thus, rather than the elemental diet protecting against the adverse effect of this helminth infection, paradoxically a more severe enteropathy has resulted. The enhanced enteropathy associated with *Giardia muris* infection could be demonstrated only by studies of epithelial cell kinetics, but again showed a further increment in epithelial cell turnover rate in the elemental diet fed animals as compared with infected normal diet controls.

We have not yet established the mechanism for this increased mucosal damage. It is conceivable that either the presence of high concentrations of nutrients within the gut lumen, or reduced competition for available nutrients by the gut flora, improves the nutrition of the parasites themselves and thereby increases their virulence. Alternatively, reduced small intestinal motility may render the final stage of expulsion of immune damaged parasites less efficient than normal. A further mechanism to be considered is an alteration in antiparasite immune responses. We have previously examined the effects of elemental diet on the immune status of otherwise normal mice, and, although we found that the animals had normal cell mediated immune capacity, they had an impaired capacity to make antibodies, at least to the antigen used for the experiments, bovine serum albumin (Ferguson *et al.*, 1978). There is now evidence that the thymus dependent immune response contributes to the enteropathy of parasite infections (Ferguson and Jarrett, 1975; Roberts-Thomson and Mitchell, 1978; Manson-Smith *et al.*, 1979). Thus relative enhancement of the cell mediated component of antiparasite immunity, as would be produced in elemental diet animals, will theoretically result in greater tissue damage (Ferguson, 1978). When the humoral component of the immune response is impaired, and when non-specific effector mechanisms

such as motility are abnormal, such an enhanced local cell mediated immune response may nevertheless be ineffectual as an antiparasite mechanism.

Whatever the reason, we have clearly demonstrated for two intestinal infections of mice that the host parasite relationship is altered by the long-term use of an elemental diet. This results not only in enhanced tissue damage but almost certainly enhanced mal-absorption and, in addition, prolongation of the course of infection with the helminth *Nippostrongylus brasiliensis*. It will be important to establish the mechanisms involved in order to determine if these observations are of clinical relevance to man. For the moment our findings should merely be borne in mind if elemental diets are used in persons potentially infected with helminth or protozoal parasites.

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