

## Immunological effects of yogurt addition to a re-nutrition diet in a malnutrition experimental model

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**SUMMARY.** The therapeutic and preventive effects of yogurt and lactic acid bacteria on diseases such as cancer, infection and gastrointestinal disorders are well documented. The aim of this research was to study the effects of different doses of yogurt addition after milk re-nutrition diet, on the recovery of the intestinal barrier and mucosal immune function. Experiments were performed on groups of mice, malnourished and re-nourished with milk during 7 d, and mice with diet supplemented with yogurt for 2, 5 and 7 consecutive d. Nutritional parameters such as weight gain, serum total protein, and the number of IgA, IgM and IgG B cells of the small intestine were determined. We also quantified intraepithelial leukocytes, mastocytes and goblet cells, and performed structural and ultrastructural studies on the small intestine. We observed that 5 d of yogurt feeding was the optimal dose for improving gut barrier function and mucosal immune system in a malnutrition model. This effect was not observed with milk re-nutrition. Although the results were better for 5 d of yogurt, addition for 7 d also showed beneficial effects. Yogurt feeding in our model did not impair any gut functions. These results suggest that yogurt addition after a re-nutrition diet gives better recovery of intestinal function than the re-nutrition diet usually recommended. Although these results were obtained in an animal model, they indicate that consumption of yogurt by malnourished children might accelerate the restoration of gut function.

**KEYWORDS:** Malnutrition, re-nutrition, yogurt, intestinal immunity.

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The interaction of nutrient deficiencies and immune status has been the focus of increasing research in the last two decades. Nutrients derived from dietary proteins and fats as well as micronutrients, vitamins, and minerals impact with immune cells systematically in the circulating blood, regional lymph nodes, and specialised immune system of gastrointestinal tract, and this will affect the bodies defence mechanisms (MacDermott, 1993).

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The importance of gastrointestinal defences, in particular mucosal immune function and barrier activity against endogenous microbes and toxins, is well known, due to the increasing recognition that the intestinal tract is an important immunological organ.

Evidence from experimental models and clinical Kwashiorkor suggest that nutritional deficiencies, especially protein-energy malnutrition (PEM) result in an increased risk of gastrointestinal infection. This can be attributed in part to impaired immune response (Chandra & Waxhaw, 1993; Cunningham-Rundles, 1994; Chandra, 1997). Essentially all forms of immunity have been shown to be affected by PEM. Lymphoid tissues show a significant atrophy, in particular the size of thymus.

The intestinal barrier of the malnourished host is impaired and may lead to increased absorption of dietary and other environmental antigens (Lunn *et al.* 1991).

There is no doubt about the impairment of immunity in PEM. However, it is possible to restore all gastrointestinal functions, regenerate mucosa and mucus production, and reduce the load on the local immune system by appropriate re-feeding (Castillo *et al.* 1991). It was demonstrated that the presence of a number of growth factors and hormones in the milk of various species, including human and bovine, together with proteolytic activity in the gastrointestinal tract (Meisel & Bockelman, 1999), have a beneficial effect on the host, suggesting a potential role in the re-nutrition process.

Potential beneficial effects of consumption of yogurt and dairy products containing lactic acid bacteria (LAB) on human health have been extensively reported. Animal studies have demonstrated that the ingestion of fermented products enhances innate and acquired immunity. In addition to being a nutrient-dense calorie source containing high quality protein, absorbable calcium, phosphorus, magnesium, potassium, riboflavin and vitamin A, yogurt can be considered a 'functional food'. This is because it supplies minor components that have been shown to play a role in decreasing the risk of certain diseases, particularly gastrointestinal disorders such as infantile diarrhoea, and increasing the host resistance to bacterial infection, gastro-enteritis, and constipation (Savaiano *et al.* 1984; Hitchins *et al.* 1985; Martini *et al.* 1987; Perdigón *et al.* 1994; Puri *et al.* 1996; Meydani & Ha, 2000).

In previous studies, we demonstrated, using an experimental model of malnutrition, that yogurt improved the protective mechanisms in the gut. However, after mucosal recovery by an adequate re-feeding, care should be taken to avoid harmful effects at the intestinal level, which may occur by an over-stimulation of the atrophied mucosa by malnutrition (Agüero *et al.* 1996). The aim of this research was to study, in a malnutrition experimental model, the effect of different doses of yogurt addition after a re-nutrition milk diet, on the recovery of the integrity of the intestinal barrier and mucosa immune functions.

#### MATERIALS AND METHODS

##### *Animals*

Weaned BALB/c mice from a closed colony of the breeding unit kept at CERELA Institute were malnourished for 21 d by being fed a protein-free diet (PFD) supplemented with vitamins, mineral and essential fatty acids in order to fulfil nutritional requirements. At the end of this period, animals that weighed 35–55% less than a group of well-nourished control animals, were selected for experiments. The well-nourished group (W-N) received balanced conventional diet administered



*Haematological and serum total protein determinations*

Haematological and serum total protein determinations were made in all experimental and control groups (five mice per determination). Peripheral blood was recovered by cardiac puncture. Hematocrit (HTO) and number of leukocytes were determined by the hematocytometric method. PMN and lymphocytes populations were differentiated on smears stained with Giemsa solution. Total protein concentration was determined using the Bradford technique (1976).

*Tissue sections*

The small intestine from five mice per group was removed at the end of each treatment period and processed by modified Saint-Marie's technique (Saint-Marie, 1962). Briefly, tissues were fixed in 95% ethanol for 24 h at 4 °C, dehydrated in three changes of absolute alcohol and cleared by passing through three consecutive baths of xylene at 4 °C for 45 min. The tissue was embedded in paraffin at 56 °C for 3–6 h. Sectioning was carried out and tissue sections (3–4  $\mu\text{m}$ ) were placed on glass slides.

*Number of IgA, IgG and IgM secreting cells*

Numbers of IgA, IgG and IgM secreting B cells were determined on samples from the small intestine of five animals per group by the direct immunofluorescence technique performed using the respective monospecific antibodies ( $\alpha$ ,  $\gamma$  and  $\mu$ -chain specific) conjugated with fluorescein isothiocyanate (FITC; Sigma, Saint Louis, Missouri 63103, USA). Histological samples were incubated with 0.1 ml of different antibodies at 1/60 dilutions for IgA, and 1/100 for both IgG and IgM for 30 min at 37 °C in a humidified chamber. They were washed three times with 0.01 M-phosphate-buffered saline (PBS), pH 7.2. Slices were mounted in glycerol:PBS. The number of positive fluorescent cells was expressed as number of secreting cells/10 fields (magnification 100 $\times$ ).

*Number of intraepithelial leukocytes (IEL), mast cells, and goblet cells*

For IEL, histological slices were stained with hematoxylin-eosin and mast cells and goblet cells were stained with 10 g Alcian Blue 8GX/l (Merck, F. R. Germany, Darmstadt D-6100) in 30 g acetic acid/l, 5 g Safranin O/l (Sigma) in 0.01 M-HCl. This was performed as described by Koretou (1988). The number of cells was expressed per 10 fields (magnification 100 $\times$ ). Determinations were made in five mice of each group.

*Preparation of samples for electronic microscopy for ultrastructural studies*

At the end of each treatment period, the mice (five of each group) were sacrificed by cervical dislocation. Peyer's patches and small intestine were carefully removed. Tissues were fixed in 400 g formaldehyde/l and 100 g glutaraldehyde/l phosphate buffer, pH 7.2. Specimens were then washed in sodium phosphate buffer and fixed in 100 g  $\text{OsO}_4$ /l, dehydrated in ethanol, cleared in propylene oxide and finally embedded in low-viscosity medium. Thin sections were stained with saturated uranyl acetate in 50% ethanol and 4% citrate. Sections were examined by transmission electron microscopy and the micrographs were produced at 4900 or 12 800 magnification.

Specimens of control groups of mice were processed in the same way those experimental groups.

Table 1. Effect of yogurt feeding for 2, 5 or 7 d on body weight of malnourished (M-N) and re-nourished (Re7d) mice

(Values are means  $\pm$  SD for  $n = 10$  mice per group)

Yogurt	Body Weight (g)					
	Days of yogurt administration					
	2		5		7	
	M-N	Re7d	M-N	Re7d	M-N	Re7d
Undiluted	10.9 $\pm$ 1.7	14.5 $\pm$ 1.7	14.4 $\pm$ 0.9	20.0 $\pm$ 1.6 <sup>2**</sup>	18.8 $\pm$ 0.7 <sup>1**</sup>	19.0 $\pm$ 1.6 <sup>2*</sup>
Diluted (v/v)	9.1 $\pm$ 1.5	15.4 $\pm$ 1.5	12.9 $\pm$ 1.4	18.6 $\pm$ 0.4 <sup>2**</sup>	19.3 $\pm$ 1.3 <sup>1**</sup>	17.0 $\pm$ 1.7 <sup>2*</sup>

Values for well-nourished control group = 25.8  $\pm$  1.5 g; re-nourished (Re7d) group with 7 d milk.

<sup>1</sup> Significantly different from M-N group (before yogurt administration) = 10.4  $\pm$  1.2 g.

<sup>2</sup> Significantly different from Re7d group (before yogurt administration) = 12.1  $\pm$  1.5 g.

\*  $P < 0.05$ ; \*\* $P < 0.01$  (ANOVA and Student's test).

### Statistical Analysis

Data were summarised using descriptive statistics such as the mean and standard deviations by the Student's test. Statistical comparisons of the treatment versus control groups were analysed by the ANOVA test.

## RESULTS

### Body weight

Body weight of M-N and Re7d control groups was significantly less ( $P < 0.01$ ) than that of the W-N control group. A significant increase ( $P < 0.01$ ) was observed after feeding undiluted yogurt to the M-N + Yo group for 7 d and to the Re7d + Yo group for 5 d, compared with the W-M and Re7d groups, respectively. A similar effect was seen with diluted yogurt feeding when the body weights of both M-N and Re7d groups were compared. These results are shown in Table 1.

### Haematological and total protein

The effects on the haematological response are expressed in Table 2. We showed that after undiluted and diluted yogurt feeding the hematocrit values increased significantly ( $P < 0.01$ ) only in the re-nourished groups (Re7d + Yo and Re7d + 1/2Yo) but this did not reach those obtained with the W-N group. Results showed a significant ( $P < 0.01$ ) decrease in the number of white cells in the M-N and Re7d control groups without treatment compared with W-N. The Re7d + Yo group produced a slight increase in this parameter, which was significant at 5 and 7 d. In the Re7d + 1/2 Yo animals we also observed a significant increase in relation to the controls. Malnutrition induced a slight decrease in the percentages of PMN and lymphocytes while the re-nutrition with milk produced a slight enhancement compared with the W-N group (see Table 2). Modifications in the percentages of PMN and lymphocytes were observed in M-N + Yo group after 2 d, where the percentage of PMN increase significantly; lymphocytes decreased significantly compared with M-N groups. Determinations of total protein are shown in Table 3. In the M-N group the level of total protein was significantly decreased ( $P < 0.01$ ) compared with the W-N control group. The Re7d control group showed a slight

Table 2. *Effect of yogurt feeding on the haematological values of malnourished (M-N) and re-nourished (Re7d) mice*(Values are means  $\pm$  SD for  $n = 5$  per group)

		Haematological parameters							
		Hematocrit (%)		White cells/ $\mu$ l		PMN (%)		Lymphocytes (%)	
Yogurt fed groups	Days of feeding	M-N	Re7d	M-N	Re7d	M-N	Re7d	M-N	Re7d
Undiluted	2	42 $\pm$ 1.0	48 $\pm$ 1.1 <sup>2</sup>	3283 $\pm$ 80	3000 $\pm$ 90	24 $\pm$ 1.0 <sup>1</sup>	26 $\pm$ 0.8	76 $\pm$ 2.0 <sup>1</sup>	74 $\pm$ 0.8
	5	39 $\pm$ 1.9	50 $\pm$ 1.0 <sup>2</sup>	3405 $\pm$ 90 <sup>1</sup>	3716 $\pm$ 129 <sup>2</sup>	14 $\pm$ 1.5	10 $\pm$ 1.6	86 $\pm$ 1.1	90 $\pm$ 1.0
	7	39 $\pm$ 0.7	51 $\pm$ 1.1 <sup>2</sup>	4500 $\pm$ 81 <sup>1</sup>	4400 $\pm$ 100 <sup>2</sup>	10 $\pm$ 0.8	14 $\pm$ 1.0	90 $\pm$ 0.9	86 $\pm$ 1.1
Diluted (v/v)	2	46 $\pm$ 1.0	46 $\pm$ 1.2 <sup>2</sup>	3216 $\pm$ 80	3366 $\pm$ 90	18 $\pm$ 2.0	15 $\pm$ 0.7	82 $\pm$ 0.5	85 $\pm$ 0.9
	5	38 $\pm$ 1.0	48 $\pm$ 0.9 <sup>2</sup>	3233 $\pm$ 93	3538 $\pm$ 123	10 $\pm$ 0.9	14 $\pm$ 1.2	90 $\pm$ 1.1	86 $\pm$ 1.0
	7	41 $\pm$ 1.1	50 $\pm$ 1.0 <sup>2</sup>	3283 $\pm$ 95	5550 $\pm$ 80	15 $\pm$ 1.0	13 $\pm$ 0.9	85 $\pm$ 1.0	87 $\pm$ 1.1
Groups prior to yogurt feeding									
M-N group		42 $\pm$ 1.0 <sup>3</sup>		2800 $\pm$ 106 <sup>3</sup>		11 $\pm$ 0.9		89 $\pm$ 1.1	
Re7d group		39 $\pm$ 0.9 <sup>3</sup>		3200 $\pm$ 106 <sup>3</sup>		20 $\pm$ 1.5		80 $\pm$ 1.4	
Well-nourished control		55 $\pm$ 10		4800 $\pm$ 100		15 $\pm$ 1.0		82 $\pm$ 0.9	

M-N: malnourished group; Re7d: re-nourished group with 7 d of milk.

<sup>1</sup> Significantly different from M-N group ( $P < 0.01$ ).<sup>2</sup> Significantly different from Re7d group ( $P < 0.01$ ).<sup>3</sup> Significantly different from well-nourished control ( $P < 0.01$ ) (ANOVA and Student's test).

Table 3. *The effect of yogurt feeding of malnourished (M-N) and re-nourished (Re7d) mice on serum total protein concentration*

(Values are means  $\pm$  SD for  $n = 5$  per group)

Yogurt fed groups	Days of feeding	Total protein (g/l)	
		M-N	Re7d
Undiluted	2	38 $\pm$ 3	39 $\pm$ 1
	5	39 $\pm$ 7	41 $\pm$ 9
	7	39 $\pm$ 8	37 $\pm$ 8
Diluted (v/v)	2	41 $\pm$ 9	38 $\pm$ 3
	5	37 $\pm$ 8	39 $\pm$ 7
	7	40 $\pm$ 7	39 $\pm$ 8
Groups prior to yogurt feeding			
Malnourished group		40 $\pm$ 8 <sup>1</sup>	
Re-nourished group		49 $\pm$ 9 <sup>1</sup>	
Well-nourished control		61 $\pm$ 4	

<sup>1</sup> Significantly different from well-nourished control ( $P < 0.01$ ). No significant variations were observed between treated M-N and Re7d groups compared with their respective controls.

increase of this parameter but was still lower than the W-N control. Mice fed with yogurt showed no significant variation.

#### *Number of IgA, IgG and IgM secreting cells*

When we analysed the IgA cells associated with the lamina propria of the small intestine we saw that in the undiluted and diluted yogurt groups, the values were significantly high compared with the M-N group and after 7 d they were even greater in the M-N + 1/2 Yo group than in the W-N control (Table 4). The re-nutrition diet increased the number of IgA cells up to the values of W-N control. Feeding of undiluted yogurt (Re7d + Yo) did not induce further increase but Re7d + 1/2 Yo produced a slight increase, which was significant at 7 d of treatment relative to the Re7d group. The effect of yogurt administration on IgG and IgM secreting cells in both M-N and Re7d groups did not induce significant differences. Moreover, the number of these cells remained lower than for the W-N group (Table 4).

#### *Number of intraepithelial leukocytes (IEL), mast cells, and goblet cells*

Treatment with yogurt produced a decrease in the number of IEL at 5 d feeding; this was significant with undiluted yogurt (M-N + Yo; Re7d + Yo). After 7 d treatment values increased in comparison to both the M-N and Re7d groups. However, the significantly lower values ( $P < 0.005$ ) with the malnutrition diet, did not reach those of the W-N control. When we determined the number of goblet cells, we observed a slight increase when yogurt was added to the malnutrition and re-nutrition diets. In the M-N group, this enhancement was significant at 5 and 7 d feeding for M-N + 1/2 Yo group. The significant decrease ( $P < 0.01$ ) seen in mast cells in the M-N group compared with the W-N controls did not vary, even between the different treatments (Table 5).

#### *Histological and ultrastructural studies*

We determined by histological observation of hematoxylin-eosin-stained slices of the small intestine that the malnutrition diet (Fig. 1b) produced a decrease in the size and number per area of the villi in comparison to W-N controls (Fig. 1a). Re-feeding with milk produced a marked recovery in the intestinal structure (Fig. 1c); the effect was more evident in the Re7d + 1/2 Yo group at 5 d (Fig. 1d).

Table 4. Number of IgA, IgG and IgM secreting cells present in the small intestine of malnourished (M-N) and re-nourished (Re7d) mice treated with undiluted and diluted yogurt feeding

(Values are means  $\pm$  SD for two sections of small intestine from each of five experimental and control mice)

		Number of Ig secreting cells/10 fields					
		IgA		IgG		IgM	
Yogurt fed Groups	Days of feeding	M-N	Re7d	M-N	Re7d	M-N	Re7d
Undiluted	2	73.2 $\pm$ 2.5 <sup>1</sup>	90.8 $\pm$ 2.9	21.7 $\pm$ 1.9	27.6 $\pm$ 1.7	15.0 $\pm$ 1.7	26.2 $\pm$ 0.7
	5	85.1 $\pm$ 3.7 <sup>1</sup>	84.8 $\pm$ 3.6	23.3 $\pm$ 1.4	30.4 $\pm$ 4.5	21.1 $\pm$ 1.2	19.3 $\pm$ 1.9
	7	88.0 $\pm$ 2.6 <sup>1</sup>	88.0 $\pm$ 2.6	26.7 $\pm$ 0.9	29.1 $\pm$ 5.3	23.7 $\pm$ 3.2	22.3 $\pm$ 3.1
Diluted (v/v)	2	65.7 $\pm$ 3.4 <sup>1</sup>	78.7 $\pm$ 3.0	26.7 $\pm$ 0.5	25.5 $\pm$ 2.5	17.2 $\pm$ 4.9	22.3 $\pm$ 0.9
	5	86.8 $\pm$ 2.5 <sup>1</sup>	84.9 $\pm$ 2.1	33.5 $\pm$ 5.9	29.9 $\pm$ 2.3	20.7 $\pm$ 3.7	20.3 $\pm$ 2.0
	7	97.9 $\pm$ 3.0 <sup>1</sup>	99.3 $\pm$ 3.1 <sup>2</sup>	26.2 $\pm$ 0.7	26.8 $\pm$ 4.2	22.6 $\pm$ 3.6	22.0 $\pm$ 1.0
Groups prior to yogurt feeding							
Malnourished group		43.7 $\pm$ 3 <sup>3</sup>		22.0 $\pm$ 5.1 <sup>3</sup>		16.1 $\pm$ 3.0 <sup>3</sup>	
Re-nourished group		79.6 $\pm$ 2.7		27.7 $\pm$ 4.2 <sup>3</sup>		29.2 $\pm$ 0.7	
Well-nourished control		86 $\pm$ 2.8		43.7 $\pm$ 1.4		37.3 $\pm$ 5.7	

IgA, IgG and IgM secreting cells were determined by immunofluorescent test.

<sup>1</sup> Significantly different from M-N group ( $P < 0.01$ ).

<sup>2</sup> Significantly different from Re7d group ( $P < 0.01$ ).

<sup>3</sup> Significantly different from well-nourished control ( $P < 0.01$ ) (ANOVA and Student's test).

Table 5. Number of intraepithelial leukocytes (IEL), mastocytes and goblet cells present in the small intestine of malnourished (M-N) and re-nourished (Re7d) mice treated with undiluted and diluted yogurt feeding

(Values are means  $\pm$  SD for two sections of small intestine from each of five experimental and control mice)

		Number of IEL, mastocytes and goblet cells/10 fields					
Yogurt fed Groups	Days of feeding	IEL		Mastocytes		Goblet cells	
		M-N	Re7d	M-N	Re7d	M-N	Re7d
Undiluted	2	43.5 $\pm$ 3.2	63.7 $\pm$ 2.9 <sup>2</sup>	1.2 $\pm$ 0.9	1.0 $\pm$ 0.3	37.9 $\pm$ 4.5	43.9 $\pm$ 6.9
	5	29.7 $\pm$ 2.7	36.8 $\pm$ 3.0 <sup>2</sup>	2.5 $\pm$ 1.1	2.2 $\pm$ 0.7	41.0 $\pm$ 4.3 <sup>1</sup>	44.4 $\pm$ 5.7
	7	54.2 $\pm$ 3.5	71.3 $\pm$ 3.9 <sup>2</sup>	3.5 $\pm$ 1.1	1.2 $\pm$ 0.2	49.0 $\pm$ 6.1 <sup>1</sup>	44.8 $\pm$ 5.1
Diluted (v/v)	2	45.2 $\pm$ 2.9	50.5 $\pm$ 1.8	1.3 $\pm$ 0.5	0.5 $\pm$ 0.3	34.7 $\pm$ 4.7	37.5 $\pm$ 3.3
	5	32.2 $\pm$ 4.0	47.0 $\pm$ 4.1	3.9 $\pm$ 0.9	1.5 $\pm$ 0.8	41.7 $\pm$ 5.7 <sup>1</sup>	37.5 $\pm$ 1.7
	7	34.3 $\pm$ 3.1	82.0 $\pm$ 2.8 <sup>2</sup>	1.3 $\pm$ 0.9	0.7 $\pm$ 0.3	43.7 $\pm$ 2.0 <sup>1</sup>	47.0 $\pm$ 1.6
Groups prior to yogurt feeding							
Malnourished group		36.3 $\pm$ 3.4 <sup>3</sup>		2.7 $\pm$ 0.7 <sup>3</sup>		23.3 $\pm$ 5.8 <sup>3</sup>	
Re-nourished group		48.5 $\pm$ 5.0		2.7 $\pm$ 0.9 <sup>3</sup>		38.6 $\pm$ 5.8	
Well-nourished control		50.8 $\pm$ 2.7		14.5 $\pm$ 1.5		45.5 $\pm$ 4.8	

Number of IEL, mastocytes and goblet cells were determined by hematoxylin-eosin and alcian blue-safranin staining respectively.

<sup>1</sup> Significantly different from M-N group ( $P < 0.01$ ).

<sup>2</sup> Significantly different from Re7d group ( $P < 0.01$ ).

<sup>3</sup> Significantly different from well-nourished control ( $P < 0.01$ ). (ANOVA and Student's test).

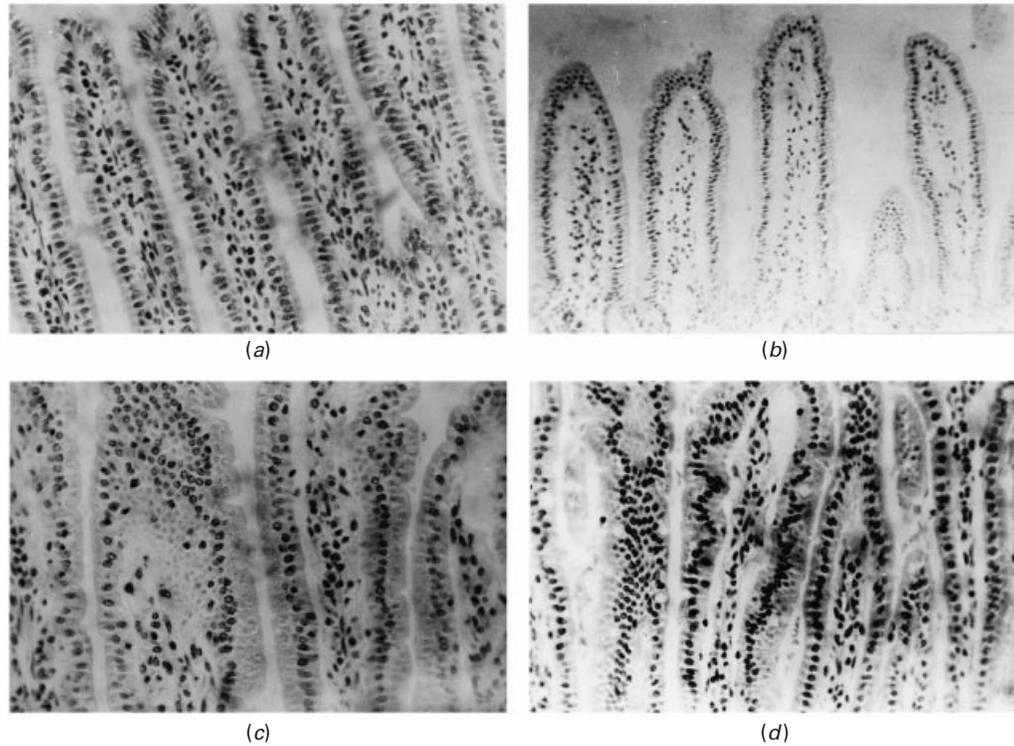


Fig. 1. Photograph of hematoxylin-eosin staining of small intestine. (a) Well-nourished control (40 × magnification). (b) Malnourished group (40 × magnification). (c) Re-nourished group (40 × magnification). (d) Re-nourished mice treated with diluted yogurt during 5 d (40 × magnification).

By electron microscopy of the small intestine of the M-N group (Fig. 2*b*), it was seen that there was an increase in the number of microvilli compared with the W-N controls (Fig. 2*a*), and they were longer and narrower than the villi in the Re7d group (Fig. 2*c*) and in yogurt-fed mice. In the Re7d+1/2 Yo group we observed improvement of the epithelial cells (Fig. 2*d*).

#### DISCUSSION

Therapeutic effects of yogurt on diseases such as cancer, infections, gastrointestinal disorders and asthma have been described previously. Most of these studies provide strong evidence for the hypothesis that yogurt consumption, particularly in immunocompromised hosts, may enhance immunity.

We demonstrated, using an experimental model of malnutrition in the mouse, that the diet of re-nutrition supplemented with yogurt is beneficial to improve nutritional and immunological parameters.

The components of yogurt responsible for the effects, which bolster host-defence mechanisms, have not been fully defined (De Simone *et al.* 1993). It is believed that LAB are essential for yogurt to exert its immunostimulatory effects and that LAB cell walls contain the main immunomodulatory components (Takahashi *et al.* 1993). In the same way, it is well known that non-bacterial milk components and components derived from milk fermentation may also contribute to the immunostimulatory activity of yogurt (Matar *et al.* 2000). We used both undiluted and diluted yogurt feeding to avoid in our malnutrition model an over-stimulation of the gut-

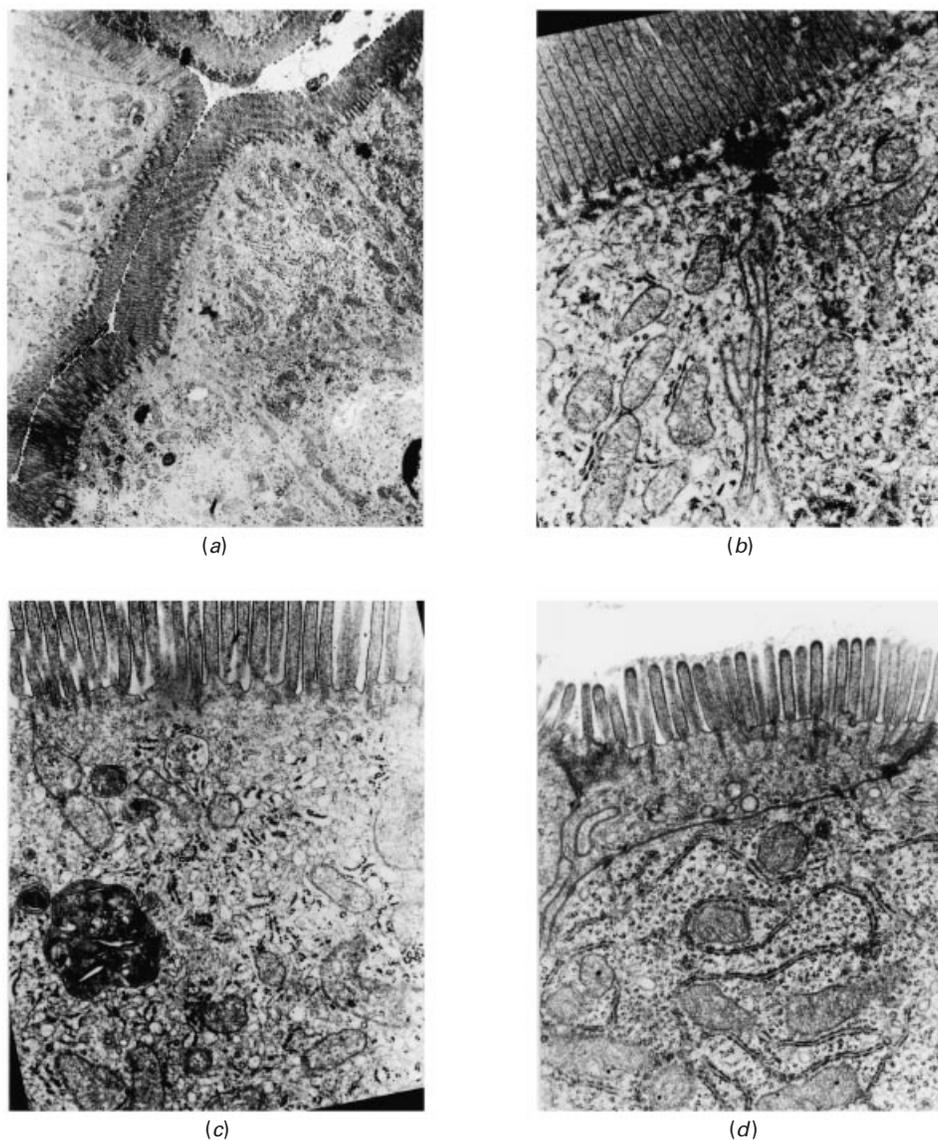


Fig. 2. Transmission microphotograph of epithelial cell of small intestine. (a) Well-nourished control ( $4\ 900\times$  magnification). (b) Malnourished group ( $12\ 800\times$  magnification). (c) Re-nourished group ( $12\ 800\times$  magnification). (d) Re-nourished mice treated with diluted yogurt during 5 d ( $12\ 800\times$  magnification).

associated immune system for the different bacterial and non-bacterial components. We demonstrated that the 5 d of diluted yogurt feeding (Tables 1, 2, 3, 4, 5 and Figs. 1, 2) was the optimal dose for improving the intestinal barrier and the mucosal immune system. This conclusion was reached mainly on the basis of the changes in the number of IgA cells (Table 4).

If an antigen overcomes the non-specific host-defence system, both the humoral and the cell-mediated response are activated. Many studies have demonstrated strong correlations between titers of specific S-IgA antibodies in secretions and resistance to infection (Onorato *et al.* 1991; Perdigon *et al.* 1991). In order for S-IgA

antibodies and IgA B cells from the lamina propria of the intestine to provide protection, they must recognise the surface of the pathogen even if it has been altered by luminal enzymes. We have considered that yogurt might also provide a better barrier against different intestinal infectious agents by increasing in the number of IgA cells in the lamina propria of the intestine (Lamm *et al.* 1996) and subsequently enhancing of the production of a specific immunoglobulin against an antigen. In addition, it is known that the abundance of locally produced IgA is probably crucial for immunological homeostasis within the lamina propria. It has been shown that S-IgA exhibits a remarkable capacity to de-granulate eosinophils (Abu-Ghazaleh *et al.* 1989). Therefore, we thought that the beneficial effect of yogurt feeding might become harmful for the intestinal epithelium if the enhancement of the number of IgA cells was highly significant (Brandtzaeg *et al.* 1993).

Within the intestinal epithelium there is a large population of leukocytes, which are mostly accounted for lymphocytes (intraepithelial lymphocytes, IELs). Although the functions of IELs are unclear, some possibilities are cytotoxicity, lymphokine secretion, regulation of renewal of mucosal epithelium, and tolerance (Cerf-Bensussan *et al.* 1991). In this study (Table 5), the early increase of IEL may be due to enhanced permeability caused by malnutrition for antigenic products, which produce activation of these cells. By microscopic examination, we showed that yogurt induced qualitative proliferate activity of enterocytes (Fig. 1*d*), which could result in recovery and preservation of villous height and barrier function from the beginning of the diet. Therefore, we thought that the decrease in the number of IEL observed at 5 d supplementation (Table 5) may be counterbalanced by the opposing sequestration of immune cells. Furthermore, mucosal lymphocyte movement is regulated by a series of receptors and counter-receptors that are located on lymphocytes and high-endothelial venules. Retention of T cells within the intraepithelial space is very efficient and stable (Famularo *et al.* 1997; Perdigón & Oliver, 2000). The recovered epithelium should be more selective for the cell population interspersed between the epithelial cells of the small and large intestine and the subsequent enhancement of IEL may be as a consequence of a powerful immune activation. This could be supported by the observation that there was a small increase in the number of goblet cells in both groups. An increase in goblet cells has been claimed to reflect local irritation of the intestine (Keren *et al.* 1975). Other hypotheses have suggested that the increasing production of mucus from goblet cells could be considered beneficial because it is known that it protects epithelial cells from digestion by enzymes produced by the intestinal flora and by pancreatic and biliary juices. It could also protect against adherence of enteropathogenic organisms, whereas other components in the mucus, including lysozima, secretory IgA, and lactoferrin, have antibacterial activity.

Mast cells, which are widely distributed throughout the body and are found in abundance in the mucosal tissues, contain a large number of bioactive mediators such as histamine, proteases and leukotrienes. They also play an important role in the innate immunity (Wedemeyer *et al.* 2000). In our model we did not observe significant change with yogurt feeding. Inhibition of inappropriate activation of mast cells would play a crucial role in maintaining homeostasis in mucosal immunity.

We also determined that yogurt addition in a re-nutrition diet helps the recovery of the integrity and function of the intestinal barriers as was observed in the electron microscopic studies, where the epithelial cells showed a prominent endoplasmic reticulum. These extended cisternums suggest an important synthesis of protein (Fig. 2*d*).

It is not surprising that protein deficiency is observed to interfere with resistance to infection, because most immune mechanisms are dependent on cell replication or the production of active protein compounds and protein cannot be synthesised without a balance of essential amino acids. In addition, differentiation and maturation of lymphoid cells depends on a series of intracellular biochemical events involving signal transduction molecules subsequent to receptor-ligand interactions. It is hypothesised that these events are highly dependent on the presence and function of appropriate enzymes, macronutrients, and micronutrients.

Our results strongly suggest that the addition of a re-nutrition diet with yogurt is a good practice. We also demonstrated in a malnutrition process that feeding of yogurt does not induce harmful effects. Even though these results were obtained in an animal model, and the effects of probiotic bacteria in humans have not been well established, the indication is that the consumption of yogurt by malnourished children will accelerate of recovery of gut function

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