concentration of the samples was expressed in percentage in relation to the pattern serum. The absolute values of total proteins of each groups were used as witnesses in relation with those values of gamma globulins as a way of allowing the comparison between the groups studied. The statistical analysis of the samples was made by the t-test method. The concentrations of total proteins studied during the cycle did not have significant variations: the values were found in the range of 5.47 to 8.04 mg/ml. The highest values of total protein in the vaginal fluids of experimental animals was found in the castrated and estrogen-treated animals after 8 days of treatment. In this group the protein concentration was found in a range of 3.48 to 12.74 mg/ml (table). A considerable decrease of the gamma globulin fraction of the vaginal fluids was found in the samples obtained during the estrus in comparison with the values found in diestrus ($p < 0.001$). The vaginal fluids of the mice in group II studied 3 and 8 days after estrogen treatment showed a significant decrease ($p < 0.001$) of gamma globulins fraction in relation to the non-treated castrated animals. The lowest values were found after 8 days of the estrogen therapy (table).

Previously, we had found that the plasma cells in the lamina propria of the mice vaginal mucosa show a cyclical and experimental variations[16]. This phenomenon had also been found in hamster[17] and in women[18] and it is apparently controlled by estrogens. The results of the present report show cyclical and experimental variations of the gamma globulin fraction in the protein content of the mouse vaginal fluids. It is of interest to point out that the greatest content of gamma globulins found in the diestrus and castrated mice coincide with the greatest number of plasma cells in the lamina propria in the vaginal mucosa. Our results suggest that the variation of gamma globulins found in the vaginal fluids is related in part by the presence of vaginal plasma cells and influenced by estrogens.

Relation between of the total proteins and gamma globulins content in the mice vaginal fluids

Group	Total proteins (mg/ml)	Gamma globulins c/o pattern serum
Diestrus	7.00 ± 1.06	5.54 ± 1.42
Proestrus	8.04 ± 1.91	4.24 ± 1.21
Estrus	5.47 ± 0.42	1.16 ± 0.20
Metaestrus	5.99 ± 2.02	3.36 ± 1.03
Castrated	3.48 ± 0.94	2.51 ± 0.93
Castrated + estradiol 3 days	6.66 ± 1.76	2.43 ± 0.97
Castrated + estradiol 8 days	12.74 ± 2.52	2.99 ± 0.60
Castrated + estradiol 32 days	5.32 ± 1.27	1.93 ± 0.46

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Vitamin C and the immune response

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Summary. The inclusion of vitamin C in the drinking water of BALB/c mice was without effect on the humoral antibody response to sheep red blood cells and bacterial lipopolysaccharide. However, there was a significantly increased cell-mediated immune response as determined by increased T-lymphocyte responses to concanavalin A. This might suggest a mechanism, along with interferon enhancement, for the possible protection by vitamin C against some viral infections.

Previous reports[2,3] have suggested that the participation of vitamin C in protection against some viral infections may be in the enhancement of interferon production. We have reported an increased response to interferon induction in mice fed a diet containing vitamin C[2] and have also observed a similar phenomenon in mouse cell cultures[3]. There has been some evidence recently that interferon may have a modulating effect on the immune response[4]. It was therefore of interest to determine whether ascorbic acid might also play a role in the mediating of the immune response.

Materials and methods. BALB/c mice, males 2.5–4.5 months of age, were used except where indicated. Experimental animals were fed L-ascorbate (250 mg%) in their drinking water ad libitum, and similar sets of control animals remained on untreated water. Vitamin C and

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control mice were immunized i.p. with sheep red blood cells (SRBC). 4 and 5 days later mice were killed, and the number of antibody or plaque forming cells (PFC) in the spleen was determined[5]. For carrying out the assay in spleens of lipopolysaccharide (LPS, Difco, E. coli 055:B5) immunized animals, SRBC employed in the substrate medium were first coated with LPS which had been heated at 100 °C for 2 h[6].

With regard to mitogenic response studies, mouse spleens were removed and prepared as described previously[7]. Briefly, nylon column-filtered spleen lymphocytes were cultured at a density of 2×10^6/ml in 25 mM HEPES-buffered medium 199 with 2.5% decomplemented fetal calf serum and added antibiotics. A concentration of 2 μg/ml concanavalin A (con A, Sigma, St. Louis, MO) was used in the experimental cultures. $^5/_{100}$ ml of medium containing 1.0 μCi of triated thymidine with specific activity of 6.7 Ci/mM (New England Nuclear, Boston, MA) was added to the cultures 24 h prior to harvesting at 72 h of culture at 37 °C. The cell cultures were terminated and prepared for scintillation counting as previously described[7]. Radioactivity of the samples was determined in a Packard Tri-Carb liquid scintillation spectrometer and the mitogenic response for each individual experiment expressed as the difference in radioactivities between stimulated and unstimulated cultures.

Endogenous colony forming cell assays[8] were performed by exposing mice to 400–600 R whole body x-irradiation (Picker-Vanguard, 71 R/min at 277 kvp and 15 ma with a half-value layer of 1.9 mm Cu). 9 days later, spleens were harvested, placed in Bouin's fixative, and the colonies counted. Data is presented as the mean ± standard error of the mean. The Student t-test is employed to compare experimental results and the results considered to be significantly different when p values < 0.05 are obtained.

Results and discussion. The possibility that vitamin C, earlier demonstrated to enhance interferon formation[2,3], might also play a role in modulating the immune response was investigated. In this regard, there did not appear to be any significant effect on the responsiveness of the bone marrow-derived lymphocyte (B-cell) as determined by humoral antibody responses to sheep red blood cells and to bacterial lipopolysaccharide. As shown in table 1, there was little effect on spleen antibody plaque formation in BALB/c mice after drinking vitamin C for periods of 9 to 67 days prior to SRBC immunization.

SRBC is a thymus-dependent antigen, that is, for the induction of antibody response a collaboration between thymus-derived lymphocyte (T-cell) and B-lymphocyte is necessary, the former providing a helper function to the antibody precursor B-cell. In contradistinction, the response to lipopolysaccharide antigen is T-lymphocyte independent. The data in table 2 indicate that the antibody response to LPS, uniquely a B-lymphocyte response, was not significantly different in the vitamin C and control animals.

Endogenous colony formation in the spleen, a measure of the hemopoietic stem cell population in active growth cycle[9], is also noted with a number of strains of mice to parallel the extent of the humoral immune response[10]. Enumeration of colony forming units (CFU) in the spleens of mice irradiated at 400 R, 500 R or 600 R (table 3) showed no significant differences between vitamin C treated and control mice, in accord with the observed antibody responses (tables 1 and 2).

The mitogen con A stimulates T-lymphocytes selectively and may be used as a sensitive probe to detect mouse splenic T-cells. In such experiments in which spleen lymphoid cells were stimulated in vitro with con A (table 4), cells harvested from animals on a vitamin C regimen for 2–8 weeks showed significantly greater thymidine incorporation than controls, indicative of enhanced T-cell activity.

T-lymphocytes participate in cell-mediated immune (CMI) processes. These include the release of a variety of soluble effector molecules, or lymphokines, which are

Table 1. Effect of vitamin C on antibody response to SRBC in BALB/c mice*

Experiment	Treatment	Days on vitamin C before immunization	PFC/spleen $\times 10^{-3}$ mean ± S.E.M.
I	Vitamin C	9	163.7 ± 4.359
	control		146.2 ± 17.21
II	Vitamin C	26	175.1 ± 55.90
	control		160.7 ± 38.40
III	Vitamin C	67	86.2 ± 7.93
	control		73.6 ± 6.09

*Mice were placed on a vitamin C regimen (250 mg%) at 3.5–4.5 months of age. After the indicated number of days, animals were injected i.p. with 0.20 ml of a 1 : 10 suspension of washed sheep red blood cells (SRBC) and vitamin C treatment continued. Direct spleen plaque forming cells were assayed on days 4 or 5. Each value represents the mean for 3–5 mice. Differences between vitamin C and control mice were not significant ($p>0.05$).

Table 2. Effect of vitamin C on the response of BALB/c mice to immunization with E. coli lipopolysaccharide*

Experiment	Treatment	Days on vitamin C before immunization	PFC/spleen $\times 10^{-3}$ mean ± S.E.M.
I	Vitamin C	9	11.9 ± 1.69
	control		15.6 ± 1.10
II	Vitamin C	17	23.3 ± 2.20
	control		27.4 ± 4.08
III	Vitamin C	29	25.9 ± 5.85
	control		24.7 ± 3.02

*Mice were placed on a vitamin C regimen (250 mg%) at 2.5–3.5 months of age. After the indicated number of days, animals were injected intravenously with 5 μg E. coli lipopolysaccharide and vitamin C treatment continued. Direct spleen plaque-forming cells were assayed on days 4 or 5 using LPS-coated SRBC as substrate. Each value represents the mean for 3–5 mice. Differences between vitamin C and control mice were not significant ($p > 0.05$).

Table 3. Endogenous spleen colony formation (CFU) in BALB/c mice treated with vitamin C*

X-ray dose (R)	Vitamin C Spleen wt (mg)	CFU/spleen	Control Spleen wt (mg)	CFU/spleen
400	60.5±2.46	22.4±5.26	64.3±4.15	18.2±3.63
500	36.5±2.41	2.4±0.59	43.2±1.30	5.2±1.76
600	35.1±2.72	0.50±0.22	30.1±1.40	0.20±0.17

*Mice were started on a regimen of 250 mg % vitamin C in the drinking water at 2.5 months of age. Four weeks later animals were exposed to the designated x-ray dose and spleen colony formation and spleen weights measured 9 days later. Differences were not significant between vitamin C and control mice ($p > 0.05$).

Table 4. Effect of vitamin C on response of spleen lymphoid cells to con A*

Experiment	Treatment	cpm Unstimulated	con A	Δ cpm	Δ cpm mean ± S.E.M.	Significance
			2 weeks			
I	Vitamin C	827 ± 49	12866 ± 264	12039	11692 ± 283	
		609 ± 42	11955 ± 127	11346		
						$p < 0.025$
	Control	121 ± 5.5	1447 ± 52	1326		
		426 ± 17	5778 ± 178	5352	3403 ± 1163	
		340 ± 8.5	3873 ± 340	3533		
			4 weeks			
II	Vitamin C	399 ± 6.4	41353 ± 731	40954		
		1250 ± 31	53984 ± 521	52734	42490 ± 4784	
		682 ± 40	34465 ± 712	33783		
						$p < 0.05$
	Control	613 ± 18	30277 ± 342	29664		
		341 ± 25	21460 ± 335	21119	21068 ± 4311	
		436 ± 27	12856 ± 342	12420		
			8 weeks			
III	Vitamin C	1427 ± 8.5	56724 ± 1544	55297		
		879 ± 29	57079 ± 399	56200	47666 ± 7003	
		456 ± 20	31957 ± 261	31501		
	Control	387 ± 16	6179 ± 198	5792		$p < 0.05$
		485 ± 48	8996 ± 90	8511	12975 ± 5089	
		573 ± 21	25197 ± 45	24624		

*BALB/c mice were placed on a vitamin C regimen (250 mg% in drinking water) at 2.5 months of age. At 2 weeks (males) and at 4 and 8 weeks (females) spleen lymphoid cells were harvested for con A stimulation. Values presented are of assays for individual mice carried out in groups of 2–3 vitamin C and control animals. The different time points represent 3 separate experiments. Assays of ^{3}H-thymidine incorporation were performed in triplicate in each case.

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probably responsible for effecting CMI[11]. For example, entrapment of macrophages by such lymphokines as macrophage inhibitory factor (MIF) and heightened activation by macrophage activation factor (MAF) result in more ready ingestion and degradation of infectious agents. Thus, the cell-mediated immune response is considered to provide a mechanism for enabling the infected host to rid itself of viruses and other intracellular parasites. Conceivably, then, the enhancement of interferon synthesis and the augmentation of T-lymphocyte activity would suggest a dual role for vitamin C in its putative protective participation against viral disease.

The effect of levamisole on phosphodiesterase activity

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Summary. Phosphodiesterase activity of mouse liver homogenates was estimated in presence and absence of levamisole. The enzyme activity was 1394 and 1399 nmoles/mg protein/30 min respectively. Our data show that levamisole does not affect the phosphodiesterase activity.

Levamisole, a widely used anthelminthic drug, was found to restore host defense mechanisms by stimulating phagocytes and lymphocytes when they are defective, and it has a potential therapeutic value as an immuno-modulating agent in domestic animals and in man[1]. In man, encouraging results have been reported under a wide range of conditions, many of which are to unknown etiology, but all with suspected defects in host defense mechanisms[2].

The mode of action of levamisole is not known and many hypotheses have been formulated. The variety of cells and the multitude of functions that are affected make it likely that levamisole influences a basic mechanism common to

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