

# Vitamin A Supplementation Improves Macrophage Function and Bacterial Clearance during Experimental Salmonella Infection (42888)

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**Abstract.** The effects of additional but nontoxic amounts of vitamin A on susceptibility to salmonella infection was studied by comparing rates of bacterial clearance and phagocytosis. Forty-eight male Lewis rats were divided into a treatment group receiving a total of 6000 units of vitamin A palmitate weekly for 5 weeks and a control group was given an equal volume of saline. After completion of the treatment regimen, one-half from each group were infected intraperitoneally with  $10^5$  *Salmonella typhimurium*; the other half received intraperitoneal injection of saline. At this time no differences in weight gain were noted and all animals were sacrificed within 2 weeks. At 72 hr after bacterial challenge, all saline-treated control animals displayed bacteremia. Cultures of liver and splenic homogenates were positive in 89 and 100% of infected control animals vs 0 and 44% for treated animals during the first week of infection. Kupffer cell, peritoneal, and splenic macrophages of the vitamin A-treated group had greater phagocytic activity than controls as assessed by the percentage of cells ingesting yeast particles and by the number of particles ingested (phagocytic index). These results suggest that vitamin A in moderate amounts may benefit the host's response to infection by enhancing phagocytic cell function.

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Vitamin A, a fat-soluble vitamin is important in maintaining the functional integrity of epithelial and mucosal surfaces and in the production of mucous secretions. Vitamin A deficiency, a major world health problem, associated with visual disturbances, has also been implicated in increasing host susceptibility to a variety of bacterial, rickettsial, viral, and parasitic infections (1, 2). Supportive findings indicate that vitamin A influences immune system function (3–6). Modest increases in dietary vitamin A have been reported to enhance host resistance to challenge with bacterial and fungal microorganisms (7–9). The mechanisms by which vitamin A modulates host susceptibility to infection remain unclear, although it has been reported that retinoids given at low concentrations suppress both Fc receptor-mediated binding of macrophages and subsequent phagocytosis (10). Increased doses of vitamin A have been shown to alter lysosomal

membranes (11), act as an adjuvant (4, 12), and exert a positive effect on cell-mediated immunity (3–5).

The mechanism for nonspecific resistance to salmonella infections in experimental animals has been primarily attributed to cell-mediated factors (13–15), although some studies have shown that humoral antibody plays an important role (16–18). The protective mechanism to intracellular pathogens such as *Listeria* and *Salmonella* may be attributed to a proliferating cell population of monocytes and macrophages (19). Macrophages can kill or inhibit bacteria as well as the growth of certain tumor cells. The mechanisms, although not clear, may be due to the cell's production and release of macromolecular substances or oxygen radicals that are mediators for inflammation and tumoricidal activity (20–22). Fixed tissue macrophages have been shown to be involved in clearance of bacteremia and in initial killing (19). The liver, for example, has the highest number of fixed macrophages (Kupffer cells) in the body and these cells play an important role in host defense against bacterial infection (23). More important in controlling infection are the bone marrow-derived monocytes, the cells primarily responsible for limiting early multiplication of organisms in the liver and spleen (24). In this report we hypothesized that vitamin A

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induces resistance to infection in a nonspecific manner by enhancing phagocytic cell function, especially of the reticuloendothelial system. This study is an investigation of the effects of vitamin A supplementation on host resistance to experimental infection by assessing the parameters of blood clearance and macrophage activity.

### Materials and Methods

**Animals.** Weanling male inbred Lewis rats (Charles River Laboratories, Wilmington, MA) received at 21 days of age were housed individually in cages with wire mesh bottoms and fed standard lab chow<sup>1</sup> and water *ad libitum* throughout the experimental period. All animals were maintained in the animal care facility at a room temperature of 20–22°C.

**Infecting Agent.** *Salmonella typhimurium* type LT2 (provided by Dr. A. DeMaria, Boston City Hospital, Boston, MA) grown in beef heart infusion broth was washed and quantitated, serially diluted, and administered to the rats. Quantitative counts performed on nutrient agar was used to determine actual numbers of organisms administered.

**Vitamin Preparation.** Water-miscible vitamin A palmitate (United States Biochemical Corp., Cleveland, OH) adjusted for test concentration in units was dissolved in sterile pyrogen-free saline and administered to control and experimental animals.

**Experimental Design.** Forty-eight 21-day-old male Lewis rats were divided into two groups of 24 each. One group received intraperitoneal doses of 6000 units of vitamin A palmitate in 0.5 ml of saline weekly (single injections of 3000 units on Tuesdays and 3000 units on Thursdays) for 5 weeks for a total of 30,000 units of vitamin A. The other group received intraperitoneally equal volume injections of saline weekly for 5 weeks. After completion of treatment regimen, one-half of the vitamin A group and one-half of the control group were infected intraperitoneally with a sublethal dose of 10<sup>5</sup> colony-forming units of *S. typhimurium*. The remaining half from each group received an intraperitoneal injection of saline. Animals were observed daily and weighed throughout the course of experiment. At intervals of 3, 5, 7, and 12 days after infection, three animals from each group were sacrificed under ether anesthesia and the following parameters were measured: animal and organ weight (spleen and liver); serum retinol, hematocrit, CBC, and WBC differential; bacterial clearance of blood, spleen and liver; and phagocytosis by morphologic macrophage assay.

<sup>1</sup> Diet (#5001) was obtained from Ralston Purina Co., St. Louis, MO. The percentage of composition was as follows: protein, 25; fat, 6; fiber, 4.5; ash, 7; nitrogen-free extract, 47.5; calcium, 1.3; phosphorus, 0.9; magnesium, 0.2; potassium, 1; and sodium, 0.3. The diet also contained the following vitamins and minerals in parts per million: iron, 325; manganese, 100; copper, 15; cobalt, 0.10; iodine, 2; carotene, 6; thiamin, 10; riboflavin, 8; niacin, 100; pantothenic acid, 15; choline, 1700; pyridoxine, 4.3; vitamin a, 10 IU/g; and vitamin D, 5 USP units/g.

**Bacterial Clearance.** Spleens and livers of animals sacrificed at intervals were removed aseptically, weighed, and portions homogenized individually in tissue grinder tubes containing sterile phosphate-buffered saline. Serial 10-fold dilutions were cultured on blood agar plates. Colony counts were used to calculate the geometric mean of viable bacteria per organ. Blood for bacteremia studies was obtained by cardiac puncture at time of sacrifice and cultured on nutrient agar plates.

**Peripheral Blood.** Blood samples for hematologic parameters prior to infection were obtained by retro-orbital sinus technique. Blood obtained by cardiac puncture at time of sacrifice was used following infection.

**Peritoneal Macrophages.** Peritoneal cells were harvested by washing the peritoneal cavity with cold phosphate-buffered saline prior to time of sacrifice, centrifuged, suspended in tissue culture medium (RPMI 1640; GIBCO, Grand Island, NY), and counted. Viability was determined by trypan blue dye exclusion.

**Spleen Cells.** Homogenates were washed and cells separated by passing through sterile fine mesh stainless steel screen and suspending in RPMI 1640 medium supplemented with 2 mM/ml L-glutamine, 1% penicillin-streptomycin (GIBCO), and 10% fetal calf serum (FCS) (Sterile Systems Inc., Logan, UT). After passing through glass-wool filters, cells were washed, resuspended in medium, counted, and determined for viability by trypan blue dye exclusion.

**Kupffer Cells.** Macrophages from liver were obtained using a modified method described by Seglen (25) and Toth *et al.* (26). Briefly, isolation was performed by *in situ* perfusion of the liver with calcium-free buffer. The organ was removed, weighed, and minced into small pieces. Fragments were digested for 1 hr in a warm buffer solution consisting of collagenase types II and IV (Sigma Chemical Co., St. Louis, MO) and incubated under constant stirring in a 37°C 10% CO<sub>2</sub> incubator. The suspension was filtered through gauze, further digested in enzyme solution, washed several times with balanced salt solution, and layered in a gradient with metrizamide (Sigma Chemical Co.). Cells were collected, washed, and counted and viability was determined by trypan blue dye exclusion.

**Phagocytosis.** Macrophages were assayed by modified methods described by Spitalny (27), Cole (28), and Lemaire *et al.* (29). Fetal calf serum was heat inactivated at 56°C for 30 min. Hanks' balanced salt solution (GIBCO) was added to make 15% FCS-Hanks' balanced salt solution. Yeast particles (Fleischmann's dry powder) were opsonized in FCS-Hanks' balanced salt solution for 30 min in a 37°C CO<sub>2</sub> incubator. Peritoneal, splenic, and liver macrophages were incubated with opsonized FCS-yeast on 22 × 22 number 1 glass coverslips in a CO<sub>2</sub> incubator at 37°C for 45 to 60 min. After washing the nonadherent cells, coverslips were stained with hematoxylin and eosin and then mounted

on glass slides for microscopic examination. A minimum of 50 macrophages were counted over a random number of microscopic fields and the percentage of macrophages phagocytizing yeast cells and the number of yeast cells ingested per macrophage were tabulated. A phagocytic index (PI) was determined by dividing the number of ingested yeast cells by the number of phagocytizing macrophages.

**Statistical Analysis.** Data were expressed as means  $\pm$  SEM. Statistical significance was determined by analysis of variance (ANOVA). Where data was complete for ANOVA, Tukey's multicomparison procedure was used. For ANOVA with incomplete data, estimated effects were compared through *t* statistics with Bonferroni's adjustment used to assure an overall significance level of 0.05.

## Results

**Total Body and Organ Weight.** Table I depicts the various groupings used in the study: I, supplemented with vitamin A and then infected; II, supplemented with vitamin A and not infected; III, control group not supplemented with vitamin A but infected; and IV, control group not supplemented with vitamin A and not infected. Animals in both control (III and IV) and test (I and II) groups showed a significant increase in weight prior to infection. Food consumption by the animals was observed to be similar among the groups. Seventy-two hours following intraperitoneal injection of  $10^5$  colony-forming units of *S. typhimurium*, mean weight is significantly greater for Group IV than for Groups II or III. All 12 animals in Group I continued to gain weight. Nine of 12 infected control group animals (III) lost weight and had significantly lower body weight ( $P < 0.0001$ ) than the noninfected control group (IV). For subsequent days, animals from each group were weighed and the change noted. The number of animals diminished as three per group were sacrificed for study at each time period. Group I animals (infected

treated) over the course of infection displayed a net gain of nearly 30 g whereas Group III animals (infected nontreated) showed a net gain of only 1.2 g.

The spleens of the infected control group were significantly heavier ( $P < 0.0001$ ) than the vitamin-treated and the nontreated, noninfected groups throughout the course of infection (Table II). Liver weight per 100 g body wt did not significantly vary with either group and/or day, although the infected controls were slightly heavier than the treated group throughout the course of infection.

**Hematology.** Serum retinol levels on one animal from each group were performed over the course of infection and the values are shown in Table III. If analyzed by groups, the values, although statistically greater for the supplemental groups (I and II), are still within or slightly above the normal reference range. Hematocrit readings on all animals were within the normal range. Significant differences were seen in the WBC population following completion of the treatment regimen. The vitamin A-supplemented groups (I and II) had a significantly higher percentage of circulating neutrophils and a significantly lower percentage of lymphocytes than the nontreated control groups (III and IV) (Table IV). A marked leukopenia (WBC count less than 3500/cu mm) was seen in the infected control group (III) 72-hr postinfection. The infected treated group (I) demonstrated an increased percentage of neutrophils over the noninfected treated (II) and control groups (IV). Thereafter, no significant changes occurred among the infected groups until Day 7 when the percentage of neutrophils increased for the infected control group (III) (Day 7:  $17.3 \pm 1.8\%$  vs Day 0:  $7.7 \pm 3.1\%$ ,  $P < 0.001$ ). Throughout the course of infection, the vitamin A-treated group (II) continued to show a higher percentage of neutrophils over the nontreated control group (IV) (Day 5: Group II,  $13.3 \pm 4\%$  vs Group IV,  $8.4 \pm 3\%$ ; Day 7: Group II,  $20.8 \pm 3.2\%$  vs Group IV,  $9.5 \pm 6.1\%$ ).

**Table I.** Mean Animal Weights in Grams during Course of Treatment and Infection<sup>a</sup>

Group <sup>b</sup>	Before vitamin A administration	After vitamin A administration and before infection	After infection			
			Day 3 (wt change)	Day 5 (wt change)	Day 7 (wt change)	Day 12 (wt change)
I	68.4 $\pm$ 4.5	298.8 $\pm$ 11.2	310.2 $\pm$ 10.7 (+11.4)	318.3 $\pm$ 13.5 (+8.1)	325.7 $\pm$ 15.4 (+7.4)	327.7 $\pm$ 11.4 (+2.0)
II		298.3 $\pm$ 9.7	302.4 $\pm$ 10.9 (+4.1)	308.8 $\pm$ 12.6 (+6.4)	319.2 $\pm$ 14.5 (+10.4)	330.7 $\pm$ 13.1 (+11.5)
III	70.8 $\pm$ 4.7	302.8 $\pm$ 10	291.6 $\pm$ 19.4 <sup>c</sup> (-11.2)	306.3 $\pm$ 15 (+14.7)	313.8 $\pm$ 16.1 (+7.3)	304.0 $\pm$ 15.5 <sup>d</sup> (-9.6)
IV		307.3 $\pm$ 15	315.6 $\pm$ 23.5 <sup>e</sup> (+8.2)	321.0 $\pm$ 23.8 (+5.4)	327.7 $\pm$ 25 (+6.7)	351.7 $\pm$ 15.9 (+24.0)

<sup>a</sup> Animals placed into two groups of 24 were weighed before given the first administration of vitamin A and weighed after completion of treatment regimen but before infection (day 0). Animals in four groups of 12 were weighed 72 hr after infection (Day 3) and the change in weight from Day 0 recorded. For subsequent intervals, remaining animals from each group were weighed and the change in weight from the previous period recorded. The number of animals diminished as three per group were sacrificed for study at each time period. Values are means  $\pm$  standard deviation of respective groups.

<sup>b</sup> I, vitamin A-treated and infected; II, vitamin A only; III, control and infected; IV, control only.

<sup>c</sup> Significant at  $P < 0.001$  from Group IV.

<sup>d</sup> Significant at  $P < 0.001$  from Groups II and IV.

<sup>e</sup> Significant at  $P < 0.001$  from Groups II and III.

**Table II. Effect of Vitamin A and Infection on Organ Weight<sup>a</sup>**

Day	Group <sup>b</sup>	Animal wt (g)	Spleen wt (g)	Spleen wt/100 g body wt	Liver wt (g)	Liver wt/100 g body wt
3	I	312.3 ± 9.6	0.71 ± 0.08	0.23 ± 0.03	12.09 ± 0.55	3.88 ± 0.19
	II	301.0 ± 10.4	0.67 ± 0.09	0.22 ± 0.02	11.91 ± 1.23	3.96 ± 0.48
	III	271.7 ± 19.6	1.57 ± 0.19 <sup>c</sup>	0.58 ± 0.08 <sup>c</sup>	11.54 ± 0.58	4.25 ± 0.11
	IV	321.3 ± 17.0	0.68 ± 0.02	0.21 ± 0.02	16.13 ± 3.12	5.00 ± 0.80
5	I	321.3 ± 10.3	0.76 ± 0.14	0.23 ± 0.04	12.25 ± 1.11	3.81 ± 0.25
	II	302.3 ± 11.9	0.75 ± 0.09	0.25 ± 0.04	13.14 ± 2.84	4.33 ± 0.78
	III	307.7 ± 18.5	2.19 ± 1.47 <sup>c</sup>	0.73 ± 0.51 <sup>c</sup>	13.29 ± 0.40	4.33 ± 0.27
	IV	321.7 ± 20.5	0.76 ± 0.10	0.24 ± 0.04	12.76 ± 1.31	3.96 ± 0.18
7	I	330.7 ± 19.8	0.69 ± 0.08	0.21 ± 0.03	14.07 ± 0.85	4.26 ± 0.31
	II	321.3 ± 17.9	0.70 ± 0.11	0.21 ± 0.02	12.49 ± 0.91	3.90 ± 0.41
	III	322.7 ± 6.1	2.44 ± 1.32 <sup>c</sup>	0.76 ± 0.11 <sup>c</sup>	14.21 ± 0.90	4.41 ± 0.25
	IV	315.3 ± 29.3	0.64 ± 0.01	0.21 ± 0.02	12.94 ± 3.11	4.07 ± 0.64
12	I	327.7 ± 11.4	0.75 ± 0.04	0.23 ± 0.01	12.66 ± 0.21	3.87 ± 0.12
	II	330.7 ± 13.1	0.69 ± 0.18	0.21 ± 0.05	13.40 ± 0.55	4.05 ± 0.09
	III	304.0 ± 15.5	1.06 ± 0.45	0.35 ± 0.14	13.33 ± 2.64	4.36 ± 0.65
	IV	351.7 ± 15.9	0.75 ± 0.12	0.21 ± 0.03	14.56 ± 5.16	4.17 ± 1.57

<sup>a</sup> Weights in grams expressed as mean ± SEM of three animals selected for sacrifice per group for each time interval.

<sup>b</sup> I, vitamin A and infection; II, vitamin A only; III, control and infection; IV, control only.

<sup>c</sup> Significantly higher ( $P < 0.0001$ ) from other three groups.

**Table III. Serum Retinol Levels in mcg/dl during Course of Infection with *Salmonella typhimurium*<sup>a</sup>**

Day	Group I <sup>b</sup>	Group II <sup>b</sup>	Group III <sup>b</sup>	Group IV <sup>b</sup>
3	104	96	35	36
5	63	99	42	63
7	93	125	32	66
12	123	104	42	54
Mean	95.8 ± 25.1 <sup>c</sup>	106 ± 13.1 <sup>c</sup>	37.6 ± 5.1	54.8 ± 13.5

Note. Normal reference values: 30–95 mcg/dl.

<sup>a</sup> Serum retinol levels performed by RBP-end point radioimmunoassay at Smith Kline Clinical Laboratories (Waltham, MA) on one animal from each group over course of infection.

<sup>b</sup> I, vitamin A-treated and infected; II, vitamin A only; III, control and infected; IV, control only.

<sup>c</sup> Significant at  $P < 0.05$  from Group III.

<sup>d</sup> Significant at  $P < 0.05$  from Group IV.

**Bacterial Clearance.** All infected control animals displayed bacteremia at 72-hr postinfection. Circulating bacteria were cleared from the circulation of this group after 1 week (Fig. 1). All infected treated animals were clear of infection in the blood throughout the 2-week period. Numerous colonies were grown from splenic homogenates of infected controls during the first week of infection. Decreasing numbers were noted after Day 3 and total clearance by Day 12. By contrast, spleens from infected but vitamin A-treated animals provided fewer colonies of *Salmonella* initially and none by Day 7 postinfection (Fig. 2A). Liver homogenates were cultured and were negative for all infected treated animals (Fig. 2B). Bacterial growth was detected in eight of nine (89%) of the infected controls (III) during the first week of infection. Thereafter, no bacteria was detected in any of these animals.

**Macrophage Assay.** The PI for all macrophage types studied—peritoneal, splenic, and Kupffer—was greater for the vitamin A-treated groups (I and II) than the controls (III and IV) in all except a few instances (Day 3: peritoneal, Group II, 2.9 vs Group IV, 3.2; Day 5: Kupffer, Group II, 3.5 vs Group IV, 3.6). Group I (infected treated) in all but two instances had higher values than the control group (IV), and at these two points the values were equal (Day 3: peritoneal, Groups I and IV, 3.2; Day 5: Kupffer, Groups I and IV, 3.1). As can be seen in Figure 3, by Day 5 Group I and II animals had significantly greater mean PI for splenic macrophages than on Day 3 ( $P < 0.0001$ ). The mean PI for peritoneal macrophages on Day 7 was significantly greater than that of Day 3 or Day 7 ( $P < 0.0001$ ). Figure 4 shows that the mean percentage of phagocytizing cells was greater for the vitamin-treated animals than for the controls. The phagocytic capacity of spleen cells and peritoneal macrophages from infected vitamin A-treated animals was significantly greater than the other groups. The mean phagocytic index and percentage of ingestion of phagocytic cells from the infected nontreated group (III) were significantly lower than the macrophages from other groups during the course of infection.

## Discussion

We have shown that vitamin A supplementation in nontoxic amounts may be beneficial in infectious episodes with intracellular pathogens. In a series of earlier experiments (unpublished data) we examined the effects of administering vitamin A intraperitoneally to rats in small doses over 4 consecutive days and challenging with different doses of *S. typhimurium* and

**Table IV.** Effect of Vitamin A and Infection on Circulating Cells<sup>a</sup>

Day	Group <sup>b</sup>	Hematocrit (%)	WBC (mm <sup>3</sup> )	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
0	I	47.4 ± 8.6	10,709 ± 4000	13.9 ± 6.4 <sup>c</sup>	83.7 ± 7.0	1.5 ± 0.9
	II					
	III	47.7 ± 5.1	12,610 ± 4400	7.9 ± 3.0	89.9 ± 3.3 <sup>c</sup>	1.8 ± 1.1
	IV					
3	I	34.7 ± 9.3	10,963 ± 4257	23.0 ± 2.5	74.0 ± 3.1	2.8 ± 1.0
	II	41.0 ± 4.0	11,090 ± 4552	12.7 ± 4.9	85.3 ± 4.6	1.7 ± 0.6
	III	39.7 ± 2.5	3,035 ± 2008 <sup>e</sup>	28.7 ± 4.1	68.0 ± 6.3	3.3 ± 1.3
	IV	39.7 ± 3.1	12,806 ± 2844	6.3 ± 1.9	89.7 ± 4.9	1.5 ± 0.4
5	I	41.3 ± 1.5	11,867 ± 4038	16.5 ± 10.8	80.8 ± 9.4	2.2 ± 1.6
	II	40.3 ± 10.7	12,867 ± 4524	13.3 ± 4.0	83.5 ± 4.1	1.5 ± 0.5
	III	32.3 ± 7.2	9,000 ± 3811	12.4 ± 6.0	84.7 ± 11.6	2.5 ± 0.5
	IV	38.3 ± 1.2	8,200 ± 2307	8.4 ± 3.0	80.5 ± 8.2	0.7 ± 0.3
7	I	36.0 ± 8.2	10,708 ± 5461	15.0 ± 5.6	84.2 ± 5.6	0.8 ± 0.3
	II	37.0 ± 2.0	11,123 ± 5206	20.8 ± 3.2	77.3 ± 3.9	1.3 ± 0.4
	III	38.0 ± 1.7	8,040 ± 3553	17.3 ± 1.8	81.7 ± 2.5	0.8 ± 0.6
	IV	34.3 ± 9.3	8,016 ± 3106	9.5 ± 6.1	88.2 ± 4.9	1.7 ± 1.2
12	I	36.7 ± 11.0	9,019 ± 3055	14.5 ± 4.5	82.3 ± 4.6	1.8 ± 0.5
	II	39.0 ± 5.3	7,390 ± 3810	18.7 ± 4.1	79.3 ± 4.2	1.6 ± 0.8
	III	34.7 ± 6.7	12,555 ± 3258	15.2 ± 3.4	83.2 ± 5.4	1.4 ± 0.9
	IV	37.0 ± 3.5	6,708 ± 1684	8.6 ± 2.5	90.1 ± 3.8	1.2 ± 0.3

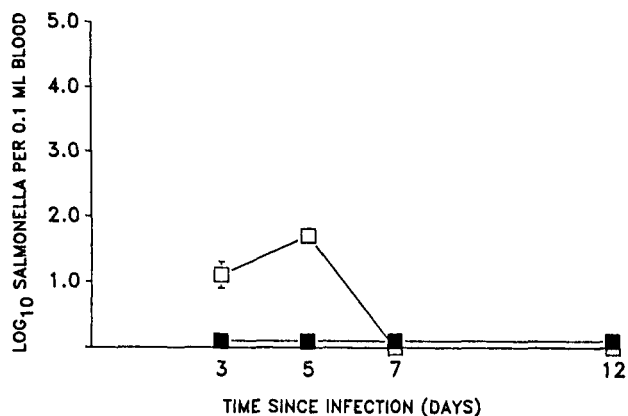
<sup>a</sup> Values at Day 0 are the mean percent of 24 animals per group of two; at Days 3, 5, 7, and 12 mean percent of three animals per group of four.

<sup>b</sup> I, vitamin A and infection; II, vitamin A only; III, control and infection; IV, control only.

<sup>c</sup> Significantly greater ( $P < 0.0001$ ) from Groups III and IV.

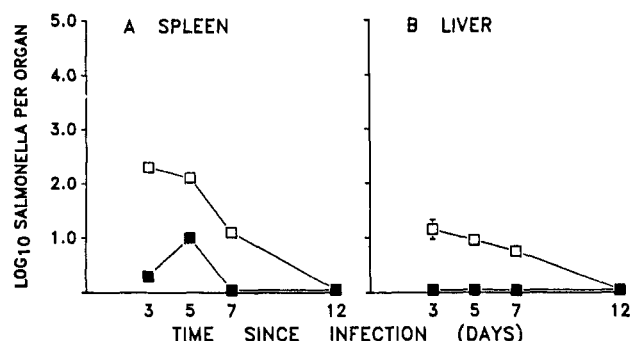
<sup>d</sup> Significantly different ( $P < 0.0001$ ) from Groups I and II.

<sup>e</sup> Significantly lower ( $P < 0.0001$ ) from Groups I, II, and IV.



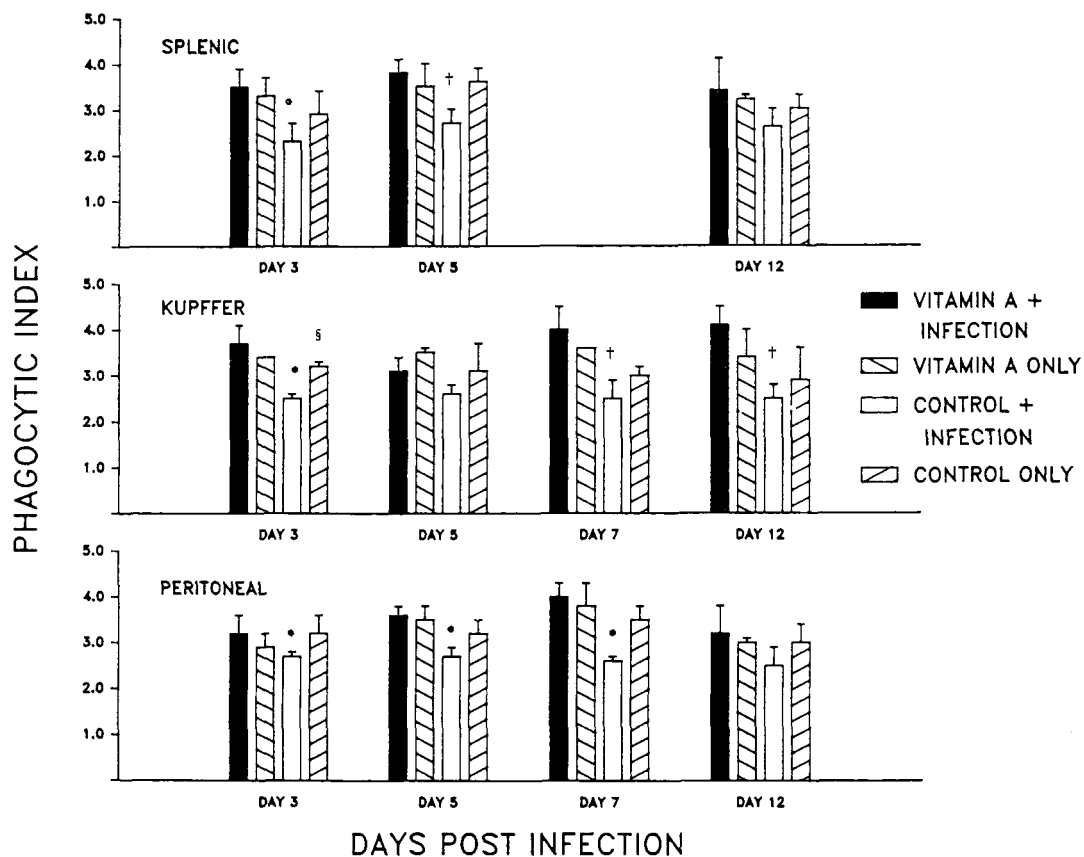
**Figure 1.** Clearance of *S. typhimurium* in blood of Lewis rats given vitamin A intraperitoneally (■) and saline intraperitoneally (□) over 5 weeks and then infected intraperitoneally with  $10^5$  organisms. Viable counts were performed on three rats per group per interval following final injection. SEM for Day 5 of vitamin A group was smaller than the size of the symbol.

*Listeria monocytogenes* both intraperitoneally and intravenously. In a follow-up study two different doses of vitamin A was administered intraperitoneally over a prolonged period before intraperitoneal challenge with salmonella. Animal survival, bacterial clearance in blood and spleen, and peritoneal and splenic macrophage activity were assessed. This study, in which more parameters were examined as well as an evaluation of



**Figure 2.** Clearance of *S. typhimurium* in spleen (A) and liver (B) of Lewis rats given vitamin A intraperitoneally (■) and saline intraperitoneally (□) over 5 weeks and then infected intraperitoneally with  $10^5$  organisms. Viable counts were performed on three rats per group per interval following final injection. Spleen: SEM for Days 3, 5, and 7 of vitamin A group and SEM for Days 3 and 5 of saline group were smaller than the size of the symbol. Liver: SEM for Days 5 and 7 of vitamin A group were smaller than the size of the symbol.

the functional activity of isolated Kupffer cells, confirmed the results from our previous experiments. Vitamin A-treated animals were healthier, displayed rapid clearance of bacteria from blood and spleen, and demonstrated enhanced macrophage phagocytic activity over infected nontreated animals. Vitamin A-treated animals infected with *Salmonella* continued to gain weight whereas infected nontreated animals lost weight.



**Figure 3.** Phagocytic index for splenic macrophages, Kupffer cells, and peritoneal macrophages over course of infection. Results are mean  $\pm$  SEM of three animals per group expressed as an index of phagocytosis derived by the number of yeast cells ingested per number of phagocytizing macrophages. \* Significantly lower than Groups I, II, and IV ( $P < 0.0001$ ). † Significantly different from Groups I and II ( $P < 0.0001$ ). § Significantly lower than Group I ( $P < 0.0001$ ). All statistical significance by ANOVA.

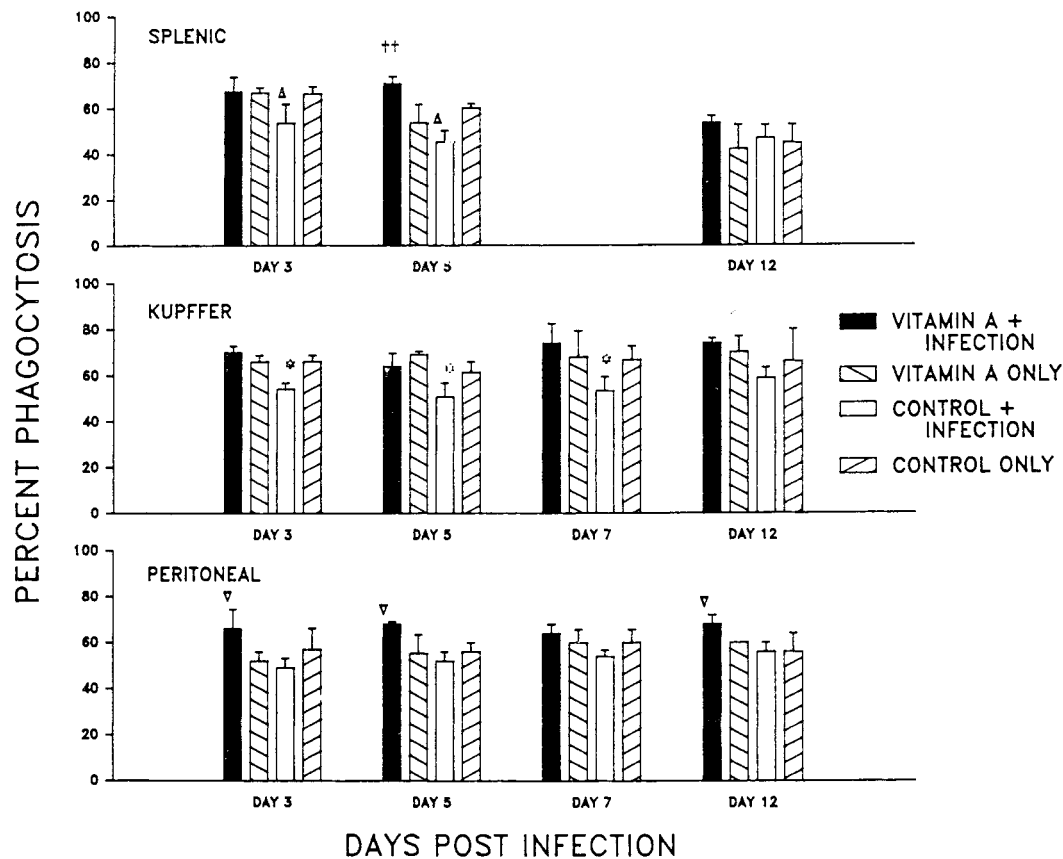
This weight gain may in part be due to the vitamin's role in reducing infectivity, thereby enhancing the body's catabolic processes, and to its function of maintaining epithelial surfaces, normal keratinization, and possible role in bone formation (30).

The effect of vitamin A on hematopoiesis has not been studied in detail, although one study has shown a significant decrease in circulating WBC and in the percentage of circulating lymphocytes in vitamin A-deficient rats (31). Our study showed an increase in the circulating neutrophils in vitamin A-treated animals before and after infection. This increase in cells and results showing that all infected vitamin A-treated animals exhibited a total clearance of bacteria in the blood throughout the course of infection indicates a positive effect of vitamin A's ability to enhance the functional capacity of phagocytes. Preliminary data (not shown) from our laboratory using the method of luminol-enhanced chemiluminescence, a sensitive and simple method for detecting activated oxygen species generated by phagocytes, suggests that this enhanced clearing of bacteria from the blood may be due to the vitamin playing a role in the oxygen radical generating capability of neutrophils by increasing myeloperoxidase activ-

ity. The marked leukopenia at 72 hr in the nontreated infected rats is a characteristic finding in salmonella infection.

There are strain differences in host resistance against salmonella in rats. The Lewis rats used are susceptible to salmonella infection and have been used in other studies for such purposes. Investigators from this laboratory using *S. typhimurium* LT2 in the Lewis strain of rat calculated a  $LD_{50}$  of  $10^9$  organisms. Several experiments by others (32-34) using this particular organism in mice calculated  $LD_{50}$  between  $10^5$  and  $10^6$ . In our earlier studies using challenge doses of  $10^6$ ,  $10^7$ , and  $10^8$  organisms given intraperitoneally to rats, morbidity was seen with  $10^7$  and some mortality with  $10^8$  organisms. Although administering differing doses of organism intravenously via tail vein demonstrated a greater bacterial load in the blood, results were similar to previous experiments—clearance in blood and spleen occurred more rapidly in vitamin-treated animals than in controls.

In previous experimental studies, vitamin A administered in large doses over several consecutive days either intraperitoneally (7) or orally (8) prior to bacterial or fungal infection has been associated with improved



**Figure 4.** Percentage of splenic macrophages, Kupffer cells, and peritoneal macrophages ingesting yeast particles in random number of fields. All data expressed as means  $\pm$  SEM of three animals per group. \* Significantly lower than Groups I, II, and IV ( $P < 0.0001$ ). † Significantly greater than Group II ( $P < 0.0001$ ). ‡ Significantly lower than Groups I and IV ( $P < 0.0001$ ). ∇ Significantly greater than Groups II and III ( $P < 0.0001$ ). All statistical significance by ANOVA.

survival. However, the high doses administered were shown to be toxic to the animals (8, 9). Our preliminary experiments giving vitamin A in lower doses to rats over 4 consecutive days did increase resistance to both *Salmonella* and *Listeria* organisms without the toxicity of hypervitaminosis. Best results of enhanced resistance were obtained by moderate administration of the vitamin given intraperitoneally over a course of 5 weeks with 6000 units given weekly. Indeed, serum retinol levels indicate that, although the supplemented group had expected higher levels than the nonsupplemented group, there was no toxicity from excess vitamin A in any of the treated groups. Vitamin A was given in the water-miscible palmitate form as this preparation has been reported to be effective both intraperitoneally and orally compared with the acetate form which was effective only on oral administration (9). It should be noted that the vitamin A preparation contained antioxidants butylated hydroxyanisole and butylated hydroxytoluene, but these stabilizing agents were in such trace quantities that any effect on biologic systems would be insignificant.

Decreased susceptibility of the host to infection as a result of rapid clearance of bacteria by the reticulo-

endothelial system of treated animals appears to substantiate our findings. Cultures of blood, splenic, and liver homogenates were positive for 44%, 100%, and 89% of infected nontreated animals 0%, 44%, and 0%, respectively, for the infected treated group during the first week of infection. The increase in phagocytosis of the treatment group as indicated by the phagocytic index and percentage of reticuloendothelial system cells phagocytizing yeast particles support previous assumptions that vitamin A acts to enhance phagocytic cell function (7, 8). Furthermore, our results are in concurrence with a recent study which showed that vitamin A-deficient rats infected with *Escherichia coli* had marked impairment of host defense as evidenced by depressed blood clearance and decreased phagocytic activity of polymorphonuclear leukocytes (35).

Infection with *S. typhimurium* clearly depresses phagocytic activity as indicated by the significantly lower phagocytic index and percentage of phagocytizing ability of the challenged nontreated animals. The increase in phagocytic capacity of the vitamin A-treated group may be due to increased activity of the cells and not to just an increase in number. One possible explanation might be that vitamin A plays a role in the

production of lymphokines that stimulate macrophages to increase the oxygen metabolic activity, thus enhancing their bactericidal activity. Alternatively, it is suggested that vitamin A may enhance the production of specific antibodies and thus increase the opsonizing capacity of reticuloendothelial system macrophages as a prerequisite for phagocytosis.

Based on our findings vitamin A improves the functional capability of macrophages and enhances the functional integrity of the reticuloendothelial system and therefore may play an important role as an immunomodulator in the host's response to infection. The results from our study suggests a beneficial role of vitamin A supplementation and possible use as a therapeutic agent during experimental salmonella infection.

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