Effect of Dietary Phosphorus on Blood Phosphorus and Leukocyte Levels in Experimental Salmonellosis[1] (36909)

R. L. DOAK,[2] G. B. GARNER, P. F. HUEBNER, AND B. L. O'DELL[3]

Department of Agricultural Chemistry, University of Missouri, Columbia, Missouri 65201

Previous work in this laboratory (1) has shown an inverse relationship between dietary phosphorus level and the susceptibility of guinea pigs to experimental infection with virulent *Salmonella typhimurium*. Mortality rate was significantly reduced as the phosphorus level was increased from 0.4 to 1.0%. The mechanism by which the higher level of phosphorus increases resistance to salmonellosis is not known. One possibility is that it maintains a pool of readily available phosphorus which is essential for production of phagocytic cells. Another is that 0.4% of phosphorus is inadequate for guinea pigs and the deficient animals are more susceptible to salmonellosis.

This investigation was initiated to determine the effect of dietary phosphorus on the number of peripheral leukocytes and the level of blood inorganic phosphorus before and after Salmonella infection. Another objective was to establish whether or not the basal phosphorus level (0.4%) was adequate for noninfected guinea pigs.

Materials and Methods. Female guinea pigs, 7–11 wk of age and weighing 450–500 g, were used. The basal diet contained 0.4% phosphorus and was essentially the same as the casein based diet used by Nabb and O'Dell (1). It contained the following major constituents: 30% casein, 44.1% sucrose, 15% cellulose, 4% mineral premix, 1.9% potassium carbonate, 0.5% magnesium oxide, 0.2% DL-methionine, 4% soybean oil, 0.1% choline chloride, 0.2% ascorbic acid and other vitamin supplements. The diet supplied the following milliequivalents of ions per 100 g of diet: sodium, 5; potassium, 40; and phosphate, 22. The high phosphorus diet contained 1.0% phosphorus (56 mEq/100 g of diet). The additional phosphorus was supplied by H_3PO_4 or KH_2PO_4 added at the expense of sucrose and potassium carbonate so as to maintain the potassium level constant. Animals were fed the respective diets for at least 2 wk prior to their inoculation with Salmonella. Feed and water were supplied *ad libitum*. Additional ascorbic acid (60 mg/animal) was given orally every other day during an infection trial.

The virulent organism, *S. typhimurium*, was from the same stock culture used previously (1). It was identified by serological typing and was stored at —20° after lyophilization. To prepare the inoculum, cultures grown in trypticase soy broth for 18 hr were harvested by centrifugation at 8700*g* for 15 min at 4°. The organisms were resuspended, washed once by centrifuging in sterile 0.15 *M* NaCl, and finally resuspended in sufficient sterile 0.15 *M* NaCl to give 80% transmittance at 650 nm in a Spectronic 20 colorimeter. This suspension was diluted 1:50 and 0.5 ml was injected intraperitoneally. By plating on both tryptose and brilliant green agar, 0.5 ml was estimated to contain between 2.5×10^5 and 2.5×10^6 viable organisms. The inoculum as injected gave reactions typical of Salmonella when cultured on triple sugar iron agar, lysine iron agar, brilliant green agar, Salmonella-Shigella agar, and urea broth.

Blood was collected from a toe of the hind foot at desired intervals before and during

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[2] Present address: Poultry Disease Research Center, 953 College Station Road, University of Georgia, Athens, GA 30601.

[3] To whom correspondence should be addressed.

the trial to determine inorganic phosphorus concentrations, as well as total and differential leukocyte counts. Inorganic phosphorus was measured by the method of Fiske and Subbarow (2). Total peripheral leukocyte counts were performed with a Coulter model B electronic particle counter, and blood smears were stained with Wright's stain for differental counts.

Results. The effect of four different levels of dietary phosphorus on the growth rate in normal guinea pigs indicates that the basal level (0.4%) was adequate to support the maximal growth rate (Table I) in noninfected animals. The highest level of phosphorus depressed the growth rate slightly.

Food consumption was essentially the same in both dietary groups before infection. Guinea pigs fed 0.4% phosphorus consumed an average of 5.9 g ($N = 15$) of diet/day/100 g body weight while those fed 1.0% phosphorus consumed 5.8 g ($N = 15$). Food consumption decreased slightly in both dietary groups as the infection progressed. During the first 10 days after infection the consumption of basal diet was 5.2 g and of high phosphorus diet, 5.7 g. During the next 10-day period the respective values were 5.0 and 5.4 g.

Increasing the dietary phosphorus level from 0.4 to 1.0% lowered the mortality rate from 56 to 10% and increased the mean survival time (Table II). The effect on mortality was highly significant statistically ($p < 0.005$).

The concentration of inorganic phosphorus in the blood of noninfected guinea pigs reflected the level of dietary phosphorus. In one series, noninfected animals fed 0.4% phosphorus had an average blood phosphorus of 5.2 ± 0.12 mg/100 ml ($N = 45$) whereas those fed 1.0% phosphorus had 6.3 ± 0.22 mg ($N = 45$). In another series the animals were infected and blood phosphorus followed over a period of 4 wk. As shown in Fig. 1 blood phosphorus levels declined in both dietary groups as a result of infection, but dropped to a markedly lower level in guinea pigs fed 0.4% phosphorus. The difference between the blood phosphorus concentrations was greatest at the eighth day after infection when those fed 0.4% phosphorus reached the lowest level. The mean blood phosphorus values at this time were 4.0 and 5.7 mg/100 ml in the basal and high phosphorus animals, respectively. Later the levels rose in both groups but did not return to preinfection levels. The concentration of inorganic phosphorus was maintained at significantly higher levels throughout the infection period in animals that received the higher dietary phosphorus level.

As shown in Table III the number of cir-

TABLE I. Dietary Phosphorus Level and Growth Rate in Noninfected Female Guinea Pigs.[a]

Dietary phosphorus (%)	Av daily gain (4 wk) (g)
0.4	5.2 ± 0.20
0.6	4.5 ± 0.23
0.8	4.9 ± 0.23
1.0	4.2 ± 0.19[b]

[a] Phosphorus was supplied as monosodium phosphate. There were at least 20 animals/group and the starting weights average 340 g.

[b] Significantly different ($p < .01$) from those fed 0.4% phosphorus; otherwise no significant differences.

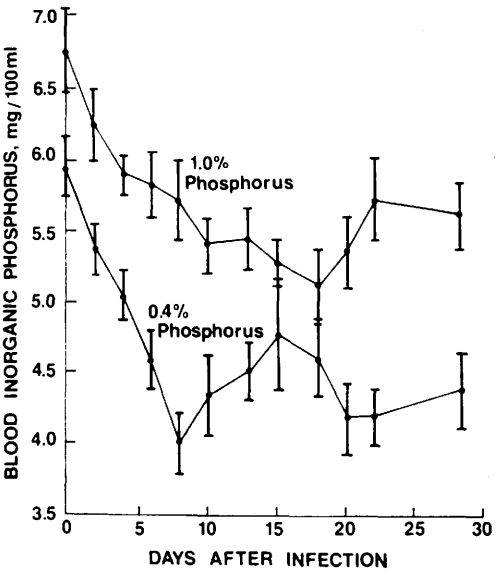

FIG. 1. The effect of *S. typhimurium* infection on level of blood inorganic phosphorus in guinea pigs fed 0.4 and 1.0% dietary phosphorus. Each point represents 14 or 15 animals and the bars indicate standard error of the mean.

TABLE II. Dietary Phosphorus Level and Mortality Rate in Guinea Pigs Infected with *Salmonella typhimurium*.[a]

	Dietary phosphorus	
	0.4%	1.0%
Dead/infected (no.)	33/59	6/63[b]
Mortality (%)	56	10
Mean time (days) to death	14.0 ± 1.5	18.5 ± 4.4

[a] The data are pooled from 3 separate trials. The duration of the trials varied from 36 to 42 days, a time sufficient to insure that survivors were no longer in jeopardy.

[b] Chi-square analysis showed a statistically significant difference between groups, $p < .005$.

culating leukocytes/mm^3 of peripheral blood, observed at intervals before and after infection, was directly related to the dietary phosphorus level. The first count was made approximately 5 wk before infection, a time when the guinea pigs had been on their respective diets only 2 days. The increase in leukocyte count, which occurred in both groups during the period before infection, may be an age effect. In any case, animals fed the higher level of phosphorus showed a larger increase in circulating leukocytes than those fed the basal diet, 75% compared to 25%. As in the case of the blood inorganic phosphorus pattern, after infection the difference in numbers of circulating leukocytes became even more pronounced. During the first week postinfection a slight leukopenia developed, particularly among the animals fed 0.4% phosphorus. Subsequently the leukocyte count increased in both dietary groups. However, guinea pigs fed the higher phosphorus diet had a significantly higher ($p < 0.01$) count. In noninfected guinea pigs fed the basal phosphorus diet distribution of leukocytes was 74% lymphocytes, 24% neutrophils, 1% monocytes, and 1% eosinophils. This pattern was not altered by phosphorus level or by infection. Thus in this experiment the change in the total number of leukocytes before and after an infection was not associated with any particular leukocyte type.

Discussion. This study confirms the earlier observation (1) that a level of dietary phosphorus higher than that required for maximal growth rate of guinea pigs reduces mortality caused by experimental salmonellosis. Conceivably the protective effect could be the result of correcting a phosphorus deficiency in the basal diet. This does not seem to be the case since noninfected guinea pigs fed the basal diet grew at a normal rate. The higher phosphorus level did not stimulate growth rate but, in fact, somewhat depressed it. The fact that the mean time to death was 12 to 15 days among the animals fed 0.4% phosphorus suggests that phosphorus exerts its protective effect during the first 2 wk after infection.

The protective action appears to be related to the decrease in blood inorganic phosphorus which is observed immediately after infection. The dramatic lowering of blood phosphorus suggests that there is less phosphorus available for critical functions such as production of new phagocytic cells, activation of existing reticuloendothelial cells, or production of opsonin. The lower blood phosphorus might be the result of lowered food consumption, reduced phosphorus absorption or increased phosphorus excretion via the urinary or gastrointestinal tract. Reduced intake does not account for the effect since food consumption was lowered only slightly after an infection, but no attempt was made to evaluate the effect on excretion or absorption of phosphorus.

Other explanations for the lower blood phosphorus observed after infection include

TABLE III. Dietary Phosphorus and Peripheral Leukocyte Count Before and After Infection.

Time (days)[a]	Total leukocyte count[b]	
	0.4% P	1.0% P
−35[c]	3700 ± 190 (21)[d]	3900 ± 270 (22)
−1	4650 ± 190 (30)	6850 ± 290 (30)
+8	3850 ± 370 (28)	6550 ± 430 (28)
+21	5650 ± 540 (21)	9000 ± 350 (29)

[a] Time relative to infection.

[b] Mean number per cubic millimeter of blood ± standard error of mean.

[c] Guinea pigs were 5 wk of age and had received the respective diets for only 2 days.

[d] Number of animals.

an increased metabolic demand for phosphorus or its redistribution. Redistribution of phosphorus due to infection would be analogous to the effect of endotoxin on the concentration of other ions in the blood. Serum zinc (3) and plasma iron concentrations (4) in rats are decreased significantly after endotoxin administration. Studies with ^{65}Zn and ^{59}Fe have shown a redistribution of these ions with a higher concentration in the liver (5). In any case the animals fed the higher phosphorus level in the present study maintained more nearly normal blood phosphorus levels throughout the infection period. A higher blood phosphorus level may have been directly beneficial to the animals or simply reflected the beneficial effect of higher phosphorus intake. The data suggest that the metabolism of phosphorus was increased during an infection and that the higher intake more nearly saturated tissue demand.

The higher number of circulating leukocytes in guinea pigs fed 1.0% phosphorus suggests that bone marrow activity is involved in the interaction of dietary phosphorus and infection. As Prost and Riemann (6) pointed out, both the endotoxin produced and the organism itself play a role in Salmonella infections. Endotoxin administration causes an early leukopenia followed by a pronounced leukocytosis involving mainly neutrophils (7, 8). The leukocytic response induced by endotoxin appears to involve both hyperplasia of bone marrow stem cells (9) and an increased rate of leukocyte release from the bone marrow compartment to the blood (10, 11). The number of circulating leukocytes, especially neutrophils, has been correlated with host resistance and susceptibility to bacterial infections (12, 13, 14).

The leukocyte response to endotoxin during an infection may require more phosphorus than the available pool can supply and thus extra dietary phosphorus is needed. It appears that the phosphorus level needed for maximal bone marrow activity is greater than the level for maximal growth rate in noninfected animals. Endotoxin is probably the primary stimulus for leukocytosis during an infection and it appears from the data that the phosphorus level in the diet is correlated with numbers of leukocytes found in the blood. The higher leukocyte counts may be beneficial for the favorable outcome of the infection and thus partly responsible for increased survivorship in animals fed high phosphorus.

Summary. Guinea pigs were fed purified diets containing either 0.4 or 1.0% phosphorus. The basal level was adequate to support maximal growth in noninfected guinea pigs. The higher phosphorus level slightly depressed the growth rate. Animals were inoculated with *Salmonella typhimurium* and observed for a period of 6 wk. The mortality rate was significantly lower among animals fed 1.0% P. Noninfected guinea pigs fed the higher phosphorus level had both a higher level of inorganic phosphorus and more circulating leukocytes in the blood. After infection the differences between dietary groups were magnified. Although blood inorganic phosphorus dropped in both groups as a result of infection, those fed the higher phosphorus level maintained a significantly higher concentration. Bone marrow response as reflected by total leukocyte counts was greater in animals fed the higher phosphorus level. It is postulated that the protective action of high dietary phosphorus is mediated at least in part by maintenance of a pool of phosphorus required for the proliferation of cells critical to the defense mechanism.

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