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Impaired Blood Clearance of Bacteria and Phagocytic Activity in Vitamin A-Deficient Rats (41999)

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Abstract. The effect of vitamin A deficiency on the functional integrity of the reticuloendothelial system and the phagocytic capacity of circulating polymorphonuclear leukocytes was evaluated in retinoate-cycled vitamin A-deficient rats under conditions such that secondary dietary imbalances were eliminated. Kinetics of blood clearance of 2×10^7 *Escherichia coli* injected intravenously was depressed within 8 days of the withdrawal of retinoic acid; all animals were profoundly affected by Day 12 of deficiency. *In vitro,* the phagocytic activity of polymorphonuclear leukocytes was similarly affected; by Day 12 of deficiency, phagocytic capacity in all deficient animals was less than 40% of the appropriate control values ($P < 0.01$). Animals rendered vitamin A deficient by this procedure also displayed marked susceptibility to endogenous bacterial infection, as judged from the proportion of deficient rats that spontaneously developed bacteremia during the later stages of deficiency. These data together demonstrate unequivocally that reticuloendothelial and polymorphonuclear leukocytic functions are impaired in vitamin A deficiency in the absence of other dietary imbalances.

Epidemiological observations in humans and experimental studies in several species of animals over several decades have indicated a relationship between vitamin A deficiency and increased susceptibility to infection (1). Bacterial, rickettsial, viral, and parasitic diseases all occur with increased frequency, severity, or mortality when there is a concurrent vitamin A deficiency.

Mechanistically, a number of defects leading to increased susceptibility to infection have been postulated, including impaired humoral and cellular immune responses and a defective nonspecific defense mechanism (1–3). For instance, both the phagocytic and bactericidal activities of leukocytes from vitamin A-deficient children were depressed (1–4) as was the lysozyme content of those leukocytes compared to normal subjects (5). However, in most such studies, particularly those involving human subjects, it has been difficult if not impossible to delineate the effect(s) of secondary nutritional imbalances or prior infection on many of the aberrations that have been reported.

The purpose of this study was to determine the effect of simple, uncomplicated vitamin A deficiency on nonspecific resistance of rats reared by a system enabling a rapid and synchronous induction of vitamin A deficiency, thus minimizing if not totally eliminating the effect of concurrent inanition as commonly encountered in human populations or with the more classical animal rearing techniques. We found that both the kinetics of blood clearance of intravenously injected bacteria and the phagocytic capacity of polymorphonuclear leukocytes were depressed in our simple, uncomplicated vitamin A-deficient animals.

Materials and Methods. *Induction of vitamin A deficiency.* Adult Wistar rats weighing 210–260 g were rendered vitamin A deficient by the withdrawal of retinoic acid from the diet of retinoate-cycled, stringently vitamin A-deficient animals as previously described (6). In brief, male weanling rats were fed stock vitamin A-free diet *ad libitum* for 3 weeks until early weight plateau. Thereafter, they were fed a stock diet first supplemented with and then lacking in 5 μg retinoic acid/g diet in repeating 18-day:10-day cycles. After a minimum of four complete cycles, the level of retinoate supplementation was reduced to 2 μg/g diet for at least 8–10 days prior to the induction of deficiency. Animals selected as A^+ controls were given 1000 μg retinyl palmitate in 500-μg split doses 2 days (T_{-2}) and 1 day (T_{-1}) prior to retinoate withdrawal (T_0). Animals selected as vitamin A deficient

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(A^-) were given only oil placebo. Following the ultimate withdrawal of retinoic acid, both groups of animals were tube-fed twice daily with stock 18% casein vitamin A-free diet with sucrose in place of starch at 120% the daily estimated caloric maintenance requirement based on T_0 individual body weights, a level of feeding which largely prevented body weight losses in deficient animals during the 12-day duration of the experiments. The severity of vitamin A deficiency with this regimen was such that by T_{12} more than half of the A^- rats developed bacteremia involving normal gastrointestinal flora including *Proteus, Pseudomonas,* and *Acenetobacter* species. All data presented in this report were obtained from animals without detectable bacteremia as evidenced by direct blood culture at T_8 and T_{12}.

Measurement of blood clearance of bacteria. The method used to determine the kinetics of blood clearance of injected *Escherichia coli* was modified from that previously described by Benacerraf *et al.* (7). Animals were injected intravenously with an 18-hr suspension of 2×10^7 *E. coli* ATCC 25922. Samples of approximately 0.1 ml of blood were taken from the retroorbital venous plexus 2, 5, 7, 10, 15, 30, and 60 min after injection and, after appropriate dilution in trypticase soy broth (TSB), were spread on MacConkey agar. After incubation at 37° for 24 hr, the number of bacterial colonies was determined. Since the rate of removal of injected bacteria from the circulation during the initial stages of clearance follows an exponential function (7), a slope based on the first 10 min of clearance was calculated to enable quantitative comparison between A^+ and A^- animals.

After the 60-min blood sample was taken, the animals were returned to the force-feeding regimen and residual bacteria in the spleen and liver were quantitated. Groups of animals were killed 1, 3, or 5 days later, and the spleen and liver were then removed aseptically, homogenized, appropriately diluted in TSB, plated on MacConkey agar, and the number of bacteria was enumerated as above.

Phagocytic activity of polymorphonuclear leukocytes. The phagocytic activity of polymorphonuclear (PMN) leukocytes from individual A^+ and A^- rats was determined (8) on the day of retinoic acid withdrawal (T_0) and thereafter at T_8 and T_{12}. Leukocyte-rich plasma was separated from heparinized blood; leukocytes were washed and resuspended in Hanks' balanced salt solution (HBSS) at concentration of 4×10^6 cells/ml. Thereafter 10^6 leukocytes were incubated with 10^8 *E. coli* in a total volume of 0.5 ml at 37°C for 30 min. After incubation, the leukocytes were sedimented by centrifugation at 70*g* for 5 min and then washed with cold HBSS. Pellets were smeared on a microscope slide and, after air-drying, were then fixed in absolute methanol and stained with Giemsa's stain. Phagocytic activity was expressed as the number of cells containing one or more bacteria per 100 PMN examined.

Results. The data presented in Fig. 1 demonstrate an adverse effect of vitamin A deficiency on blood clearance of *E. coli* which became particularly obvious during the later stages of deficiency (T_{12}). On the day of retinoic acid withdrawal (T_0), animals receiving either retinyl palmitate (A^+) or carrier oil only (A^-) exhibited similar patterns of clearance. More than 90% of circulating bacteria were cleared from the circulation of both groups within 10 min, and by 30 min, less than 0.1% remained in circulation. In contrast, 8 days after retinoic acid withdrawal (T_8) the clearance patterns of some A^- animals deviated from those observed at T_0. In some animals, up to 10% of the injected bacteria remained in circulation 30 min after injection. By T_{12} all A^- animals displayed defective blood clearance. This impaired clearance in vitamin A deficiency was particularly apparent when one examined the slope of bacterial clearance from the blood which declined progressively as the period of deficiency was prolonged. Mean values for the slope of A^- rats changed from -0.213 at T_0 to -0.087 at T_{12}. This difference was statistically significant at the 1% level. Mean values for A^+ controls were unchanged throughout the experimental period. It seems unlikely that this defective blood clearance in A^- rats is associated with a difference in the quantity of circulating antibodies to *E. coli* since the agglutinin titers in both groups were of a similar magnitude, typical values in most animals being 1:2 or lower.

When residual bacteria in the spleen and liver of these animals were quantitated 1, 3,

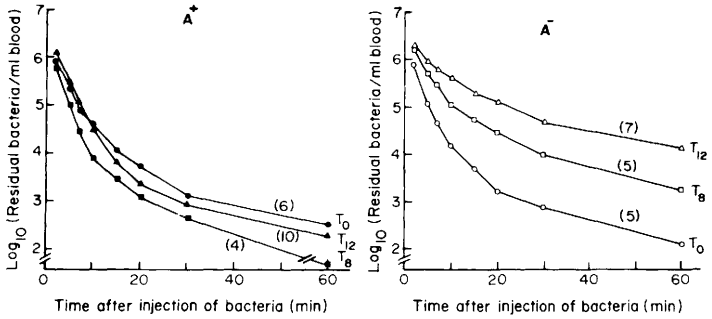

FIG. 1. Clearance of bacteria from the circulation of vitamin A-deficient (A^-) and control (A^+) rats. Animals were injected intravenously with 2×10^7 *E. coli* either on the day of retinoic acid withdrawal (T_0), or on Day 8 (T_8), or Day 12 (T_{12}) of deficiency. Residual bacteria were quantitated from blood samples taken 2, 5, 7, 10, 15, 20, 30, and 60 min after bacterial injection. Curves were plotted from mean values, the number of animals in each group being indicated in parentheses. Slope of bacterial clearance (log of residual bacteria per ml blood per min) could be calculated from the data based on the first 10 minutes of clearance.

and 5 days after *E. coli* injection, the results obtained for both A^+ and A^- animals were in accord with those noted for the blood clearance. Approximately 10^4 bacteria could be detected in either the spleen or liver of A^+ animals on Day 1; thereafter no bacteria were detected in any of these animals. Similar results were obtained whether the A^+ controls were injected at T_0, T_8, or T_{12}. In contrast, the number of bacteria in either the spleen or liver of A^- rats injected 8 and 12 days after retinoic acid withdrawal (T_8 and T_{12}) was as high as 10^5–10^6 in animals killed 1 day after injection. Similarly high values were noted in some other A^- animals killed 3 and 5 days after the injection.

Changes in phagocytic activity of circulating polymorphonuclear leukocytes isolated from individual A^+ and A^- animals were also determined at various intervals from the day of retinoic acid withdrawal. The results shown in Fig. 2 again demonstrated that vitamin A deficiency markedly depressed the phagocytic activity of PMN leukocytes in these animals. As shown in the figure, differences in the phagocytic activity between the two groups of animals were evident within 8 days of retinoic acid withdrawal ($P < 0.05$). By T_{12}, the mean phagocytic activity of A^- rats was less than half that of the A^+ controls ($P < 0.001$). However, neither the number of bacteria ingested per cell nor the total number of circulating PMN leukocytes was noticeably different between the two groups.

Discussion. The results presented demonstrate unequivocally that (1) a single nutritional deficiency involving only vitamin A adversely affects nonspecific host defense as evidenced by depressed blood clearance of bacteria and from decreased phagocytic activity of PMN leukocytes, and (2) retinoic acid can substitute for storage forms of vitamin A in the maintenance of this function.

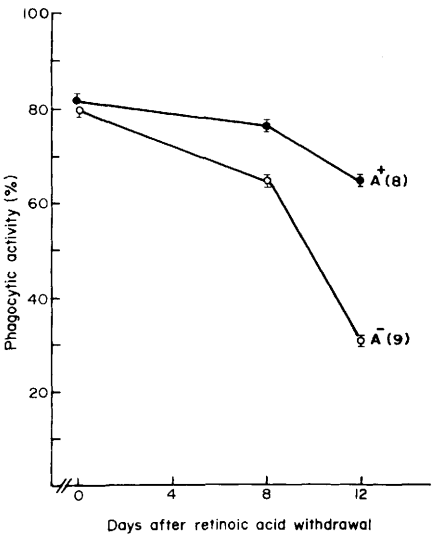

FIG. 2. Changes in the phagocytic activity of polymorphonuclear leukocytes from individual A^+ ($n = 8$) and A^- ($n = 9$) rats from the day of retinoic acid withdrawal (T_0) until Day 12 (T_{12}) of deficiency. The curves represent mean values and the bars SEM.

Furthermore, it is quite obvious from the data presented that defects associated with vitamin A depletion become progressively worse with prolongation of the deficiency state.

It is unlikely that the depressed bacterial clearance from the blood of vitamin A-deficient rats is due to a difference in opsonic activity of the serum as the natural serum agglutinins against *E. coli* in both groups were similar. It is also unlikely that such a defect is associated with the effect of vitamin A deficiency on the complement system since we previously reported complement levels of these animals are not depressed (9). In addition to a defective reticuloendothelial function, the results shown in Fig. 2 demonstrate that the phagocytic activity of the polymorphonuclear leukocytes of these animals was also depressed.

The clear results obtained in this study were made possible by the availability of vitamin A-deficient rats reared by a technique (6) which almost totally eliminates secondary inanition. The latter is unavoidable with the classical procedures commonly used in the past. The data obtained with this animal model support those obtained in a number of studies of malnourished children and animals with vitamin A deficiency (1, 4, 10). Furthermore, our conclusions based on the depletion of vitamin A are in consonance with previous reports showing that an excessive dose of vitamin A and other retinoids enhanced nonspecific resistance in mice against infections by various types of microorganisms (11, 12). It was shown also in these studies that vitamin A-supplemented animals had lower mortality and enhanced microbial clearance when compared with unsupplemented normal animals.

The impaired cellular functions reported in this study would no doubt potentiate other defects associated with vitamin A deficiency. The structural and functional integrity of the epithelial tissues is compromised in vitamin A deficiency (2, 13). Some epithelial tissue such as the trachea becomes squamatous and keratinized, thereby reducing the proportion of cells with mucociliary functions (12, 13). Reduction of mucous secretion is also known to result in an uncontrolled multiplication of bacteria, thus enhancing the probability of colonization and tissue penetration (1, 2). Increased susceptibility to infection resulting from defects in these various nonspecific factors may be further aggravated by a defective local immune response reported previously (14). Altogether, these changes would enhance the probability of tissue invasion which in the face of reduced microbial clearance and phagocytic activity could lead to a fatal outcome.

In humans, a malnourished condition involving a deficiency of only one dietary component, particularly vitamin A, rarely exists. Therefore, defects known to be associated with vitamin A deficiency generally are aggravated further by deficiencies involving other components. We have previously reported that a defective local immune system in children with protein–calorie malnutrition is more severe in those cases with concomitant vitamin A deficiency (15). Vitamin A and some of its derivatives have also been shown to be clinically useful in the treatment of some neoplastic and dermatological disorders (3, 16, 17). Among other explanations, the effectiveness of vitamin A and various synthetic retinoids may be attributed to their ability to enhance nonspecific resistance in such patients (3, 16, 18). These possibilities remain to be determined and warrant further investigation.

The functional integrity of the reticuloendothelial system has been used for the assessment of protein-colorie malnutrition (19). The results presented in this study suggest that this parameter can also be used in the case of vitamin A deficiency. Furthermore, the data suggest that it may be possible to use a simpler *in vitro* assay of PMN leukocyte phagocytic activity as an additional parameter. This possibility should be explored as it may be useful in epidemiological studies.

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