Effect of Vitamin E on the Immune Response of Hypoxic and Normal Chickens

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Chicks and hens raised on a diet enriched with 60 mg of vitamin E per lb. of feed had a significantly increased immune response as measured by the antibody plaque-forming cell test or by hemagglutination. Since the effect was much greater on hypoxic (simulated altitude exposed) chicks, a synergistic effect between hypoxia and vitamin E may be suggested.

Vitamin E, which was first recognized as a fertility factor for rats (10) and is known as a powerful antioxidant (7), apparently has other important but less known functions. Among these functions may be the involvement in the cellular development of the hemopoietic-immunopoietic system, the cells of which most likely originate from a common pluripotential stem cell (12). The involvement of vitamin E in the development and population kinetics of erythroid cells has already been demonstrated (3, 6, 8). The purpose of the present communication is to show that vitamin E is also involved in the immune response and that the effects of hypoxia and vitamin E on the immune response are synergistic. In our earlier research, we have shown that hypoxia alone increased the immune response of adult rabbits (11).

S. C. White Leghorn hens (from Colorado State University Station stock) and the chicks hatched from their eggs were maintained on a diet fortified with 60 IU (60 mg) of vitamin E per lb. of feed. The normal diet contains about 10 mg of vitamin E per lb. Seven-day-old chicks were immunized with 0.2 ml of 20% sheep red blood cell (SRBC) suspension, and half of them were exposed to 16,000 ft (4,800 m) simulated high altitude for 4 days. Each of the eight experimental groups, including a control for each parameter (vitamin E, altitude, immunization), contained 25 chicks. Antibody (AB)-producing cells were counted from five pooled spleens or bursa per group by the microslide plaque-forming cell (PFC) test (2, 4), except with undiluted guinea pig complement to increase the size and definition of the otherwise small and ill-defined plaques. After secondary immunization, the indirect PFC method was also used (9), and the hemag-

glutination (HA) titer of the serum also was measured.

The peak direct or indirect PFC count was obtained on day 4 in each treatment group and the maximal HA titer on day 7. No significant shift in the kinetics of these responses was observed due to vitamin E or altitude treatment. In the figures, these peak responses are shown.

It can be seen in Fig. 1 that vitamin E significantly increased the number of AB PFC both at ground level (1,500 m) and at 4,800 m simulated altitude level. However, the great increase in PFC in the altitude group indicated that the effect of vitamin E and hypoxia was synergistic.

The unusually high background counts in Fig. 1 as compared to those obtained by others (1) probably were due to the use of undiluted guinea pig complement and indicated a large percentage of nonspecifically reacting cells, which are most likely not precursors of PFC (5). Those cells which responded specifically to the SRBC immunogenic stimulus, however, gave significant differences within treatment groups and compared to the controls ($P < 0.05$). The background PFC counts among diet and altitude controls were not significantly different. The HA titers in these young chicks at this stage were too low to interpret correctly.

The chicks were reimmunized at age 31 days, partly to see whether a residual effect of the hypoxic shock remained and partly to see the change in the secondary response. Figure 2 shows the result of an indirect PFC test, showing that (i) the previous hypoxic shock had no effect on this secondary immune response, and (ii) the bursal response at this stage is negligible. The direct PFC counts at this stage were lower (PFC <2,000) and not significantly different among the treatment groups, indicating that the

response has shifted largely to immunoglobulin G-producing cells, measured only by the indirect PFC test. The corresponding HA titers, obtained 7 days after the secondary immunization (Fig. 3) show that not only the number of PFC but also the amount of circulating AB increases significantly with an increase of vitamin E in the diet.

The effect of vitamin E-fortified diet appears to be restricted to immunocytes, since we did not find a significant difference in hemoglobin and hematocrit values between vitamin E and normal diet groups, both at ground level (average hematocrit = 34% and average hemoglobin = 40 mg/100 ml) and at altitude (average hematocrit = 45% and average hemoglobin = 50 mg/100 ml).

To check the possibility that the vitamin E effect is not restricted to young chicks, we also immunized 1-year-old laying hens with SRBC

maintained on the same vitamin E-fortified or normal diet. We corroborated the result obtained with young chicks. The mean hemagglutination peak titer on the 7th day after immunization was 128 in the vitamin E group and 32 in the normal group; the PFC values on the 4th day were $2,900/10^6$ cells for the vitamin E group and $2,000/10^6$ cells for the normal group.

The primary HA response of chicks was measured weekly after hatching to find out the effect of maturation on this response in chicks fed three different levels of vitamin E (Fig. 4). As the HA titer rises with age the immune response always increases more with increasing levels of vitamin E in the diet. Further experiments are in progress now to find out the optimal level of vitamin E in the immune response.
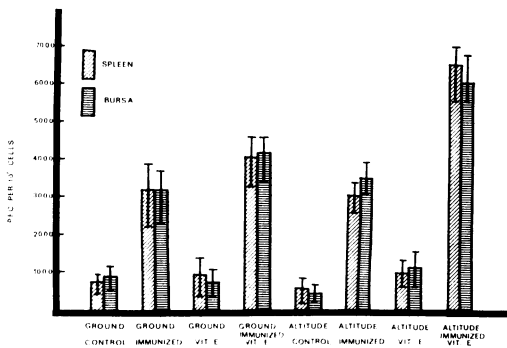

FIG. 1. Four day PFC response of chicks immunized at age 7 days with sheep red blood cells. Values are means of five organs in six parallel PFC tests. On all graphs the vertical bars represent standard deviations. The differences are significant for all immunized groups at the $P < 0.05$ level.

FIG. 3. Peak mean hemagglutination titer of chicks reimmunized at age 31 days with sheep red blood cells. Assay was taken on the 7th day after reimmunization.
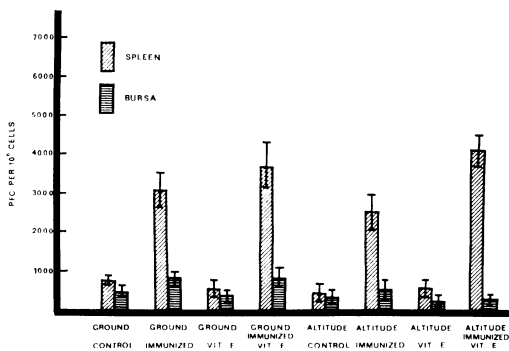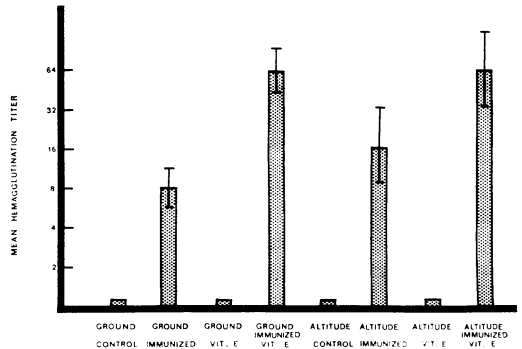

FIG. 2. Four day indirect PFC response of chicks reimmunized at age 31 days with sheep red blood cells. The plaques were developed with rabbit anti-chicken gamma globulin serum. The differences are significant for all immunized groups $(P < 0.05)$.
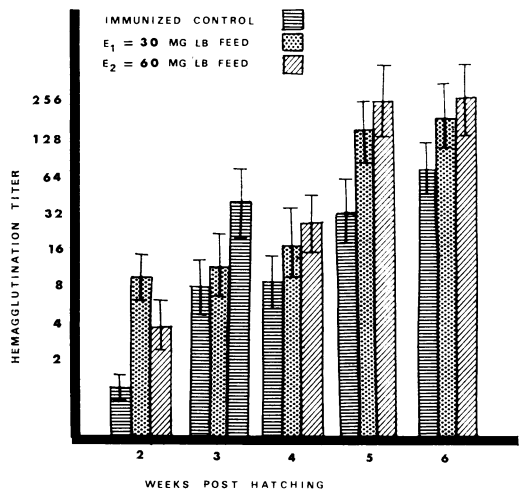

FIG. 4. The hemagglutination titer of SRBC immunized chicks as a function of age. The HA titer was measured 7 days after immunization.

In the light of these data plus the reports in the literature on the role of vitamin E in erythrocyte regulation (3, 6, 8) and the common stem cell origin of erythrocyte and lymphoid cells (12), the hypothesis of a direct involvement of vitamin E in immunocyte development and AB regulation is not unlikely, and can be tested experimentally using diets from vitamin E-deficient to fortified, and measuring quantitatively AB biosynthesis and immunocyte kinetics. The synergistic effect observed with the combined vitamin E and altitude treatment suggests that the mechanism of vitamin E action is most likely connected with its antioxidant property; vitamin E combined with hypoxia creates more reducing conditions in the electron transport chain for cellular developmental processes (3).

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