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The Effect of Vitamin A and Protein Deficiency on Complement Levels in Rats[1] (40147)

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Malnutrition is almost always associated with increased susceptibility to infection, and the infections that occur are invariably more severe and more slowly resolved than in well-nourished individuals (1, 2). Admittedly, in some nutritional deficiency states, infections caused by intracellular parasites may be less severe, depending on whether the parasites or the host are more adversely affected by the deficiency (1). Regardless of causative agent however, the infections that occur in vitamin A deficiency are always more severe and lead to a higher mortality rate (1).

It has been demonstrated recently that impaired host defense in children with protein–calorie malnutrition may be related to a defective complement system (3). A number of the children involved in the study quoted however were also vitamin A deficient. Since vitamin A deficiency is known to affect the development and function of the mucosal epithelium (4) some cells of which are involved in the synthesis of selected complement proteins (5), it occurred to us that the depressed complement levels in these children might have been partly the result of vitamin A deficiency. The purpose of the present study, therefore, was to investigate complement levels in rats reared under conditions where both the vitamin A and protein status of the animals could be precisely controlled. The data suggests that vitamin A deficiency has but a minor depressant effect during the early stages of deficiency, but that thereafter the serum hemolytic activity is enhanced. Protein deficiency on the other hand leads to an immediate and marked decrease in complement levels.

Materials and methods. Animals. Male albino rats weighing 250–300 g were used throughout. Vitamin A deficiency was induced by the withdrawal of retinoic acid from the diet of vitamin A deficient animals as previously described (6). In brief, weanling rats weighing 35–40 g were fed a vitamin A free diet for 3 weeks until growth plateau, and were then fed cyclically a diet first supplemented with and then lacking in 5 μg retinoic acid per g diet for 18 and 10 days, respectively. These monthly holding cycles were repeated until the animals were required for experimentation, a minimum of six complete supplementation–deprivation cycles being employed in the present studies. During a final 8–10 day shortened supplementation phase immediately preceding all experiments, which effectively negated all deficiency symptoms from the preceding deprivation phase, the level of retinoic acid was reduced to 2 μg per g diet in order that the onset of deficiency be better synchronized following the ultimate withdrawal of the retinoate supplemented *ad libitum* fed diet (T_0). Animals selected as A^+ controls were given 500 μg retinyl palmitate in split doses 48 hr (T_{-2}) and 24 hr (T_{-1}) prior to retinoate withdrawal. Finally, all animals were given an oral dose of 10 μg retinoic acid in oil at T_0 to negate prior differences in meal eating patterns and hence endogenous retinoate levels between the A^+ and A^- groups of animals.

Thus until T_0, all animals were fed the standard 18% casein diet *ad libitum* (6). Subsequently, all animals were tube-fed twice daily an isocaloric vitamin A free diet containing either 0% or 18% casein with sucrose in place of protein and/or starch at 100% the calculated (7) maintenance level. Effectively therefore, from T_0 onwards there were four groups of animals (five rats per group) derived from the one original *ad libitum* fed retinoic acid supplemented group: namely, A^+ and 18% casein, A^- and 18% casein, A^+ and 0% casein, and A^- and 0% casein.

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Collection of blood. Animals were bled alternately from the left and right ophthalmic venous plexus on days T_{-3}, T_0, T_4, T_8, and T_{11}. The blood was allowed to clot at 4° for 3–4 hr, after which the serum was frozen at −70° until analyzed.

Complement assays. Serum hemolytic activity was determined by radial hemolysis in gel as described by McGhee and associates (8), using guinea pig complement titrated by a conventional method (9) as standard. Instead of measuring the diameter of hemolytic plaques from photographs as originally described however, the diameters of all plaques were measured directly by calibrated magnifier at the end of each incubation period. As a check on the reliability of the method and in order to generate an adequate standard curve for each plate, a minimum of eight standards were run with every plate. Each sample was analyzed in duplicate. Analyses were repeated if duplicates differed by more than 10–15%. The complement values of the T_0 samples from the four groups of animals are shown in Table I. These values were not significantly different from one another and were comparable to the values reported for normal animals by other investigators (8). The method of McGhee and associates (8) was selected because of the desirability of sequential samplings with time in each of the four groups, which limited the volume of blood that could be taken at each sampling.

Presentation of data. To allow for differences in basal complement values among animals, individual values prior to and at various times after the withdrawal of retinoic acid were expressed as a percentage of the T_0 complement value before calculating group mean values and standard errors of the mean (SEM).

Assay for anticomplementary activity. The serum to be tested was mixed with an equal volume of pooled normal rat serum of known complement activity. The mixture was incubated at 37° for 30 min before the total hemolytic activity of the mixture was determined.

TABLE I. SERUM COMPLEMENT LEVELS (T_0) IN THE FOUR GROUPS OF ADULT ALBINO RATS.

Dietary group	Serum complement level (CH_{50}/ml)[a]
A^+ 18% casein	9.5 ± 2.8
A^+ 0% casein	9.6 ± 3.6
A^- 18% casein	10.1 ± 3.1
A^- 0% casein	10.7 ± 1.9

[a] Mean ± SE of the mean.

Results. The complement values in each of the four groups prior to, at the time of, and at various times after the withdrawal of retinoic acid and commencement of tube feeding are summarized in Fig. 1. The values observed at the time of withdrawal of retinoic acid (T_0) were not significantly different from the values at T_{-3}. In that two of the four groups received retinyl palmitate on days T_{-2} and T_{-1}, it may be concluded, incidentally, that retinoic acid is as effective as vitamin A in the maintenance of serum complement levels. The continued relative constancy of serum complement levels in the A^+ control group fed the 18% casein diet further supports this contention. Thus, the T_0 reference values represent valid points of departure for the comparison of serum hemolytic activities in the four groups subsequent to the withdrawal of retinoic acid and institution of the force feeding regimen.

Within 4 days of retinoic acid withdrawal (T_4), the complement levels of four groups differed markedly from one another. The A^- group receiving the 18% casein diet demonstrated a slight but reproducible reduction in the T_4 complement levels. The T_4 complement values of the two groups maintained on 0% casein diets were more sharply reduced regardless of vitamin A status, compared either with their own T_{-3} values ($P < 0.05$), or with the T_4 value of the control A^+ and 18% casein group ($P < 0.025$). Indeed, at all times the complement levels in the two groups maintained on 0% casein diets were lower than the corresponding values in animals maintained on 18% casein diets. This depressant effect of protein deficiency was most clearly demonstrated at the A^+ group receiving the 0% casein diet, where the complement level was significantly depressed throughout the entire 11 day period of observation. In a separate experiment, it was found that the hemolytic activity of the serum in A^+ animals fed the 0% casein diet beyond day 11 was unchanged; similar determinations were not possible with A^- animals due to the death of most of the animals by T_{14}. At least in rats,

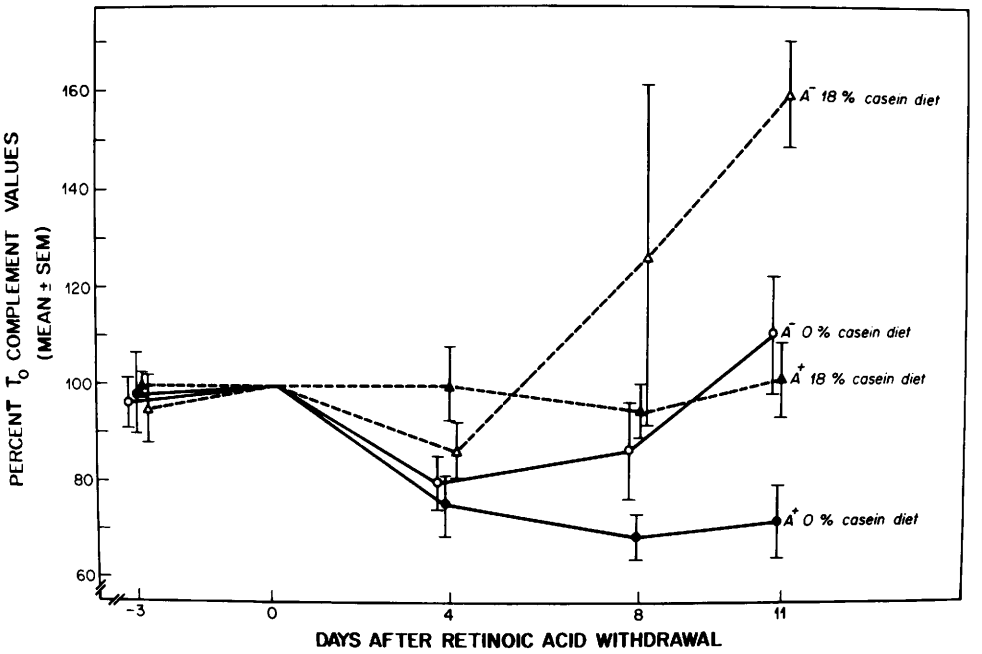

FIG. 1. Effect of vitamin A and protein status on the complement levels of rats.

therefore, protein is seemingly more important than vitamin A in maintaining serum complement levels.

One unexpected finding was the observation that following the initial reduction at T_4, the complement levels of A^- animals regardless of protein status were elevated, this effect being particularly marked in animals maintained on the 18% casein diet. This was not a laboratory artifact since the complement levels of the A^+ control group receiving the 18% casein diet were effectively unchanged during the entire period of observation. Two other experiments of closely similar design were in agreement with the data reported in Fig. 1.

Finally, an attempt was made to determine the mechanisms(s) leading to depressed complement levels in the protein deficient animals. One possible factor in the interference of serum hemolytic activity is the presence of anticomplementary activity in the serum of such animals. An experiment involving mixed normal rat serum and pooled serum obtained at T_{11} from the A^+ group fed the 0% casein diet failed however to demonstrate any circulating anticomplementary activity in these animals (Table II).

Discussion. The results shown in Fig. 1 demonstrate clearly that it is the protein component of the diet and not vitamin A that is responsible for the maintenance of serum hemolytic activity in these rats. In that protein synthesis generally is dependent on protein intake, and given the failure to detect anticomplementary activity in the sera of A^+ animals fed protein-free diets (Table II), we feel that the reduced complement levels seen in the protein deficient animals are more probably related to depressed synthesis of

TABLE II. ASSAY FOR ANTICOMPLEMENTARY ACTIVITY IN RAT SERUM.

Samples tested	Complement level (CH_{50}/ml) Expected	Observed
Pooled serum from rats fed a normal pellet diet (= normal rat serum, NRS)	—	12.8
Pooled serum from A^+ control rats fed the 0% casein diet following the withdrawal of retinoic acid from the diet		
T_{-3}	—	10.1
T_{11}	—	7.2
Mixed serum samples		
NRS + T_{-3}	11.4	9.4
NRS + T_{11}	10.0	10.4

complement rather than increased catabolism. This interpretation is supported in large part by results obtained previously in studies of children with protein–calorie malnutrition (3, 10). In that anticomplementary activity has been encountered under condition of longer term protein deprivation in humans however, it should be noted that increased catabolism of complement may be an additional factor in the depression of complement levels under such conditions (10).

The failure to demonstrate other than a transient depressant effect of vitamin A deficiency on complement levels in the present studies was unexpected in that the mucosal cells of the gastrointestinal tract are known to be involved in the synthesis of some complement proteins in man (5), and the morphological and functional integrity of epithelial tissues is reproducibly altered in vitamin A deficiency (4). In the present experiments, for instance, both the epithelial cells and the lymphoid tissues of rats reared and fed as described exhibited marked morphological changes from day 6 onwards (W. Rojanapo, M. Anzano, A. J. Lamb; unpublished observations). The marked increase in complement levels in A^- animals from T_4 onwards, particularly in animals fed the 18% casein diets, is even less readily explained. In that the half-life of rat complement (C3) is less than 1 day (11) however, any imbalance between daily synthetic and catabolic rates would lead to rapid changes in overall complement levels. Administration of a large dose of vitamin A to mice is known to suppress circulating hemolytic activity within 24 hr (12). It is also of interest that the rate of turnover of some proteins of the body appears to be depressed in vitamin A deficiency (13). It would be of considerable interest therefore to determine whether the enhanced complement levels we have observed in vitamin A deficiency are similarly due to decreased catabolism of complement proteins. Investigations along these lines may also yield information concerning the mode of action of vitamin A.

Summary. The role of protein and vitamin A in the maintenance of serum complement levels was studied using rats reared by a novel system enabling the synchronous induction of vitamin A deficiency and the stringent control of both the caloric and dietary protein input. Protein deficiency leads to a decrease in serum complement levels, whereas vitamin A deficiency enhances complement levels. Additional experiments to determine the relative rates of synthesis and catabolism of complement components in protein and vitamin A deficiency are required if the overall function(s) of these nutrients in determining serum hemolytic activities are to be fully understood.

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