INVITED REVIEW

J.Sci.Soc. Thailand, 14 (1988) 161-180

IMPAIRMENT OF SECRETORY IMMUNE SYSTEM IN VITAMIN A DEFICIENCY STATE*

STITAYA SIRISINHA

Department of Microbiology, Faculty of Science
Mahidol University, Bangkok

The synergistic interaction between malnutrition and infection has long been recognized; not only is there a higher incidence of infection, but the mortality rate for some infectious diseases is also higher in the malnourished than in the well-nourished individual.[1] Both clinical observations and experimental animal studies suggest that the malnourished subject responds to infection in a way different from the well-nourished individual.[2, 3] For example, there is a tendency for the malnourished child to develop afebrile gram-negative septicemia. Likewise, when localized infection spreads, it often does so with the development of gangrene rather than suppuration.

The underlying mechanisms by which diet may alter the interaction between host and invading agent are poorly understood and rather complex. This is not entirely unexpected as the quality and the quantity of dietary intake are not the only factors that affect the host defenses; the immunological status of the host and the metabolism of the invading agents also come into play. Because of such complications, this review will be focused on the effect of vitamin A deficiency on secretory (local, mucosal) immune system, with special emphasis on findings contributed from our group.

* Based on the Outstanding Scientist of Thailand Award Lecture, Bangkok, 18 August 1988.

EFFECT OF COMPOUND MALNUTRITION ON SYSTEMIC HUMORAL IMMUNE RESPONSE

This parameter of the immune system has been studied most frequently in children with protein-calorie malnutrition (PCM). This is not only because the humoral aspect is most thoroughly understood, but also because it can be readily assessed. The integrity of the humoral immune response in man is most often evaluated either by measuring the total levels of various classes of immunoglobulins (IgG, IgA, IgM, IgD and IgE) or by observing the increase in antibody titer following an appropriate antigenic stimulation. (e.g., tetanus toxoid, typhoid vaccine, etc).

It was reported previously that the synthesis of globulin, unlike the synthesis of albumin, was unaffected in PCM children.[4] This is consistent with reports that the total levels of immunoglobulins in these children were not depressed.[2, 5] In fact, an elevation was noted in some children at the time of admission. The latter is largely due to the chronic infection that is commonly associated with this condition. Because the total levels of immunoglobulins do not reflect the current status of the humoral immune system, a number of investigators including our group, prefer the alternative approach, i.e., analyzing antibody response to antigenic stimulation. For this purpose, we immunized the children with a typhoid vaccine and the anti-typhoid response was measured by an appropriate immunological method.[6] We found that the PCM children who had already recovered following dietary treatment responded more efficiently than the newly admitted PCM group (Fig. 1). Altogether these observations suggest that the systemic humoral immune reactivity of PCM children was impaired. However, it should be kept in mind that several factors can influence the outcome of such a study. These include the severity of the deficiency state and the previous immunological experience of the children to the antigen under study.

In addition to immunoglobulins in the serum, we also analyzed immunoglobulins in the secretions of PCM children. The reason behind this approach was that these children often had surface infections (e.g., diarrhea, respiratory tract infections)[1, 7, 8] and secretory (mucosal, local) antibody is known to serve as a first line of defense against these infections.[9] Results presented in Figure 2 show that the mean level of nasal wash fluid IgA of these children was significantly depressed compared with that of well-nourished controls.[10] Subsequently, several other groups of investigators have confirmed our observations.[11-15] For example, Chandra[11] reported that, not only the total IgA levels, but also the titers of specific IgA antibody produced in response to measles and poliovirus vaccines in the nasopharyngeal secretions of malnourished children, were depressed. A similar observation was also noted in the tears of other malnourished children.[12] In contrast to the secretory IgA (SIgA), the admission level of serum IgA of our malnourished children was

markedly elevated.[10] It was observed further that, in these malnourished children, the elevated mean serum IgA level gradually declined following dietary treatment (Fig. 3), while the depressed mean SIgA level slowly rose, approaching that of the normal children several months later (Fig. 4). A reversed relationship between the secretory IgA and serum IgA (but not other serum immunoglobulin isotypes) in these children suggested that there was a defective transport of serum IgA from blood or tissue fluid to external body secretions. Similar results were recently reported in protein-deficient mice.[16] Moreover, when the data from our PCM children was analyzed further, it appeared that the depressed SIgA level in our PCM children was more severe in those individuals also suffering from vitamin A deficiency. These observations triggered our interest in the role of vitamin A in the integrity of the secretory (mucosal, local) immune system.

INFLUENCE OF VITAMIN A STATUS ON THE IMMUNE SYSTEM

The association of vitamin A with the structural and functional integrity of the immune system has long been suspected but the underlying molecular mechanism for such an association is not well understood. Vitamin A is known for its functions in vision, growth, reproduction and cellular differentiation.[17, 18] The function of vitamin A in vision appears to be different from that involved in other functions. In fact, the existence of more than one metabolic function has been suggested from the presence of two functionally active metabolic forms of vitamin A, i.e., retinol and retinoic acid.[17] The latter finding is consistent with the availability of two different intracellular binding proteins for vitamin A transport, i.e., cytosol retinol binding protein for vitamin A and cytosol retinoic acid binding protein for retinoic acid.[17] At a cellular level, vitamin A functions through an action on either the nucleus or the cytoplasm. Within the nucleus, vitamin A may alter the expression of genetic information to control cellular differentiation. Inhibition of neoplastic transformation can also be attributed to its ability to modulate gene expression in the nucleus.[18] At an extranuclear site, it may function at the cell surface. At a molecular level, vitamin A is known for its role in regulation of the glycosylation of newly formed glycopeptides and glycolipids.

With respect to its influence on the immune system, vitamin A is well known for its property as an "anti-infective" agent. Although non-specific host defense including phagocytosis and intracellular killing may be involved, our attention will be directed at its function on specific host response to stimulation by various microbial agents. Vitamin A deficiency is known to be associated with depressed immunity to various types of infections and with increased susceptibility to tumor induction.[18-21] The incidence of certain types of cancer has been reported to be high in a population with low vitamin A levels. In these individuals, depressed delayed hypersensitivity, humoral response, natural killer cell activity and lympho-proliferative response have been reported.[19, 22, 23] On the other hand, supplementa-

tion of diet with excess vitamin A or some of its analogues can enhance these immune functions.[19] Although the immunopotentiating property of vitamin A has been recognized for quite some time,[23-26] the underlying molecular mechanism of vitamin A on the immune system has not yet been fully elucidated. In recent years, its immunoregulatory action has been suggested to be associated with an elevated number of helper T cells[27] and enhanced interleukin-2 production.[28] More recently, Smith and Hayes[29] showed that inefficient clonal expansion of responding B lymphocytes might be responsible for contrasting impairments of IgM and IgG responses observed in vitamin A deficient mice. They showed that the IgG response was affected earlier and to a much greater extent than the IgM response. The data presented was consistent with the notion that a functional helper T cell defect may account for a less efficient clonal expansion of antigen-responsive B cells in these deficient animals. However, such a conclusion must be made with caution as several groups of investigators, including ours, have data suggesting that the influence on the magnitude and the isotype of antibody produced depends in part on the antigens used.

Epidemiological studies and clinical observations in humans and experimental studies in animal models clearly show that vitamin A deficiency is associated with increased susceptibility to, and severity of, infection by virtually all types of micro-organisms and parasites.[1, 19] This is particularly obvious for upper respiratory tract infections and diarrhea. Although the interaction is rather obvious, the underlying mechanism at a molecular level is lacking. The obstacle in studying this problem is probably related to the fact that it is rather difficult to study vitamin A deficiency in humans, as only rarely can one find these individuals in the absence of other nutritional deficiencies. In the presence of multiple nutritional deficiencies, neither the underlying mechanisms for increased susceptibility to infection nor the immuno-regulatory function can be assigned to vitamin A, as protein, trace elements, and other vitamin deficiencies also influence immunocompetence of the host.[30-32] Moreover, uncomplicated vitamin A deficiency state is difficult to induce in animals as the classical procedure involves long term feeding of the animals with a vitamin A deficient diet, which is almost always accompanied by secondary inanition.

In humans, vitamin A deficiency is often assessed by serum vitamin A level and/or ocular lesions (e.g., xerophthalmia and blindness). Using these criteria, a majority of studies show a parallel between hypovitaminosis A and increased frequency and severity of infections (for more extensive review, see references 1 and 19). It is obvious from these reviews that both microbial infections and parasitic infestations are more common in humans with hypovitaminosis A and animals with severe vitamin A deficiency state. However, restoration of vitamin A status may or may not reverse the condition as impaired intestinal absorption of vitamin A caused by concomitant intestinal infections cannot be ruled out. Therefore, from

human studies it is difficult, if not impossible, to attribute the underlying defect to vitamin A deficiency state. If vitamin A has a role in humoral and cell-mediated immune responses, in phagocytosis and other non-specific components including complement, a well controlled experimental animal model would allow a more precise identification of the affected cell type and the underlying molecular mechanism for the role of the vitamin in cellular functions. In the experiments to be described, we employed rats rendered vitamin A deficient by a protocol enabling the induction of vitamin A deficiency with minimal secondary inanition.[33, 34] Such a protocol (Fig. 5) involved a cyclic retinoic acid supplementation-deprivation feeding schedule (18:10 days). At least 4 such cycles were required to deplete the animals of all their vitamin A reserves. With this protocol, the normal growth of animals, as determined from weight gained, was supported totally by retinoic acid and not vitamin A. The animals would then become vitamin A deficient within 4-6 days after the withdrawal of retinoic acid from their diet.[34] The vitamin A sufficient group serving as controls was given an excess does of retinyl palmitate by stomach tube. Both groups were subsequently maintained on retinoic acid-free diet until the experiment was terminated. The advantage of our rearing system is the ability to determine very precisely the exact time of onset of the deficiency state. However, one possible drawback that should be kept in mind is the integrity of the lymphoid tissues of these retinoic acid-supported rats. It was reported recently that retinoic acid may be inferior to retinol in supporting the growth of lymphoid tissues but both forms were equally effective in restoring these defects.[35]

Employing the retinoic acid rearing system described above, our data clearly demonstrated that uncomplicated vitamin A deficiency was undoubtedly associated with a decreased resistance to infection, as shown by a higher incidence of bacteremia caused by bacteria of the normal intestinal flora of these animals.[36] The impaired bacterial clearance was most likely associated with a defective phagocytic function. The latter is probably the result of an inherent cellular defect and not of depressed opsonic activity in the serum as the complement activity of these animals was not different from that of normal controls.[37] Other lines of evidence reported by several other groups of investigators also indicated a defective phagocytosis at a cellular level.[1, 26, 31] These non-specific defects at a systemic level may contribute to a shortened life span of vitamin A deficient animals.[38, 39] Our data is also consistent with the observations that the life span of vitamin A deficient animals could be extended if these animals were maintained in a germ-free environment.[40, 41] An additional possible factor that may contribute to this phenomenon is a failure of the local mechanism(s) to contain these indigenous bacteria within the gut lumen, thus allowing a more than normal degree of invasion into the underlying tissue. Although such a localized defect could be attributed to a defective physical barrier of the epithelial cells whose integrity is known to be influenced by vitamin A status, and to the quality and quantity of mucins produced by the

goblet cells of these animals,[42] an impaired local immune system should also be suspected.

EFFECTS OF VITAMIN A DEFICIENCY ON THE LOCAL IMMUNE SYSTEM

Different lines of evidence suggest that vitamin A deficiency is associated not only with an impaired non-specific host defense (as discussed in the previous section) but also with a malfunctioning of the mucosa-associated lymphoid tissues (MALT) whose major role is to provide specific defense at all mucosal surfaces. It has been observed that the incidence of mucosal surface infections, e.g., diarrhea, upper respiratory tract infections and, to a lesser extent, genitourinary tract infections, is high in populations with hypovitaminosis A.[19] It is also not uncommon to find these infections to be more severe than those encountered in vitamin A sufficient population. The apparent defective local immune response in vitamin A deficiency can be attributed to an improper development of MALT or of other peripheral lymphoid tissues. To simplify the discussion, Table 1 shows a sequence of events occurring following an exposure of immunocompetent lymphocytes to incoming antigen. The function of these lymphocytes is completed following the differentiation of lymphoblasts into plasma cells which in turn synthesize and secrete antibodies of any immunoglobulin isotype (e.g., IgM, IgG or IgA) into blood circulation. In order to transport some of these antibody isotypes to their destination in any external body secretion, these antibodies must first recognize and complex with a membrane glycopeptide (secretory component---SC) synthesized by either epithelial cells lining mucosal lumens or hepatocytes.[9, 43] Therefore, the integrity of these cells with regard to their ability to synthesize a functional SC is a prerequisite for an efficient transportation of dimeric IgA (or other polymerized immunoglobulins) to various body secretions, e.g., intestinal fluid, saliva, tears and mammary gland secretions. The following circumstantial evidences implicate a defective local immune function in a vitamin A deficiency state.

1. In contrast to other dietary deficiency states, vitamin A deficiency is almost always associated with increased incidence and severity of infections caused by virtually all types of infective agents.[1]

2. The infections that occur in this deficiency state are predominantly associated with those occurring at mucosal surfaces.[7, 8, 19, 20]

3. Immunological aberrations at a systemic level appear to be less obvious in uncomplicated vitamin A deficiency compared with severe protein-calorie malnutrition,[30-32, 44] yet both groups appear to be just as susceptible to mucosal surface infections, suggesting preferential damage at a more distant site or at MALT itself.

4. Deficiency of vitamin A superimposed on PCM aggravates the defect even further.[10]

5. Structural and functional integrity of the local immune system requires intact epithelial cell functions which are known to be vitamin A dependent.[17, 17a]

POSSIBLE UNDERLYING MECHANISMS FOR A DEFECTIVE LOCAL IMMUNE FUNCTION

The above discussion strongly suggests that vitamin A can influence the status of the local immune system, particularly that concerned with IgA production and secretion. Our initial interest in secretory IgA antibody led us to investigate this important problem more systematically, using our experimental vitamin A deficient animal model.

We initially observed that immunoglobulin synthesis in our PCM children was probably only moderately impaired judging from the mean levels of serum immunoglobulins.[5] In fact, it was noted that the serum IgA levels in these children were markedly elevated at the time of hospital admission.[10, 10a] In contrast to IgA levels in the serum, IgA levels in the nasal washes, also collected at the time of admission, were considerably lower than those of normal children. The elevated serum IgA levels gradually declined while the depressed secretory IgA levels slowly returned to near normal following appropriate dietary treatment.[10, 10a] Our initial observations have been subsequently confirmed by other investigators using other species of animals and human populations from other parts of the world.[15, 16] Altogether, these observations strongly suggest that, in addition to other immune defects, protein-calorie malnutrition adversely affects the integrity of the local immune system. Furthermore, when we analyzed our data more critically, it became obvious to us that such a defect was more severe in those superimposed with vitamin A deficiency.[10] The latter, therefore, is suggestive of the facts that (1) vitamin A preferentially influences the integrity of the local immune function, particularly regarding the quality and the quantity of IgA antibody produced and (2) defective transport of IgA across mucosal barriers may account for the defective secretory IgA system noted in this condition.

In order to distinguish between the different possible underlying mechanisms for a defective SIgA system, we used vitamin A deficient rats reared by the method mentioned in the previous section and fully described in detail elsewhere.[33, 34] It should be recalled that in our rearing system, the animals were fed *ad libitum* a retinoic acid-containing diet until such a time when the experiments were to be performed. Using this experimental model, we found that vitamin A deficient rats had depressed intestinal antibody response to dinitrophenylated bovine gamma globulin stimulation.[44] Although in this particular study we had no direct evidence to implicate a defective intestinal IgA system for the reduced antibody response noted above, our data on depressed total intestinal IgA levels was consistent with this notion (Fig. 6). However, we subsequently demonstrated that the depressed IgA levels in these deficient animals were in fact related to an impaired synthesis of IgA by the intestinal mucosa, as shown by an inefficient incorporation of radio-

active amino acids into newly synthesized intestinal IgA.[45] A reduced transportation of serum IgA across the hepatocytes into the bile, which then empties into the intestine (Fig. 7), lowered even further the overall IgA levels in the intestinal fluid of these animals.[46]

More recently, we attempted to narrow down further the possible underlying mechanisms for such a defect. In the experiment to be described we therefore bypassed the "M" cell function by injecting the antigens directly into the Peyer's patches of these animals.[46] Moreover, employing both T-independent *(Bacillus abortus)* and T-dependent (sheep red blood cells) antigens also allowed us to gain information regarding the effect on the immunoregulatory mechanism of these deficient animals, e.g., the integrity of T cell functions. The results presented in Figure 8 show that the primary serum antibody response to the T-dependent antigen, but not T-independent antigen, was adversely affected by vitamin A deficiency. These observations suggest that the immunoregulatory mechanism involving a T cell function may have been interfered with. This conclusion is consistent with the more recent data obtained from vitamin A deficient mice that there appeared to be a malfunctioning of helper-T cells, thereby accounting for an inefficient clonal expansion of antigen-driven B cells responsible for making the antibody isotypes that are T cell dependent.[29] In this set of experiments, a failure of antigen uptake by the "M" cells (whose development and differentiation presumably depend on vitamin A), if such existed at all, could be ruled out as we bypassed this step by depositing both antigens directly into the Peyer's patches.

Unlike the antibody responses in the serum, the antibody titers measured in the bile of these animals were markedly depressed for both antigens. This strongly indicated that the step involving the transportation of the antibodies across the hepatocytes into the bile may be adversely affected by vitamin A deficiency. Because in the rat the transport mechanism of IgA across the hepatocytes or intestinal epithelial cells involves SC which serves as membrane receptors for IgA, we therefore looked at this point for a possible defect in vitamin A deficiency. Results from our immunofluorescence studies suggest that the molecular mechanism for a defective IgA transport in vitamin A deficient rats may be related to an impaired synthesis of the secretory component by the epithelial cells.[44] Unfortunately, we did not have data regarding the SC status of hepatocytes. Nonetheless, a reduced transport of intravenously administered dimeric serum IgA into the bile of vitamin A deficient animals is consisent with our proposal.[46] This is not surprising as an impaired synthesis of SC, which is a glycopeptide, can be expected because one of the functions of vitamin A is to regulate glycoprotein synthesis. In fact, it was reported earlier that the intestinal goblet cells of rats rendered vitamin A deficient (by a cyclic retinoic acid supplementation-deprivation feeding schedule as described herein) synthesized and secreted mucins that were quantitatively and qualitatively different from those of normal animals.[42]

The diagram presented in Figure 9 summarizes the several possible underlying mechanisms by which a deficiency of vitamin A may interfere with the structural and functional integrity of the mucosal immune system.

1. *Antigen uptake, processing and presentation by "M" cells overlying the Peyer's patches.* Theoretically, these important functions can be adversely affected by vitamin A deficiency as vitamin A itself is known for its function in differentiation and maturation of epithelial cells.[17, 47] The ability of other antigen-presenting cells to perform these functions has never been evaluated systematically in vitamin A deficiency, although deranged phagocytosis and intracellular killing by phagocytic cells from these animals have been reported.[36] IL-1 production was found to be depressed in PCM,[48] but whether or not it also occurs in vitamin A deficiency has never been reported. However, our experiments designed to bypass the involvement of "M" cells failed to support the possible interference at this step.[46]

2. *Immunocompetence of mucosa-associated lymphocytes.* The immunoregulatory mechanism for mucosal localization of immunocompetent lymphocytes precommitted to IgA production is highly complicated and has been reviewed elsewhere.[9, 49, 50] Because the IgA system is T-cell dependent, it is therefore suspected that its development in the vitamin A deficiency state is adversely affected as it has been shown clearly very recently that T helper cell functions were impaired in the vitamin A deficiency state. Both thymus and bursa of Fabricius were previously shown to be poorly developed,[30, 35] and this by itself may account for a decreased number of lymphocytes in the lamina propria of these animals.[32] We also observed that the Peyer's patches of our vitamin A deficient rats were considerably smaller than those of vitamin A sufficient controls (unpublished observations). It has never been analyzed in detail whether or not the fewer lymphocytes found at these mucosal sites can bind antigens normally and be properly activated and proliferated as those from normal animals. It is possible that the alteration of membrane glycoproteins of the lymphocytes from vitamin A deficient rats, as noted previously,[51] may also include antigen receptors, which are also glycoprotein in nature. However, this possibility remains to be determined as the role of carbohydrate on the T cell antigen receptors has never been evaluated.

3. *Lymphocyte proliferation and differentiation.* It was shown recently that clonal expansion of antigen-responsive B cells of vitamin A deficient mice was rather inefficient due to dysfunction of helper T cells.[29] This is consistent with the observations that, not only the thymus and the bursa, but also the secondary lymphoid organs (i.e., spleen and peripheral lymph nodes) of these animals were atrophic and poorly developed.[30, 32] It was mentioned earlier that the Peyer's patches of our vitamin A deficient rats were small compared with the vitamin A sufficient controls. Moreover, these deficient animals had lymphopenia and abnormal ratios of lymphocyte subpopulations.[52, 53] Proliferative responses of these lymphocytes to mitogenic and antigenic stimulations have been noted by several groups of

investigators.[52-54] Such a defective response is attributable to a depressed IL-2 production as vitamin A supplementation could enhance its production.[28] Taken together with the well known functions of vitamin A in modulating gene expression, cellular differentiation and immunoregulation, we are inclined to suspect that this stage of the immune response is adversely affected by the deficiency state.

4. *Lymphocyte recirculation and tissue emigration.* Following antigenic stimulation, these mucosal lymphocytes now committed to IgA production migrate via thoracic duct to the blood, spleen and liver; then they either return to the gastrointestinal tract or localize at other distant mucosal sites.[9, 50] Several factors have been proposed to be responsible for the homing mechanism of these potential IgA-producing cells.[9, 55] In addition to the mucosal environment rich in microbial antigens and mitogens, specialized receptors for circulating lymphocytes present on high endothelium of post-capillary venules (HEV) and lymphocyte glycoprotein membrane structure necessary for the recognition of HEV receptors may also be involved, thus influencing the degree of mucosal localization of these lymphocytes.[55] In an adoptive transfer experiment, McDermott and associates demonstrated that the lymphocytes from vitamin A deficient animals migrated poorly to the lamina propria of normal animal and suggested that a defect was most likely associated with altered surface structure.[56] On the other hand, Takagi and Nakano presented evidence suggesting that altered lymphocyte migration to the mucosa in cases of vitamin A depletion may be the result of a derangement of lymphocyte-trapping mechanisms, e.g., HEV receptors, and not a change in the nature of lymphocytes *per se*.[57] Although a reduced number of IgA-containing plasma cells in the lamina propria of these animals has been occasionally reported, the data are not as convincing as has been noted for PCM.[58]

5. *Transportation of IgA to its destinations.* Because of the tight junction found in all mucous membranes, the IgA molecules cannot move freely from the site of synthesis in the lamina propria or from blood circulation to mucosal lumens. A specific transport mechanism has been recognized and this involves a binding of dimeric IgA with receptor secretory component glycopeptide synthesized by, and present on, the basolateral surface of mucosal epithelium, and, for some animal species, on the sinusoidal surface of the hepatocytes.[9, 43] The IgA-SC complex is endocytosed, transported in vesicles across the cytoplasm and finally secreted at the apical surface or, in the case of hepatic transport, at the canalicular surface. We have immunofluorescence results showing reduced staining for SC by the intestinal epithelium of vitamin A deficient rats. This is not at all unexpected as improper glycosylation of the SC synthesized by these cells may interfere with the ability to function as carrier to transport IgA to mucosal lumen.[44] We felt that the impairment involved quantitative rather than qualitative change as our evidence, though limited, suggested that the SC synthesized by these deficient

animals was immunologically indistinguishable from that synthesized by the normal controls. A reduced level of free secretory component in the tears of malnourished subjects[59] is consistent with our proposal.

CONCLUDING REMARKS

It is felt that, of these possibilities, the last two mechanisms, i.e., lymphocyte recirculation to mucosal sites and IgA transport across mucosal surfaces and hepatocytes, are the most attractive ones. Because the earlier stages of the immune response to T-independent antigens involve the production and secretion of serum antibodies which were found to be minimally affected by vitamin A deficiency, a defect of the secretory immune function should therefore involve the later stages of antibody production. For example, primary antibody responses to T-independent antigens *(Bacillus abortus)* in the serum of our vitamin A deficient rats were not different from those of normal controls, and yet secretory antibodies were found to be markedly depressed in the bile. Experiments involving *de novo* synthesis of intestinal IgA and biliary excretion of administered radioactive dimeric serum IgA are compatible with a defective transport of IgA to its destination in various body secretions. Immunofluorescent staining of the intestinal sections for IgA showed a slight reduction in the number of IgA-positive plasma cells in the lamina propria, and in the intensity of staining for SC in the apical one-third of the epithelial cells. Altogether our data suggest that an underlying molecular mechanism for a defective secretory immune system in vitamin A deficiency state is related to an aberrant transport of IgA across the mucosal surfaces or hepatocytes.

Different lines of evidence obtained by other groups of investigators also narrow down the possibilities to these two mechanisms. Nevertheless, other underlying mechanisms including impaired cellular proliferation and differentiation as well as deranged immunoregulation may also contribute to the impaired production and secretion of secretory IgA antibody. The availability of more specific reagents, e.g., monoclonal antibodies, and newer technologies currently available should allow one to distinguish between these two underlying mechanisms.

Very recently retinoic acid, an analogue of vitamin A, was shown to modulate immune responses by upregulating the expression of IL-2 receptors on proliferating T lymphoblasts[60] and by regulating their protein kinase C activity.[61] Also protein deprivation could preferentially stimulate suppressor T cells that suppress the IgA response after oral immunization[62] as well as interfering with lymphocyte migration.[63]

REFERENCES

1. Scrimshaw, N.S., Taylor, C.E. and Gordon, J.E. Jr. (1968). Interaction of Nutrition and Infection. WHO Monograph Series No. 57.
2. Suskind, R.M. (1977). Malnutrition and the Immune Response. Raven Press, N.Y.
3. Chandra, R.K. and Newberne, P.M. (1977). Nutrition, Immunity and Infection; Mechanisms of Interaction. Plenum Press, New York.
4. Cohen, S. and Hansen, J.D.L. (1962). Metabolism of Abumin and Globulin in Kwashiorkor. *Clin. Sci.* **23**, 351-359.
5. Suskind, R.M., Sirisinha, S., Edelman, R., Vithayasai, V., Kulapongs, P., Leitzmann, C. and Olson, R.E. (1978). *Host Defenses in Protein-Calorie Malnutrition.* In: The Infection-Prone Hospital Patients (Burke, J.F. and Hildick-Smith, G.Y., eds.), Little, Brown and Company, Boston, pp. 121-142.
6. Suskind, R., Sirisinha, S., Vithayasai, V., Edelman, R., Damrongsak, D., Charupatana, C. and Olson, R.E. (1976). Immunoglobulins and Antibody Response in Children with Protein-Calorie Malnutrition. *Am. J. Clin. Nutr.* **29**, 836-841.
7. Phillips, I. and Wharton, B. (1968). Acute Bacterial Infection in Kwashiorkor and Marasmus. *Br. Med. J.* **1**, 407-409.
8. Hutt, M.R.S. (1969). Malnutrition and Infection-East African Studies. *J. Trop. Pediatr* **15**, 153-155.
9. Underdown, B.J. (1986). Immunoglobulin A: Strategic Defense Initiative at the Mucosal Surface. *Ann. Rev. Immunol.* **4**, 389-417.
10. Sirisinha, S., Suskind, R., Edelman, R., Asvapaka, C. and Olson, R.E. (1975). Secretory and Serum IgA in Children with Protein-Calorie Malnutrition. *Pediatrics* **55**, 166-170.

10.a Sirisinha, S., Suskind, R., Edelman, R., Asvapaka, C. and Olson, R.E. (1974). *Secretory and Serum IgA in Children with Protein-Calorie Malnutrition.* In: The Immunoglobulin A System (Mestecky, J. and Lawton, A.R., eds.), Plenum Press, New York.,pp. 389-398.

11. Chandra, R.K. (1975). Reduced Secretory Antibody Response to Live Attenuated Measles and Poliovirus Vaccines in Malnourished Children. *Br. Med. J.* **2**, 583-585.
12. Watson, R.R., Reyes, M.A. and McMurray, D.N. (1978). Influence of Malnutrition on the Concentration of IgA, Lysozyme, Amylase and Aminopeptidases in Children's Tears. *Proc. Soc. Exp. Biol. Med.* **157**, 215-219.
13. Lim, T.S., Messiha, N. and Watson, R.R. (1981). Immune Components of the Intestinal Mucosae of Ageing and Protein Deficient Mice. *Immunology* **43**, 401-407.
14. Reddy, V., Raghuramulu, N. and Bhaskaram, C. (1976). Secretory IgA in Protein-Calorie Malnutrition. *Arch. Dis. Childh.* **51**, 871-874.
15. McMurray, D.N., Rey, H., Casazz, L.J. and Watson, R.R. (1977). Effect of Moderate Malnutrition on Concentrations of Immunoglobulins and Enzymes in Tears and Saliva of Young Colombian Children. *Am. J. Clin. Nutr.* **30**, 1944-1948.
16. McGee, D.W. and McMurray, D.N. (1988). The Effect of Protein Malnutrition on the IgA Immune Response in Mice. *Immunology* **63**, 25-29.
17. Wolf, G. (1984). Multiple Functions of Vitamin A. *Physiol. Rev.* **64**, 873-937.

17a. Zile, M.H. and Cullum, M.E. (1983). The Function of Vitamin A: Current Concepts. *Proc. Soc. Exp. Biol. Med.* **172**, 139-152.

18. Lotan, R. (1980). Effects of Vitamin A and Its Analogs (Retinoids) on Normal and Neoplastic Cells. *Biochim. Biophys. Acta.* **605**, 33-91.
19. Nauss, K.M. (1986). *Influence of Vitamin A Status on the Immune System.* In: Vitamin A Deficiency and Its Control (Bauernfeind, J.C., ed.), Academic Press, New York. pp. 207-243.

20. Sommer, A., Katz, J. and Tarwotjo, I. (1984). Increased Risk of Respiratory Disease and Diarrhea in Children with Preexisting Mild Vitamin A Deficiency. *Am. J. Clin. Nutr.* **40**, 1090-1095.
21. Moriguchi, S., Werner, L. and Watson, R.R. (1985). High Dietary Vitamin A (Retinyl Palmitate) and Cellular Immune Functions in Mice. *Immunology* **56**, 169-177.
22. Nauss, K.M., Phua, C-C., Ambrogi, L. and Newberne, P.M. (1985). Immunological Changes During Progressive Stage of Vitamin A Deficiency in the Rat. *J. Nutr.* **115**, 909-918.
23. Smith, S.M., Levy, N.S. and Hayes, C.E. (1987). Impaired Immunity in Vitamin A-Deficient Mice. *J. Nutr.* **117**, 857-865.
23a. Jurin, M. and Tannock I.F. (1972). Influence of Vitamin A on Immunological Response. *Immunology* **23**, 283-287.
24. Falchuk, K.R., Walker, W.A., Perrotto, J.L. and Isselbacher, K.J. (1977). Effect of Vitamin A on the Systemic and Local Antibody Responses to Intragastrically Administered Bovine Serum Albumin. *Infect. Immun.* **17**, 361-365.
25. Dennert, G. and Lotan, R. (1978). Effects of Retinoic Acid on the Immune System: Stimulation of T Killer Cell Induction. *Eur. J. Immunol.* **8**, 23-29.
26. Hof, H. and Emmerling, P. (1979). Stimulation of Cell-Mediated Resistance in Mice to Infection with *Listeria monocytogenes* by Vitamin A. *Ann. Immunol. (Inst. Pasteur)* **130c**, 587-594.
27. Malkovsky, M., Edwards, A.J., Hunt, R., Palmer, L. and Medawar, P.B. (1983). T-Cell-Mediated Enhancement of Host-Versus-Graft Reactivity in Mice Fed a Diet Enriched in Vitamin A Acetate. *Nature* **302**, 338-340.
28. Colizzi, V. and Malkovsky, M. (1985). Augmentation of Interleukin-2 Production and Delayed Hypersensitivity in Mice Infected with *Mycobacterium bovis* and Fed a Diet Supplemented with Vitamin A Acetate. *Infect. Immun.* **48**, 581-583.
29. Smith, S.M. and Hayes, C.E. (1987). Contrasting Impairments in IgM and IgG Responses of Vitamin A-Deficient Mice. *Proc. Natl. Acad. Sci. USA* **84**, 5878-5882.
30. Krishnan, S., Bhuyan, U.N., Talwar, G.P. and Ramalingaswami, V. (1974). Effect of Vitamin A and Protein-Calorie Undernutrition on Immune Response. *Immunology* **27**, 383-392.
31. Krishnan, S., Krishnan, A.D., Mustafa, A.S., Talwar, G.P. and Ramalingaswami, V. (1976). Effect of Vitamin A and Undernutrition on the Susceptibility of Rodents to a Malarial Parasite *Plasmodium burghei. J. Nutr.* **106**, 784-791.
32. Bang, B.G., Bang, F.B. and Foard, M.A. (1972). Lymphocyte Depression Induced in Chickens on Diets Deficient in Vitamin A and other Components. *Am. J. Pathol.* **68**, 147-162.
33. Lamb, A.J., Apiwatanaporn, P. and Olson, J.A. (1974). Induction of Rapid, Synchronous Vitamin A Deficiency in the Rat. *J. Nutr.* **104**, 1140-1148.
34. Anzano, M.A., Olson, J.A. and Lamb, A.J. (1980). Morphologic Alterations in the Trachea and the Salivary Gland Following the Induction of Rapid Synchronous Vitamin A Deficiency in the Rats. *Am. J. Pathol.* **98**, 717-732.
35. Davis, C.Y. and Sell, J.L. (1983). Effect of *all-trans* Retinol and Retinoic Acid Nutriture on the Immune System of Chicks. *J. Nutr.* **113**, 1914-1919.
36. Ongsakul, M., Sirisinha, S. and Lamb, A.J. (1985). Impaired Blood Clearance of Bacteria and Phagocytic Activity in Vitamin A Deficient Rats. *Proc. Soc. Exp. Biol. Med.* **178**, 204-208.
37. Madjid, B., Sirisinha, S. and Lamb, A.J. (1978). The Effect of Vitamin A and Protein Deficiency on Complement Levels in Rats. *Proc. Soc. Exp. Biol. Med.* **158**, 92-95.
38. Wolf, G. (1980). *Vitamin A.* In: Nutrition and the Adult: Micronutrients. (Alfin-Slater, R.B. and Kritchevsky, D., eds.), Plenum Press, New York, pp. 97-203.
39. Beaver, D.L. (1961). Vitamin A Deficiency in the Germ-Free Rat. *Am. J. Pathol.* **38**, 335-357.
40. Bieri, J.G., McDaniel, E.G. and Rogers, W.E. Jr. (1969). Survival of Germ-Free Rats without Vitamin A . *Science* **163**, 574-575.

41. DeLuca, H.F. and Suttie, J.W. (1970). The Fat Soluble Vitamins. University of Wisconsin Press, Madison.
42. Rojanapo, W., Olson, J.A. and Lamb, A.J. (1980). Biochemical and Immunological Characterization and the Synthesis of Rat Intestinal Glycoproteins Followings the Induction of Rapid Synchronous Vitamin A Deficiency. *Biochim. Biophys. Acta* **633**, 386-399.
43. Solari, R. and Kraehenbuhl, J.P. (1985). The Biosynthesis of Secretory Component and Its Role in the Transepithelial Transport of IgA Dimer. *Immunol. Today* **6**, 17-20.
44. Sirisinha, S., Darip M.D., Moongkarndi, P., Ongsakul M. and Lamb, A.J. (1980). Impaired Local Immune Response in Vitamin A-Deficient Rats. *Clin. Exp. Immunol.* **40**, 127-135.
45. Sirisinha, S. (1986). The Impact of Malnutrition on Intestinal Immunity. *Wrld. Pediatr. Child Care* **3**, 227-235.
46. Puengtomwatanakul, S. and Sirisinha, S. (1986). Impaired Biliary Secretion of Immunoglobulin A in Vitamin A-Deficient Rats. *Proc. Soc. Exp. Biol. Med.* **182**, 437-442.
47. Zile, M., Bunge, E.C. and DeLuca, H.F. (1977). Effect of Vitamin A Deficiency on Intestinal Cell Proliferation in the Rat. *J. Nutr.* **107**, 552-560.
48. Bhaskaram, P. and Sivakumar, B. (1986). Interleukin-1 in Malnutrition. *Arch. Dis. Childh.* **61**, 182-185.
49. Sirisinha, S. (1984). The Mucosal Immune System. *Asian Pacific J. Allergy Immunol.* **2**, 281-288.
50. Word, C.J., Crago, S.S. and Tomasi, T.B. (1986). Regulation of IgA Expression by Isotype-Specific T Cells and Soluble Binding Factors. *Ann. Rev. Microbiol.* **40**, 503-524.
51. Mark, D.A., Baliga, B.S. and Suskind, R.M. (1980). Vitamin A Deficiency and T-cell Immunocompetence. *Fed. Proc.* **39**, 341.
52. Nauss, K.M., Mark, D.A. and Suskind, R.M. (1979). The Effect of Vitamin A Deficiency on the in Vitro Cellular Immune Response of Rats. *J. Nutr.* **109**, 1815-1823.
53. Puengtomwatanakul, S., Verasertniyom, O., Ongsakul, M., Lamb, A.J. and Sirisinha, S. (1985). Effect of Retinoic Acid (RA) and Retinyl Palmitate (RP) Repletion on Lymphocytes of Vitamin A Deficient Rats. *Asian Pacific J. Allergy Immunol.* **3**, 67-72.
54. Majumder, M.S.I. and Abdus Sattar, A.K.M. (1987). Peyer's Patch Immune Function of Vitamin A Deficient Guineapigs. *Nutr. Rep. Intern.* **36**, 143-150.
55. Strober, W., Hanson, L.A. and Sell K.W. (1982). Recent Advances in Mucosal Immunity. Raven Press, New York.
56. McDermott, M.R., Mark, D.A., Befus, A.D., Baliga, B.S., Suskind, R.M. and Bienenstock, J. (1982). Impaired Intestinal Localization of Mesenteric Lymphoblasts Associated with Vitamin A Deficiency and Protein-Calorie Malnutrition. *Immunology* **45**, 1-5.
57. Takagi, H. and Nakano, K. (1983). The Effect of Vitamin A Depletion on Antigen-Stimulated Trapping of Peripheral Lymphocytes in Local Lymph Nodes of the Rat. *Immunology* **48**, 123-128.
58. Green, F. and Hayworth, B. (1980). Immunoglobulin-Containing Cells in Jejunal Mucosa of Children with Protein-Energy Malnutrition and Gastroenteritis. *Arch. Dis. Childh.* **55**, 380-383.
59. Watson, R.R., McMurray, D.N., Martin, P. and Reyes, M.A. (1985). Effect of Age, Malnutrition and Renutrition on Free Secretory Component and IgA in Secretions. *Am J. Clin. Nutr.* **42**, 281-288.
60. Sidell, N. and Ramsdell, F. (1988). Retinoic Acid Upregulates Interleukin-2 Receptors on Activated Human Thymocytes. *Cell. Immunol.* **115**, 299-309.
61. Isakov, N. (1988). Regulation of T-Cell-Derived Protein Kinase C Activity by Vitamin A Derivatives. *Cell. Immunol.* **115**, 288-298.
62. McGee, D.W. and McMurray, D.N. (1988). Protein Malnutrition Reduces the IgA Immune Response to Oral Antigen by Altering B-Cell and Suppressor T-Cell Functions. *Immunology* **64**, 697-702.
63. Lamont, A.G., Gordon, M. and Ferguson, A. (1988). T Lymphocyte Function in Protein-Deprived Mice. *Clin. Exp. Immunol.* **72**, 113-117.

TABLE 1 Simplified sequence of events for antibody production

Antigenic stimulation

⬇

Antigen uptake, processing and presentation
by antigen-presenting cells
(e.g. macrophages)

⬇

Lymphocyte activation

⬇

Lymphocyte proliferation

⬇

Differentiation into antibody-producing
plasma cells

⬇

Antibody secretion into blood circulation

⬇

---------- *

⬇

Transportation of antibody to its
destination
(e.g. external secretions)

* An event below the dotted line represents an additional step required for the production of secretory antibody. If the antigenic stimulation is initiated in the mucosa, following the activation step, these lymphocytes must recirculate to any mucosal site prior to becoming plasma cells in lamina propria.

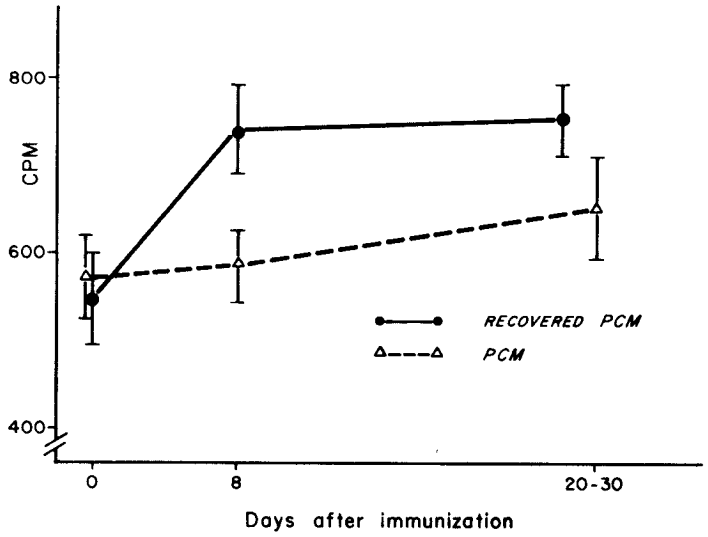

Fig. 1 Antibody response (mean ± SEM) of malnourished (PCM) and recovered PCM children to typhoid vaccine. The vaccine was given on day 0 and antibody to typhoid H antigen was determined by a modified radioimmunoprecipitation technique. (Taken from reference 6)

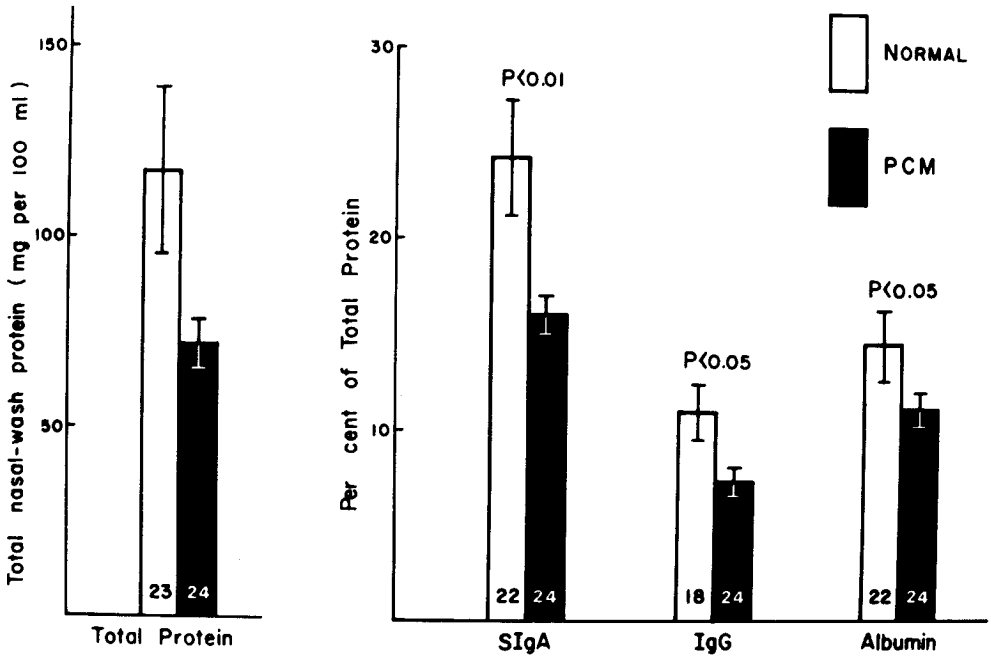

Fig. 2 Diagrammatic representations of total protein, secretory IgA (SIgA), IgG and albumin in the nasal washings of children with protein-calorie malnurition (PCM) and of well-nourished controls. Bars and lines represent mean ± SEM; the number at the base of each bar represents the number of children. Mean values were calculated from serial samples collected at intervals during the 84-day hospitalization period. (Taken from reference 10a)

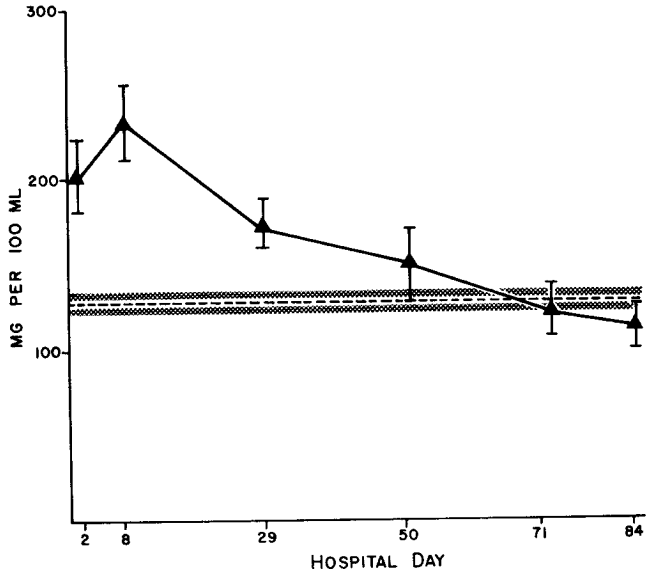

Fig. 3 Serum IgA concentrations in PCM children. Horizontal line and shaded area represent mean ± SEM from normal children of same age group and from the same geographical area. Differences from the mean of normal children at admission and on days 8 and 29 were statistically significant ($P<0.05$). (Taken from reference 6)

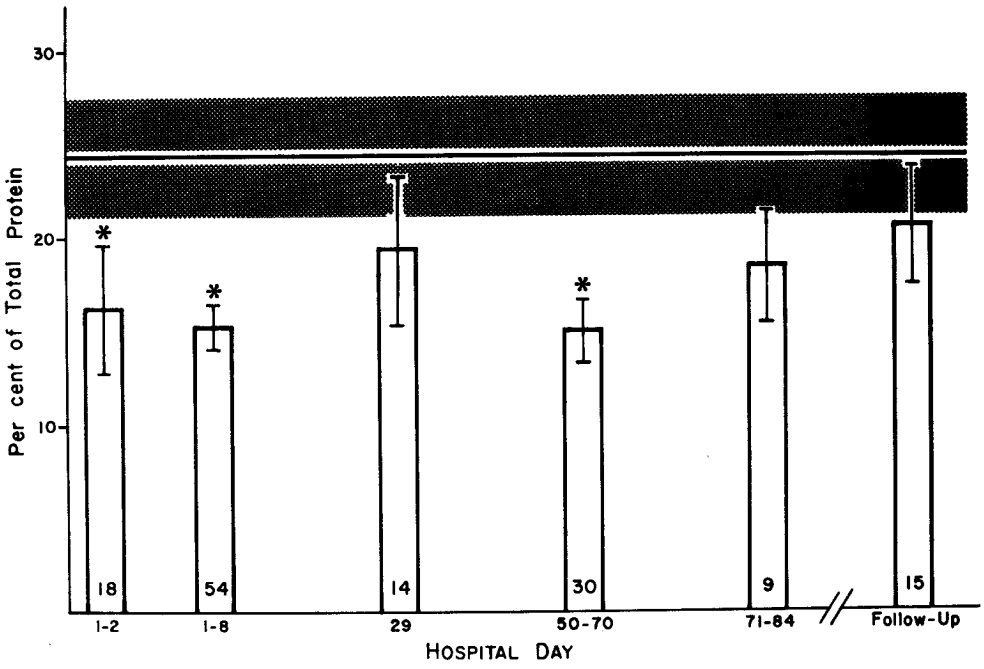

Fig. 4 Secretory IgA levels in the nasal washings of PCM children. Horizontal line and shaded area represent mean ± SEM (expessed as percentage of total nasal washing protein) from normal children of same age and from the same geographical area. Differences from the mean of normal children at admission and on days 1-2, 1-8 and 30-70 were statistically significant ($P<0.05$).

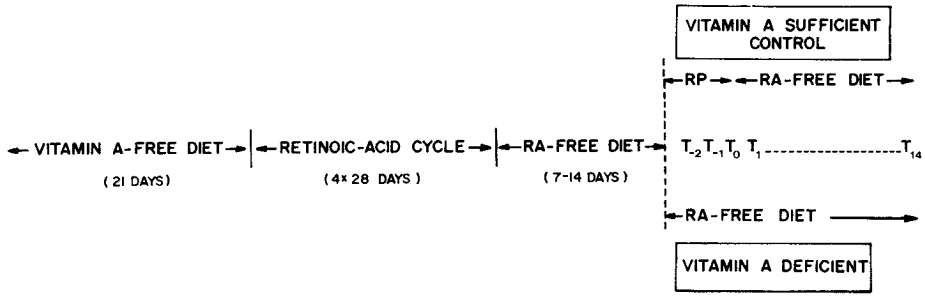

Fig. 5 Protocol for rearing vitamin A-deficient rats. Weanling albino rats were fed *ad libitum* a vitamin A-free diet for about 3 weeks before putting into retinoic acid cycle. The latter consisted of 18 days of retinoic acid (RA) supplementation and 10 days of RA deprivation. All vitamin A reserves were depleted after 4 such cycles. The animals were then fed *ad libitum* a diet containing low level RA (2 μg RA/g diet) for 1-2 weeks prior to being used in the experiment. Vitamin A-deficient rats were generated by a complete withdrawal of RA from the diet and were subsequently force-fed a RA-free diet until the end of the experiment (14 days). Vitamin A-sufficient controls were given (by stomach tube) 1000 μg of retinyl palmitate 2 days prior to the ultimate withdrawal of RA from the diet. Subsequently this control group was handled similarly to the deficient group.

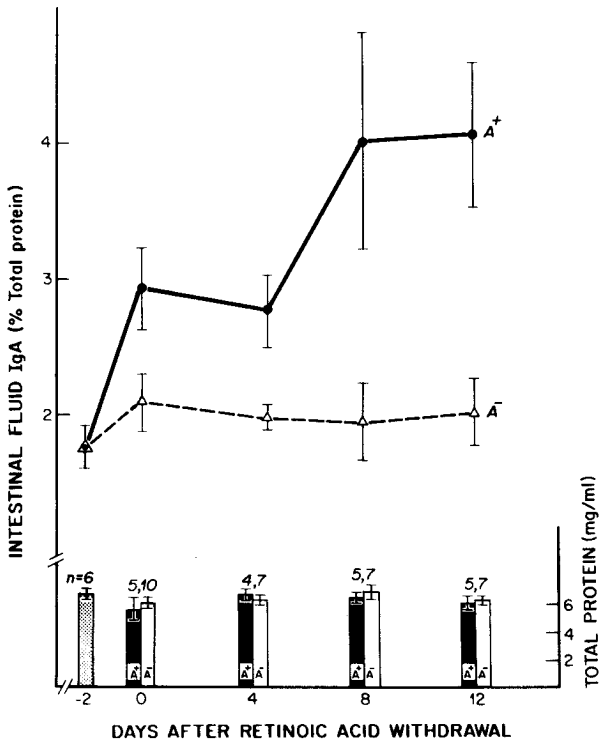

Fig. 6 Intestinal IgA levels (mean ± SEM) in vitamin A-deficient (A^-, o) and control (A^+, •) rats expressed as percentage of total intestinal fluid protein (bars and lines). (Taken from reference 44)

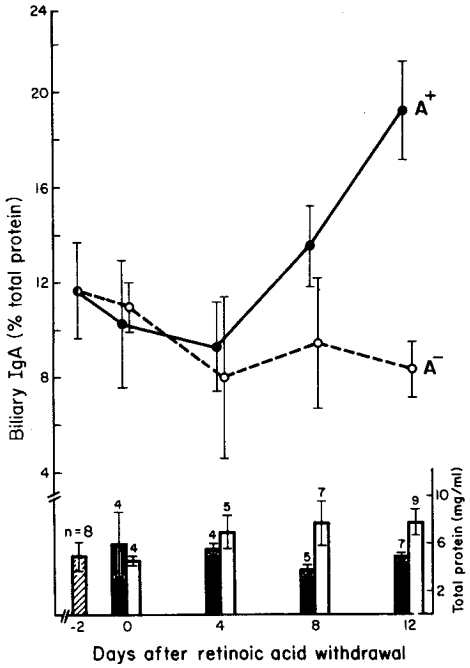

Fig. 7 Biliary IgA levels (mean ± SEM) in vitamin A-deficient (A^-, o) and control (A^+, •) rats expressed as percentage of total biliary protein (bars and lines). (Taken from reference 46)

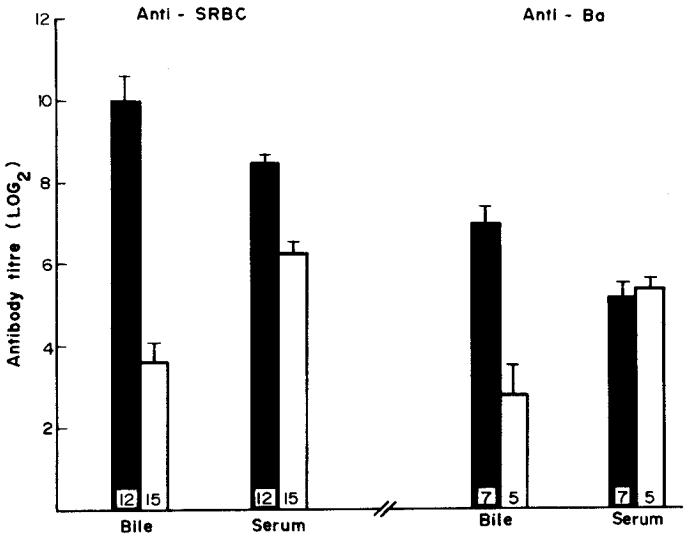

Fig. 8 The level of antibody in the bile and serum of A^- (□) and A^+ control (■) rats that received a single injection of T-dependent sheep red blood cells (SRBC) and T-independent *Brucella abortus* (Ba) antigens in the Peyer's patches. Bars and lines represent mean ± SEM; the number at the base of each bar represents the number of specimens analyzed. (Taken from reference 46)

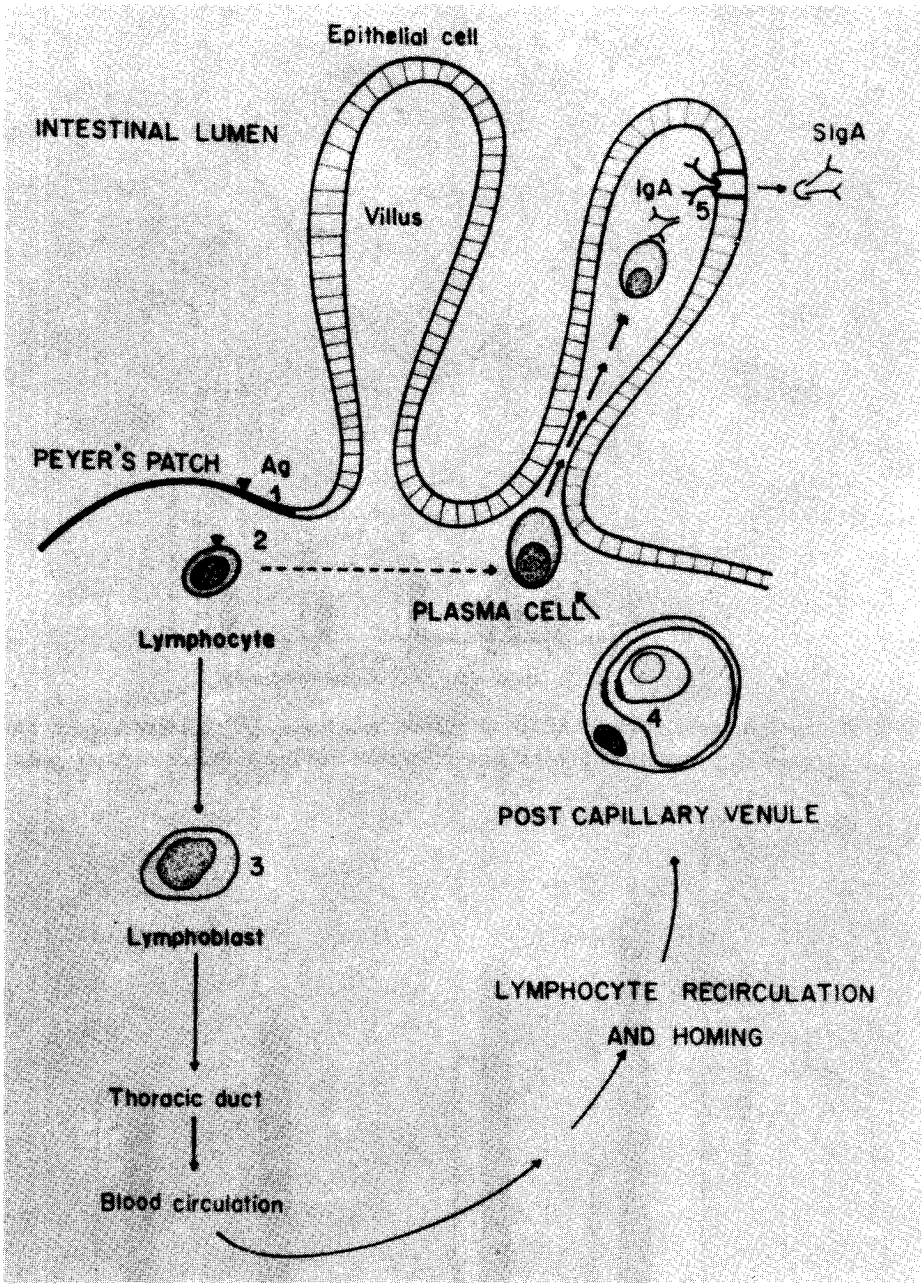

Fig. 9 Proposed mechanisms for the role of vitamin A in the development and functional integrity of secretory immune system. Vitamin A deficiency may interfer at different stages following appropriate antigenic stimulation, beginning with antigen uptake, processing and presentation by "M" cells of the Peyer's patches (Step 1), recognition of antigen by lymphocyte antigen receptors (Step 2), proliferation and differentiation of activated lymphocytes (Step 3), lymphocyte trapping and homing to mucosal sites (Step 4) and transportation of immunoglobulin across the mucous membrane to the external secretions (Step 5)