Zeitschrift für Ernährungswissenschaft

Journal of Nutritional Sciences · Journal des Sciences de la Nutrition

Band 18 Heft 4 Dezember 1979

Z. Ernährungswiss. **18**, 221–232 (1979)

ISSN 0044-264 X

Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Fed. Republic of Germany, and Institute of Hygiene and Laboratory Medicine, Municipal Hospital, Krefeld, Fed. Republic of Germany

Anti-infective properties of vitamin A*)

H. Hof and *C.-H. Wirsing*

With 6 figures and 1 table

(Received April 11, 1979)

Resistance to infection depends on sufficient and balanced nutrition. Among the numerous factors involved vitamin A seems to play an important role. 50 years ago, *Green* and *Mellanby* (1) called vitamin A the "anti-infective vitamin". The aim of the following review is to discuss if this term is indeed appropriate.

I. Influence of vitamin A deficiency

a) Effect on the mucosal penetration of infective organisms

Both in man and animals a definite vitamin A deficiency, characterized among other symptoms by xerophthalmia, will lead to an increased susceptibility to infections with various kinds of infective organisms, i.e. of bacterial, viral and parasitic origin (2–5). Rats with a definite vitamin A deficiency, which are held under conventional conditions, i.e. in microbially contaminated environments, will die because of infections. When deficient rats are reared, however, in germfree conditions, they will survive (6).

The alteration of the epithelial cell layer of mucosal surfaces (7) facilitates the penetration of germs which are the common inhabitants of these areas. Furthermore, the local immune system may also be impaired (8), so that penetration with subsequent infection may be promoted.

b) Effect on systemic defense mechanisms

Vitamin A deficiency does not only weaken local, mucosal defense mechanisms, but also interferes with systemic resistance. This is documented by experiments of *Lassen* (9) who showed that vitamin A deficient

*) Part of this report was presented at the 4th Fat Soluble Vitamin Group Meeting, Leeds (UK), April 1978

Table 1. Influence of a vitamin A free diet on body weight, spleen and thymus weight, and liver content of vitamin A (6 mice were used per group).

	Control mice	Mice fed a vitamin A free diet
Body weight (in g)	41.8 ± 1.2	37.5 ± 1.2
Spleen weight (in mg)	99 ± 9	105 ± 15
Thymus weight (in mg)	36 ± 3	39 ± 2
Vitamin A content per g of liver (in I.U.)	1480 ± 580	104 ± 42

rats were less resistant than normal controls to infection with *Salmonella* not only when these bacteria were given orally, but also when they were injected subcutaneously, thereby avoiding the mucosal passage.

It has been postulated (10) that a derangement of the reticuloendothelial system would be one reason for this susceptibility. An excellent model for investigations of the function of this mononuclear-phagocytic system is represented by the infection of the mouse with virulent germs of *Listeria monocytogenes*. The multiplication of these bacteria in spleen and liver during the first few days after intravenous infection mainly depends on the activity of this cell system (11).

Experiment 1

Male NMRI-mice (Central Institute for Laboratory Animals, Hannover, Fed. Rep. of Germany) were fed since weaning a vitamin A free diet (Vitamin-A-Mangeldiät, Intermast, Soest, Fed. Rep. of Germany). After 6 months the body weight began to decrease compared with normal control mice (table 1) which were fed a balanced diet (Basaldiät, Intermast, Soest, Fed. Rep. of Germany). The weights of the lymphatic organs, spleen and thymus, were not reduced (table 1). The chemical analysis of the liver contents of vitamin A performed with a dye reaction originally described by *Budowski* and *Bondi* (12) revealed a marked reduction in comparison to normal animals but no complete exhaustion (table 1).

This vitamin A deprivation did not exert any influence on the multiplication of intravenously injected cells of *L. monocytogenes* (Serovar 4 b). Approximately equal numbers of viable bacteria could be isolated from the spleens of vitamin A deprived mice and control animals (fig. 1). In another experiment the numbers of bacteria were determined both in spleens and livers. No significant difference was observed between both mouse groups (13).

Therefore it can be concluded that in spite of a marked reduction of vitamin A values, the mononuclear-phagocytic system was not impaired (13).

The specific immune system is likewise said to be affected by vitamin A deficiency. In hosts with manifest deficiency symptoms the lymphatic cell system is atrophic (8) and the antibody production is decreased. It has been questioned, however, whether these findings are directly related to the lack of vitamin A or are rather indirectly caused by the concomitant protein and calorie undernutrition (14).

Experiment 2

The vitamin A deprived mice (table 1) and normal control mice were injected intraperitoneally with 4×10^8 sheep erythrocytes. On several days

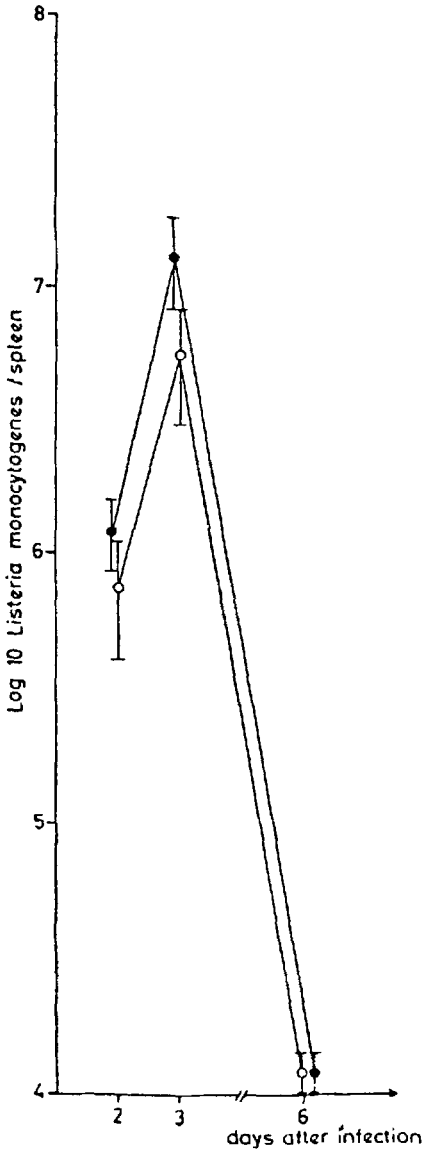

Fig. 1. Influence of vitamin A deprivation on infection of mice with *Listeria monocytogenes* (L.m.) ○ control mice fed a balanced diet: infected with 2.5×10^3 viable cells of L.m. ● mice fed a vitamin A free diet for 6 months before infection with L.m.

thereafter the numbers of antibody producing cells per spleen were tested with the plaque-method of *Jerne* (15) and a modified plaque technique (16, 17). Both the direct plaque forming cells (DPFC) (representing mainly the 19S-antibody producing cells) and the indirect plaque forming cells (IPFC) (representing the 7S-antibody producing cells) were found in approximately the same numbers in both mouse groups (fig. 2) (13).

This demonstrates that antibody production is not impaired if vitamin A is only reduced but not completely lacking.

Both results indicate that the defence mechanisms of the host are not appreciably altered in such a state of marginal vitamin A deficiency which is seen today in many different human diseases (18–21), and is much more common than manifest deficiency which is even rarely seen in peoples of developing countries (22).

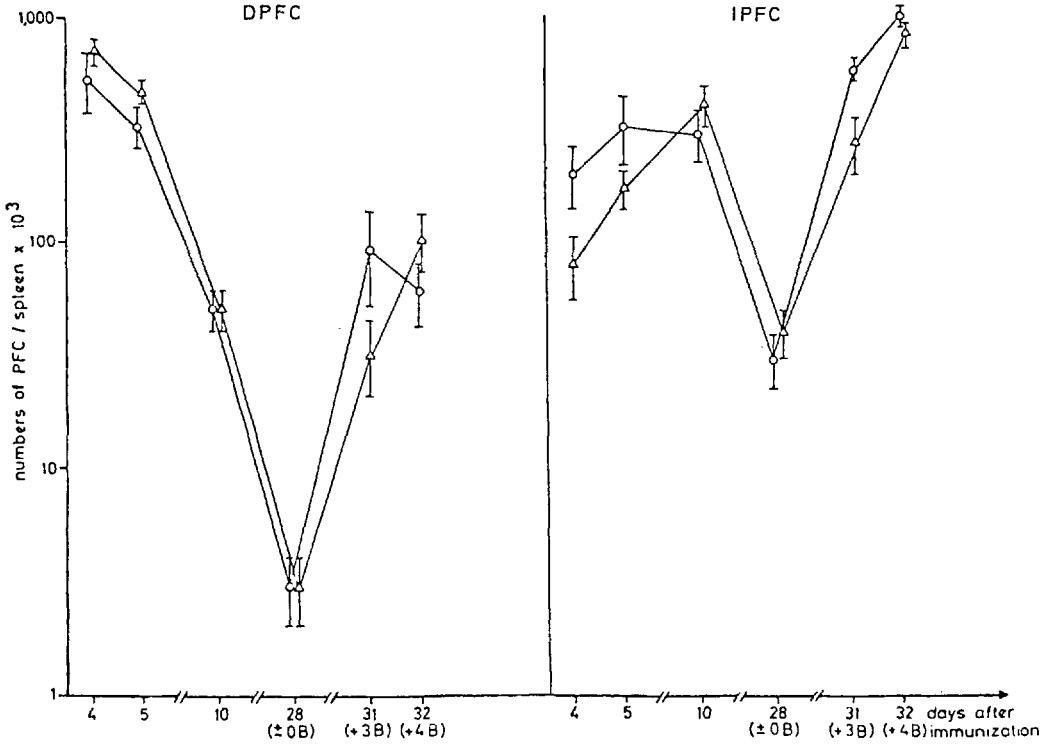

Fig. 2. Influence of vitamin A deprivation on the antibody production of mice against sheep erythrocytes (SE) as determined by the number of direct plaque forming cells (DPFC) and indirect plaque forming cells (IPFC) per spleen. ○ control mice fed a balanced diet. △ mice fed a vitamin A free diet for 6 months before immunization. All mice were treated intraperitoneally (i.p.) with 4×10^8 SE on day 0. Booster injection (B) of 4×10^8 SE i.p. on day 28.

II. Influence of massive intake of vitamin A

The fact that a serious vitamin A deficiency will damage the natural barrier against germ penetration does not necessarily support the reverse conclusion that large doses of vitamin A, given additionally to a diet

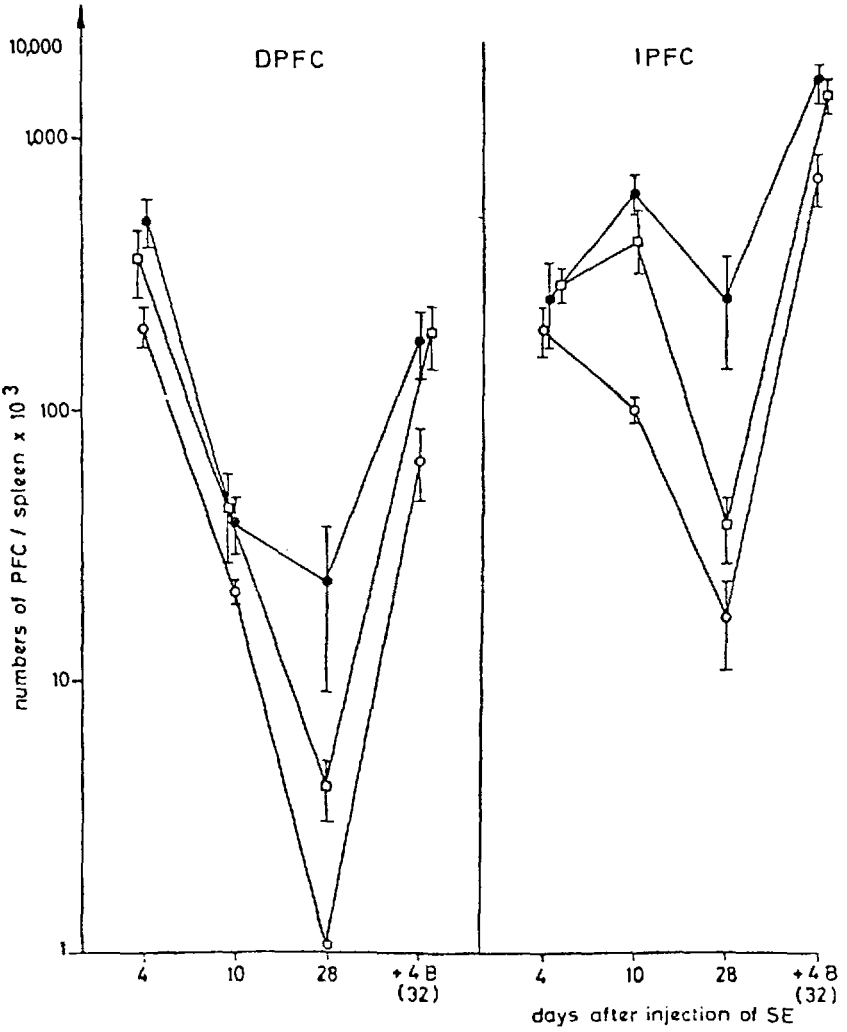

Fig. 3. Adjuvant activity of vitamin A palmitate on antibody production of mice against sheep erythrocytes (SE) as determined by the number of direct plaque forming cells (DPFC) and indirect plaque forming cells (IPFC) per spleen. ○ control mice treated intraperitoneally (i.p.) with 4×10^8 SE on day 0. Booster injection (B) of 4×10^8 SE on day 28. ● additionally to primary immunization with SE treated i.p. with vitamin A palmitate ($4 \times 7{,}500$ I.U.) on days -3, -2, -1 and 0. □ additionally to primary immunization with SE treated orally with vitamin A palmitate ($4 \times 15{,}000$ I.U.) on days -3, -2, -1 and 0 (five to six mice were used per point).

containing already adequate amounts of vitamin A, will further strengthen the mucosal line of defence.

Other systemic defence mechanisms may, however, be influenced by vitamin A treatment.

For example, vitamin A activates the lymphatic cell system (23). Furthermore it is an immunological adjuvant, at least concerning the humoral immune reaction, i.e. the antibody production (24). The adjuvant activity is fairly dependent on the pharmacological form of vitamin A. Retinol and retinal seem to be the most effective preparations (25). In most experiments, reported so far, retinyl-palmitate has been administered (26–28). Indeed, this preparation stimulates the immune reaction to non-related antigens such as sheep erythrocytes (29).

Experiment 3

The numbers of antibody producing cells per spleen after intraperitoneal injection of 4×10^8 sheep erythrocytes in normal female mice were compared with those of mice treated for 4 days before immunization with retinyl-palmitate (A-Vicotrat "aquosum"®, Heyl u. Co. Berlin, Fed. Rep. of Germany). 7,500 I.U. of vitamin A were given daily by the intraperitoneal route or 15,000 I.U. orally by means of a stomach tube, respectively.

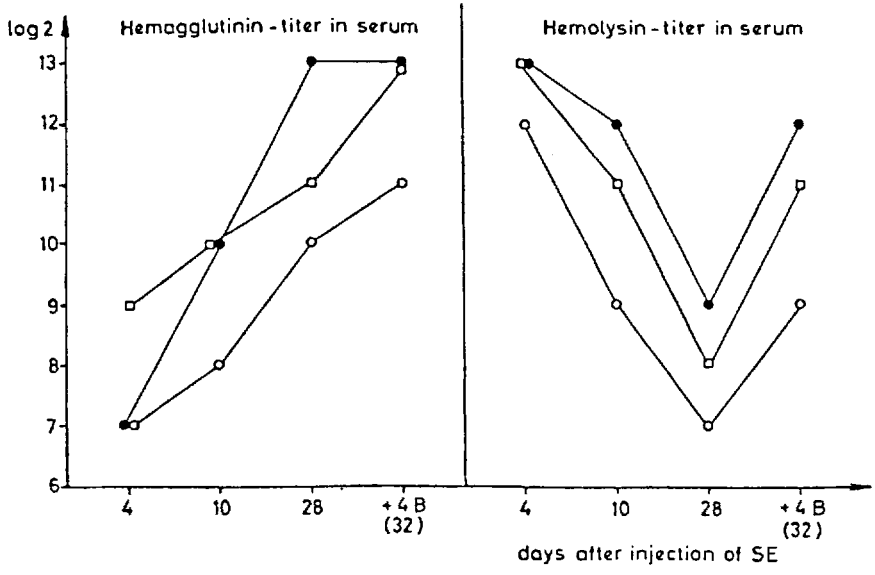

Fig. 4. Adjuvant activity of vitamin A palmitate on antibody production of mice against sheep erythrocytes (SE) as determined by the serum antibody titers. ○ control mice treated intraperitoneally (i.p.) with 4×10^8 SE on day 0. Booster injection (B) of 4×10^8 SE on day 28. ● additionally to primary immunization with SE treated i.p. with vitamin A palmitate (4 × 7,500 I.U.) on days -3, -2, -1 and 0. □ additionally to primary immunization with SE treated orally with vitamin A palmitate (4 × 15,000 I.U.) on days -3, -2, -1 and 0 (five to six mice were used per point).

In both conditions the numbers of antibody producing cells were increased (fig. 3). In the serum elevated antibody titers were found (fig. 4), too.

In comparison to other immunological adjuvants, such as killed *Bordetella pertussis* cells, the advantage of retinyl-palmitate is that this drug is not only effective when given parenterally but also on oral application (29).

Experiment 4

When instead of retinyl-palmitate another vitamin A preparation, i.e. retinyl-acetate (Vitamin A-acetat, Merck, Darmstadt, Fed. Rep. of Germany) was administered, an adjuvant effect was only seen in orally treated mice. Intraperitoneal application of this preparation was not effective (fig. 5).

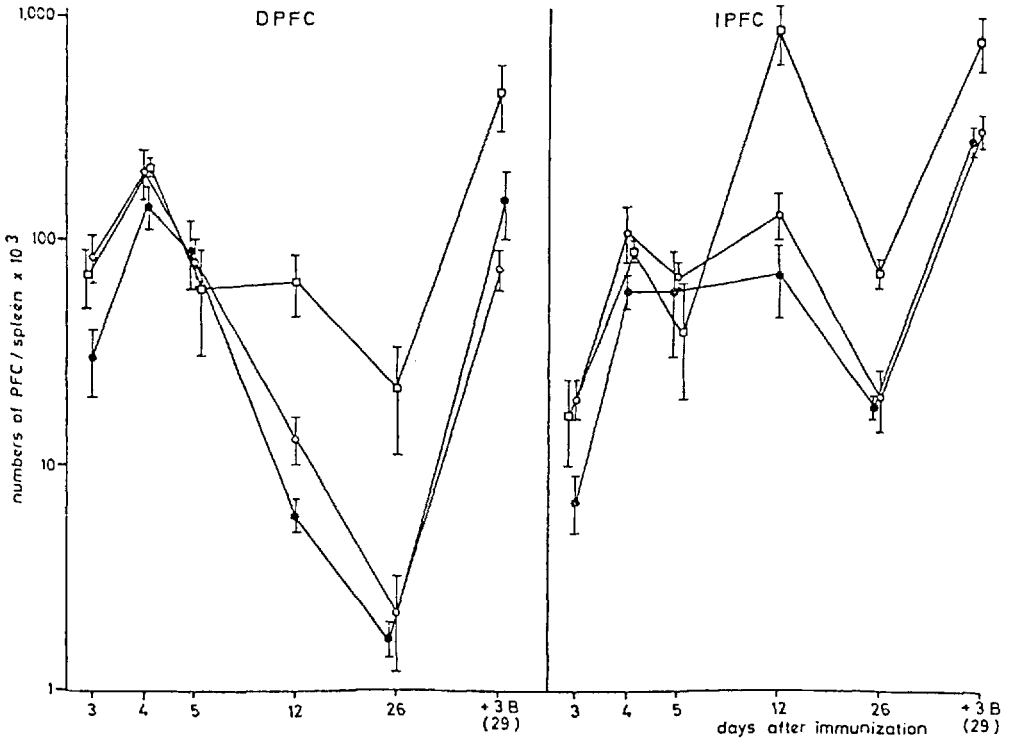

Fig. 5. Adjuvant activity of vitamin A acetate on antibody production of mice against sheep erythrocytes (SE) as determined by the number of direct plaque forming cells (DPFC) and indirect plaque forming cells (IPFC) per spleen. ○ control mice treated intraperitoneally (i.p.) with 4×10^8 SE on day 0. Booster injection (B) of 4×10^8 SE on day 26. ● additionally to primary immunization with SE treated i.p. with vitamin A palmitate ($4 \times 7{,}500$ I.U.) on days -3, -2, -1 and 0. □ additionally to primary immunization with SE treated orally with vitamin A palmitate ($4 \times 15{,}000$ I.U.) on days -3, -2, -1 and 0 (five to six mice were used per point).

A prolongation of the antibody production rather than an acceleration seems to be typical for the adjuvant activity of vitamin A.

It has to be emphasized here that all these results obtained, either with retinyl-palmitate or -acetate, were induced by rather high doses of vitamin A which produced even severe signs of hypervitaminosis. Lower doses, however, were less effective.

The reports about the effect of vitamin A on cell-mediated, i.e. T-lymphocyte-mediated, immune reactions are at present contradictory. In vitamin A treated animals the survival of heterologous skin transplants was reduced (30–32). In principle, this could be interpreted as an enhanced cell mediated immune reaction leading to an accelerated transplant rejection. Since autologous skin transplants were also rejected in hypervitaminotic animals, because of defect of neo-vascularisation of the transplant (30), non-immunological mechanisms may in this particular case also account for this effect. Retinoic acid seems to be a specific adjuvant for induction of cytotoxic T-lymphocytes but not a general T-lymphocyte mitogen or adjuvant (33). On the other hand there are reports of *in vitro* experiments indicating a decreased T-lymphocyte activity of hypervitaminotic animals (28).

An effect of vitamin A on phagocytosis also seems possible, since histologically hypertrophy of the reticuloendothelial system is seen (34). Furthermore, the instability of the lysosomal membranes (35) could possibly facilitate the killing activity of the phagocytic cells. But a definite proof of such an increased function has not yet been communicated. Neither the clearance rate of antigen from the blood stream (36, 37) nor the antigen catabolism within the cells (38) was altered in hypervitaminotic test animals.

Eventually, it could be assumed that the finding of *Cohen* and *Elin* (37) of a prolonged survival time of *Listeria*-infected animals after treatment with vitamin A may have been due, at least partially, to an enhanced macrophage activity.

For testing this possibility, the enumeration of bacteria within the spleen during the first few days after infection with a sublethal dose of *Listeria* (11) seems more convenient than the recording of mortality rates after application of high infective doses.

Experiment 5

Each day on 4 consecutive days 14,000 I.U. of vitamin A (Vitamin A-acetat, Merck, Darmstadt, Fed. Rep. of Germany) were administered to each mouse by means of a stomach tube. Thereafter, these mice and untreated controls were infected with 4.6×10^3 viable cells of *L. monocytogenes* (serovar 4 b). The numbers of bacteria recovered from the spleen few days later were markedly reduced in vitamin A treated animals (fig. 6).

Evidently, the multiplication of bacteria in the spleen had been effectively reduced. This finding supplies strong evidence that the macrophage system may be stimulated by vitamin A.

The complement system may be affected by vitamin A as well, but the results obtained so far do not allow decisive conclusions. *In vitro* the complement activity was suppressed, whereas *in vivo* – after prolonged

vitamin A application – complement levels were increased at least sometimes, in man (39) and in rats (40), but not in guinea pigs (39).

All reports mentioned so far possibly argue for beneficial consequences of vitamin A treatment on the defence mechanisms.

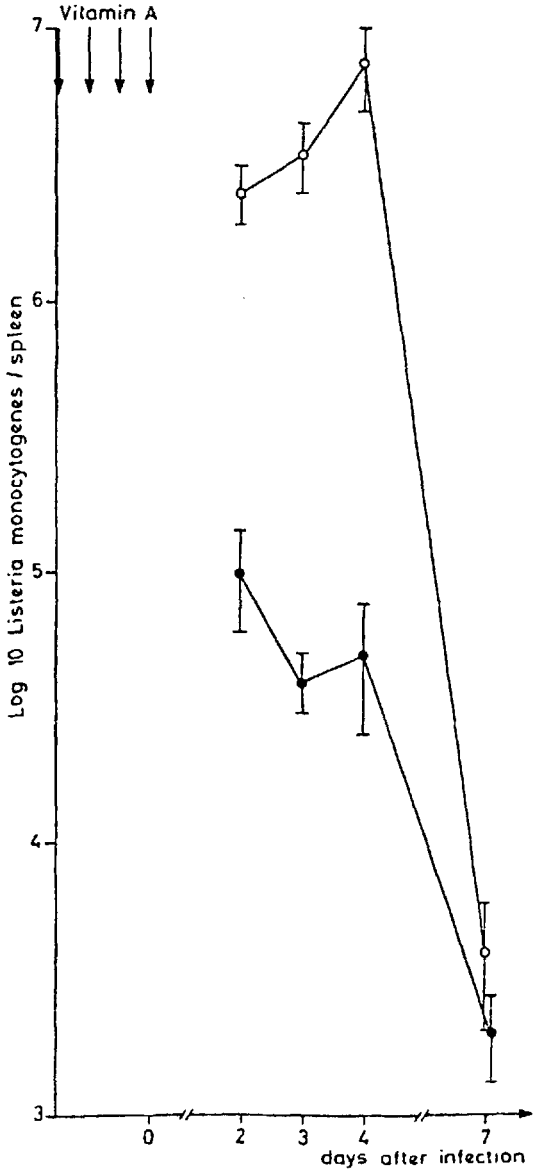

Fig. 6. Influence of vitamin A high dosage on infection of mice with *Listeria monocytogenes* (L.m.). ○ control mice, infected intravenously (i.v.) with 4.6×10^3 viable cells of L.m. on day 0. ● mice which had received orally $4 \times 14{,}000$ I.U. of vitamin A acetate before infection with L.m.

In some instances vitamin A may have, however, adverse consequences. The interferon system is impaired. Although it is generally assumed that glycoprotein synthesis is increased by vitamin A (41), the production of interferon which is a glycoprotein seems to be reduced (42). And also the *in vitro* activity of interferon is blocked (43).

So, conclusively one can say that a high dose of vitamin A may indeed have some influence on certain resistance mechanisms. This does not allow at this time, however, an enthusiastic recommendation of this vitamin for practical use against infections, because:

- the beneficial effect seems to depend on the pharmacological form and route of application,
- the beneficial effects are achieved only by high doses approaching hypervitaminotic amounts,
- the consequences of high intake of vitamin A are detrimental for some resistance mechanisms.

Summary

1. Six months after feeding a vitamin A free diet the liver content of mice was markedly reduced but not yet completely exhausted. These vitamin A deprived mice were either immunized with sheep erythrocytes or infected with *Listeria monocytogenes*. In comparison to normal control mice no significant difference was observed. This indicates that neither the immune system nor the mononuclear-phagocytic system was involved.

2. Mice treated with a high dose of vitamin A showed increased antibody production against sheep erythrocytes and also increased resistance against infection with *L. monocytogenes*. These experimental findings indicate a stimulatory effect on the immune system and the mononuclear phagocytic system. As a conclusion it is deduced that the term "anti-infective vitamin" does not hold absolutely true for vitamin A, although certain anti-infective properties cannot be denied.

Zusammenfassung

1. Sechs Monate nach Fütterung einer Vitamin-A-freien Diät erwies sich der Gehalt an diesem Vitamin in der Leber von Mäusen als erheblich reduziert, wenn auch nicht als völlig erschöpft. Diese an Vitamin A verarmten Mäuse wurden entweder mit Schaferythrozyten immunisiert oder mit *Listeria monocytogenes* infiziert. Beim Vergleich mit Kontrollmäusen wurde kein signifikanter Unterschied beobachtet. Das weist darauf hin, daß weder das Immunsystem noch das System der mononukleären Phagozyten betroffen wurde.

2. Mäuse, die mit hohen Vitamin-A-Dosen behandelt wurden, zeigten eine erhöhte Antikörperproduktion gegen Schaferythrozyten und auch eine verbesserte Resistenzlage gegenüber *L.-monocytogenes*-Infektionen. Diese Versuchsergebnisse weisen auf einen stimulierenden Effekt von Vitamin A auf das Immunsystem und das System der mononukleären Phagozyten hin. Zusammenfassend läßt sich sagen, daß der Ausdruck „antiinfektives Vitamin" nicht ganz auf Vitamin A zutrifft, wenn sich auch gewisse infektionsverhütende Eigenschaften nicht in Abrede stellen lassen.

References

1. *Green, H. N., E. Mellandby:* Vitamin A as an anti-infective agent. Brit. Med. J. **1928/II**, 691. – 2. *Aycock, W. L., G. E. Lutman:* Vitamin deficiency as an epidemiologic principle. Amer. J. Med. **208**, 389–406 (1944). – 3. *Schneider, H. A.:* Nutrition and resistance to infection. The strategic situation. Vitam. Horm. **4**, 35–70 (1946). – 4.

Clark, P. E., L. S. McLung, H. Pinkerton, W. H. Price, H. A. Schneider: Influence of nutrition in experimental infection. Bacteriol. Rev. **13,** 99–134 (1949). – 5. *Hof, H.:* Vitamin A, das „anti-infektive Vitamin?". Münch. Med. Wschr. **118,** 1485–1488 (1976). – 6. *Bieri, J. G., E. G. McDaniel, W. E. Rogers:* Survival of germfree rats without vitamin A. Science **163,** 574–575 (1969). – 7. *Wolbach, S. B., P. R. Howe:* Tissue changes following deprivation of fat soluble A vitamin. J. Exp. Med. **42,** 753–777 (1925). – 8. *Bang, B. G., F. B. Bang, M. A. Foard:* Lymphocyte depression induced in chickens on diets deficient in vitamin A and other components. Amer. J. Pathol. **68,** 147–162 (1972). – 9. *Lassen, H. C. A.:* Parathyphoid infections in avitaminotic rats with special reference to vitamin A deficiency (Copenhagen 1931). – 10. *Lawler, H. J.:* The relation of vitamin A to immunity to Strongyloides infection. Amer. J. Hyg. **34,** D, 65–72 (1941). – 11. *Mackaness, G. B.:* The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J. Exp. Med. **129,** 973–992 (1969). – 12. *Budowski, P., A. Bondi:* Determination of vitamin A by conversion to anhydrovitamin A. Analyst **82,** 751–760 (1957). – 13. *Hof, H., P. Emmerling, H. Finger, C. Wirsing, E. Karle, W. Reiners:* Einfluß von latentem Vitamin-A-Mangel der Maus auf die Bildung humoraler Antikörper gegen Schaferythrozyten und auf die Infektionsresistenz gegen *Listeria monocytogenes.* Zentralbl. Bakteriol. (Orig. A), **237,** 310–317 (1977). – 14. *Krishnan, S., U. N. Bhuyan, G. P. Talwar, V. Ramalingaswami:* Effect of vitamin A and Protein-calorie undernutrition on immune responses. Immunology **27,** 383–392 (1974). – 15. *Jerne, N. K., A. A. Nordin, C. Henry:* The agar plaque technique for recognizing antibody-producing cells. In: Cell-bound antibodies, *Amos, B.,* and *Koprowski, H.* (eds.) (Philadelphia 1963). – 16. *Wortis, H. H., R. B. Taylor, D. W. Dresser:* Antibody production studied by means of the LHG assay. I. The splenic response of CBA mice to sheep erythrocytes. Immunology **11,** 603–616 (1966). – 17. *Finger, H., P. Emmerling, H. Tusch, W. Bredt:* Einfluß von *Bordetella pertussis* auf das lymphatische Gewebe von Mäusen. III. Die Beeinflussung der Kinetik der Antikörperbildung gegen Schaferythrozyten durch *Bordetella pertussis.* Z. Immunitätsforschg. Immunobiol. **136,** 268–284 (1968). – 18. *Harris, A. D., T. Moore:* Vitamin A in infective hepatitis. Brit. Med. J. **1947/I,** 553. – 19. *Gopolan, C., P. S. Ventatachalam, B. Bharani:* Studies of vitamin A deficiency in children. J. Clin. Nutr. **8,** 833–842 (1960). – 20. *Smith, F. R., W. S. De Goodman:* The effects of diseases of the liver, thyroid, and kidneys on the transport of vitamin A in human plasma. J. Clin. Invest. **50,** 2426–2436 (1971). – 21. *Smith, F. R., B. A. Underwood, C. R. Denning, A. Varma, W. S. De Goodman:* Depressed plasma retinol-binding protein levels in cystic fibrosis. J. Lab. Clin. Med. **80,** 423–433 (1972). – 22. *Venkataswamy, G., K. A. Krishnamurthy, P. Chandra, S. A. Kabir, A. Pirie:* A nutrition rehabilitation centre for children with xerophtalmia. Lancet **1976/I,** 1120. – 23. *Taub, R. N., A. R. Krantz, D. W. Dresser:* The effect of localized injection of adjuvant material on the draining lymph node. I. Histology. Immunology **18,** 171–186 (1970). – 24. *Dresser, D. W.:* Adjuvanticity of vitamin A. Nature 217, 527–529 (1968). – 25. *Charabati, M. F., D. S. McLaren:* Action des différentes formes actives de la vitamine A sur le mécanisme immunitaire chez le rat. Experientia **29,** 343–344 (1973). – 26. *Spitznagel, J. K., A. C. Allison:* Mode of action of adjuvants: retinol and other lysosome-labilizing agents as adjuvants. J. Immunol. **101,** 119–127 (1970–. – 27. *Cohen, B. E., I. K. Cohen:* Vitamin A: adjuvant and steroid antagonist in the immune response. J. Immunol. **111,** 1376–1380 (1973). – 28. *Lapin, V., W. Rella, H. Wrba:* Effect of hypervitaminosis A on immunocompetence in rats. Österr. Z. Onk. **1,** 96–99 (1974). – 29. *Hof, H., H. Finger:* Steigerung der Immunantwort gegenüber nichtverwandtem Antigen durch oral verabreichte Adjuvantien. Der Krankenhausarzt **49,** 93–96 (1976). – 30. *Nelken, D., E. Pick, M. Gabriel, W. Bitterman, J. H. Boss:* Vitamin A-induced rejection of autografts and homografts. Nature **205,** 1022 (1965). – 31. *Floersheim, G. L., W. Bollag:* Accelerated rejection of skin homografts by vitamin A acid. Transplantation **14,** 564–567 (1972). – 32. *Felix, E. L., B. Loyd, M. E. Cohen:* Inhibition of the growth and development of a transplantable murine melanoma by vitamin A. Science **189,** 886–888 (1975). – 33.

Dennert, G., R. Lotan: Effects of retinoic acid on the immune system: stimulation of T killer cell induction. Eur. J. Immunol. **8,** 23–29 (1978). – 34. *Laubmann, W.:* Über Schädigungen durch Vitamin A. II. Histologische Veränderungen an Rattenorganen. Klin. Wschr. **12,** 1171–1174 (1933). – 35. *Dingle, J. T., J. A. Lucy:* Vitamin A, carotenoids and cell function. Biol. Rev. **40,** 422–461 (1965). – 36. *Uhr, J. A., G. Weissman, L. Thomas:* Acute hypervitaminosis A in guinea pigs. II. Effects on delayed-type hypersensitivity. Proc. Soc. Exp. Biol. Med. **112,** 287–291 (1963). – 37. *Cohen, B. E., R. Elin:* Vitamin A-induced nonspecific resistance to infection. J. Infect. Dis. **129,** 597–600 (1974). – 38. *Stark, J. M.:* Rate of antigen catabolism and immunogenicity of ^{131}J BGG in mice. II. The immunogenicity of ^{131}J BGG and adjuvant action after alteration of the metabolic rate by various means. Immunology **19,** 457–464 (1970). – 39. *Orynich, R. E., G. H. Wirtz:* Vitamin A: probe of immune complement reaction. II. Enhancement of human complement hemolysis. Immunochemistry **8,** 113–119 (1971). – 40. *Billitteri, A., Y. Raoul:* Action de la vitamine A sur le complément in vitro et in vivo. C. R. Soc. Biol. (Paris) **159,** 1882–1886 (1965). – 41. *De Luca, L., S. H. Yuspa:* Altered glycoprotein synthesis in mouse epidermal cells treated with retinyl acetate *in vitro.* Exp. Cell. Res. **86,** 106–110 (1974). – 42. *Blalock, J. E., G. E. Gifford:* Suppression of interferon production by vitamin A. J. Gen. Virol. **32,** 143–147 (1976). – 43. *Blalock, J. E., G. E. Gifford:* Inhibition of interferon action by vitamin A. J. Gen. Virol. **29,** 315–324 (1975).

Für die Verfasser:

Dr. *H. Hof,* Institut für Hygiene und Mikrobiologie, Universität Würzburg, 8700 Würzburg