larger values of these parameters, the transition becomes continuous. This analysis accounts for both the discrete transition observed in a 50 percent acetone-water mixture and the continuous transition in pure water. The former corresponds to a low τ value, whereas the latter corresponds to a high τ value. Although only the electric force was considered in describing the phase transition, other factors may play a role. The effects of inhomogeneous distributions of ions, currents, and solvent composition within the gel and electrochemical reactions occurring at the electrodes need to be considered for a complete understanding of the phenomena.

The discrete volume transition of the gel induced by an electric field can be used to make switches, memories, and mechanochemical transducers. For example, ionic gels controlled by coordinated signals from a microcomputer may be used for an artificial muscle. It may also be possible to store two- or three-dimensional images by using the local collapse and swelling of the gel.

TOYOICHI TANAKA
IZUMI NISHIO
SHAO-TANG SUN
SHIZUE UENO-NISHIO
Department of Physics and *Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge 02139*

References and Notes

1. T. Tanaka, *Sci. Am.* **244**, 124 (January 1981); *Phys. Rev. Lett.* **40**, 820 (1978).
2. ———, D. Fillmore, I. Nishio, S.-T. Sun, G. Swislow, A. Shah, *ibid.* **45**, 1936 (1980).
3. T. Tanaka, S. Ishiwata, C. Ishimoto, *ibid.* **38**, 771 (1977); T. Tanaka, *Phys. Rev. A* **17**, 763 (1978).
4. K. Dusek and D. Patterson, *J. Polym. Sci. Part A-2* **6**, 1209 (1968).
5. P. J. Flory, *Principles in Polymer Chemistry* (Cornell Univ. Press, Ithaca, N.Y., 1953).
6. We thank G. Swislow and I. Ohmine for their critical reading of the manuscript. This work has been supported by the Office of Naval Research under grant N00014–80–C–0500.

4 January 1982; revised 1 June 1982

Gestational Zinc Deprivation in Mice: Persistence of Immunodeficiency for Three Generations

Abstract. *Pregnant Swiss Webster mice were fed a diet moderately deficient in zinc from day 7 of gestation until parturition. Offspring of these mice showed depressed immune function through 6 months of age. In addition, the second and third filial generations, all of which were fed only the normal control diet, continued to manifest reduced immunocompetence, although not to the same degree as in the first generation.*

A number of nutritional factors modulate immunological function (*1*). Although initial interest in this subject was generated by the immunodeficiency observed in protein-malnourished children, it is now recognized that such individuals suffer from a wide range of nutritional deficiencies (*2*). Attention has thus become focused on the role of specific nutrients in immune responsiveness. In particular, much has been learned about the influence of dietary zinc on the maintenance of immunological function in adult animals (*3*). Zinc deficiency has been associated with impaired immune responses, particularly those mediated by T lymphocytes (*4*), and has been shown to influence the course of infectious (*5*), neoplastic (*6*), and autoimmune disease (*7*). Similar observations have been made in humans (*8*). However, relatively little is known about the role of zinc in the ontogeny of immunity. We previously showed that when mice are deprived of zinc during the suckling period, their ability to generate an effective immune response is markedly compromised (*9*). Indeed, mice only marginally deficient in zinc show decreased responses to mitogens and sheep red blood cells (SRBC's) and highly altered serum immunoglobulin profiles (*10*). We now report that moderate deprivation of zinc during prenatal life alone is also associated with a depressed plaque-forming cell (PFC) response to SRBC inoculation and with impaired development of serum immunoglobulins. In addition, these defects in immunological function persist into the second and third generations.

Pregnant young adult Swiss Webster mice were fed a zinc-deficient diet or a control diet ad libitum from day 7 of gestation (the beginning of pregnancy was designated day 0) to term. The biotin-fortified, egg white–based diet (*7*) contained 5 ppm zinc and the control diet 100 ppm zinc. The choice of these dietary levels of zinc was based on our previous studies in BALB/c and N:NIH(S) mice (*7*, *9*). Moderate deficiency was defined as a reduction in serum zinc to levels 60 to 70 percent of those in control mice (*9*). Because zinc deficiency is known to cause inanition (*10*), which also may alter immune function (*11*), pair-fed controls were also studied. During the period of zinc deprivation all the mice were maintained under conditions designed to reduce extraneous zinc contamination (*7*, *10*). At parturition, litters were culled to five pups and all dams and offspring were returned to the control diet, which they consumed until the end of the experiment. The PFC response to SRBC's and the concentration of immunoglobulin M (IgM) in serum were assessed in some offspring at 6 weeks, 10 weeks, and 6 months of age; the remainder of the offspring were mated (brothers to sisters) at 10 weeks of age to produce an F_2 generation. Because a consequence of zinc deprivation during pregnancy is impaired development of the mammary gland and reduced lactational ability (*12*), pups from zinc-deprived dams were cross-fostered to control dams and pups from control dams were cross-fostered to zinc-deficient dams. The F_2 and F_3 offspring were culled to five pups and studied at the same ages as the F_1 offspring or bred at 10 weeks of age. All F_1, F_2, and F_3 mice were thus fed the control diet throughout their lives. The only period of zinc deprivation was during gestation of the F_1 offspring.

Overt signs of zinc deficiency, such as alopecia and exfoliative dermatitis, were not observed in the zinc-deprived dams; nonetheless, other features were observed, such as decreased food intake, fewer and smaller offspring, and slightly higher neonatal mortality rates (*13*). Analysis of maternal plasma on day 17 of gestation indicated that a significant degree of zinc deficiency had been achieved, with the controls fed ad libitum and the pair-fed controls having zinc concentrations of 106.4 and 109.7 μg per 100 ml of plasma, respectively, compared to 58.0 μg per 100 ml in dams fed the 5 ppm zinc diet. These zinc concentrations were not as low as those generally seen in animals fed a diet nearly devoid of zinc (*14*), indicating that a moderate deficiency was achieved. No further differences were observed between plasma zinc levels in any groups at any age. Thus, the postnatal effects of zinc depletion during gestation were due to a defect in development rather than to a persistence of low plasma zinc.

The F_1 offspring of zinc-deficient dams had no detectable IgM at 6 and 10 weeks of age (Fig. 1A). By 6 months of age, significant amounts of IgM had appeared, but the concentrations were still below those of both control groups.

0036-8075/82/1029-0469$01.00/0

Cross-fostering of zinc-deprived pups to control dams did little to improve the low levels of serum IgM. Cross-fostering of control pups to zinc-deprived dams resulted in lower IgM concentrations, but these levels were substantially higher than those of zinc-deprived pups allowed to suckle their natural mothers, indicating the importance of prenatal zinc status and maternal lactational performance for normal immune ontogeny.

Aberrant serum IgM patterns in pups from zinc-deprived females persisted in the F_2 offspring. While serum IgM was detectable in F_2 pups from zinc-deficient dams at 6 weeks of age, its concentration remained well below that of control F_2 progeny. By 6 months, however, there was no significant difference in serum IgM concentrations between control and zinc-deprived F_2 progeny. In F_3 pups, serum IgM concentrations were lower than in the controls at 6 weeks of age, but were similar to control values by 10 weeks. Cross-fostering of zinc-deprived F_1 offspring to control dams promoted a more rapid development of serum IgM in F_2 and F_3 progeny, with normal levels being achieved by 10 weeks of age. Cross-fostering of control F_1 pups to zinc-deprived dams had virtually no impact on F_2 and F_3 offspring.

The F_1 offspring of zinc-deficient dams showed a markedly lower PFC response to SRBC immunization at 6 weeks than did controls fed ad libitum (Fig. 1B). Pair-fed control pups also showed a lower total PFC response to SRBC's than did the other controls, but there was no significant between-group difference in plaques formed per 10^6 splenic white blood cells. Cross-fostering of the deficient pups to control dams did little to alter the deficient PFC response at 6 weeks of age. Control pups nursed by dams deprived of zinc during pregnancy also showed a depressed PFC response to SRBC's, although it was not as low as that of zinc-deprived offspring.

The F_2 offspring of zinc-deprived mice continued to demonstrate a profoundly depressed response to SRBC inoculation. The F_2 offspring of pair-fed controls also had a lower total PFC response to SRBC, but, once again, there was no between-group difference in plaques per 10^6 white blood cells. As with the F_1 offspring, cross-fostering did little to alter the immunodeficiency observed in F_2 pups of zinc-deprived dams. In contrast, F_2 offspring of control pups cross-fostered to zinc-deficient dams showed a normal response to SRBC inoculation. The F_3 offspring of zinc-deprived dams continued to manifest a deficient PFC response to SRBC's, although it was not as depressed as that of the F_1 and F_2 offspring. Therefore, while the degree of immunodeficiency produced by zinc deprivation during the latter two-thirds of gestation was partially ameliorated over generations of adequate nutriture, it persisted to a significant extent into F_3 offspring.

An animal's ability to recognize and respond to thymus-dependent antigens, such as heterologous erythrocytes, seems to be especially vulnerable to nutritional deficits (*9, 11, 15*). The depressed PFC response in zinc-deprived animals could be due to faulty B cell mutation, defective T cell–mediated guidance of B cell maturation, inability to recognize and bind the antigen because of low affinity, inability to respond to the antigen once recognition has occurred, or defective function of an accessory cell type required for antigen recognition. In mice postnatally deprived of zinc, the B cell response to mitogens is modestly reduced (*10*), perhaps indicating an immaturity of B lymphocytes or an inability of those cells to respond to antigens. In addition, zinc concentration affects the mobility of surface immunoglobulins in vitro (*16*); this may alter the ability of the B cell to patch, cap, and subsequently synthesize and secrete immunoglobulins. Finally, defective placentation may allow maternal IgM to cross the placenta, a process known to inhibit development of the apparatus necessary for IgM synthesis (*17*).

Production and secretion of IgM seem particularly affected by nutrient deficiency (*18*). In humans deprived of protein early in life there is prolonged depression of serum immunoglobulin levels and of

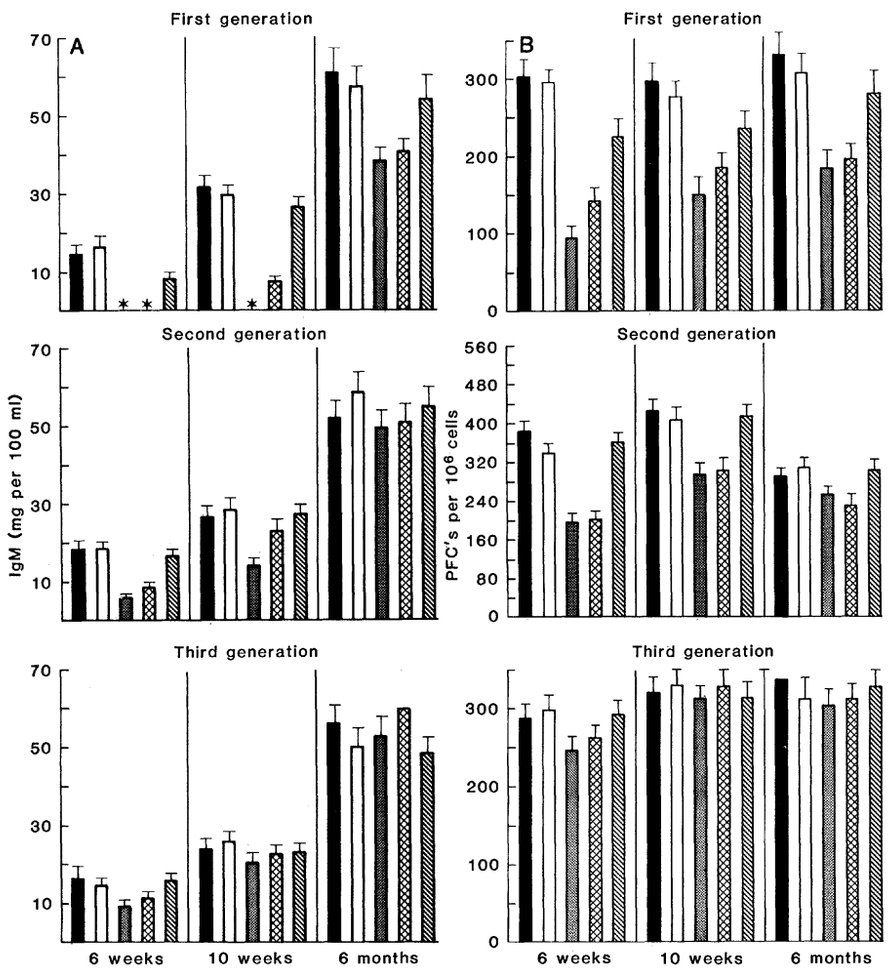

Fig. 1. (A) Concentrations of IgM in serum in F_1, F_2, and F_3 offspring of mice fed a diet moderately deficient in zinc during the last two-thirds of pregnancy and in controls, as determined by radial immunodiffusion. Solid bars represent control offspring of dams fed the 100 ppm zinc diet ad libitum; open bars, offspring of pair-fed controls; shaded bars, non-cross-fostered offspring of dams given the zinc-deficient diet; cross-hatched bars, offspring of zinc-deprived dams, cross-fostered to dams fed the 100 ppm zinc throughout pregnancy; and hatched bars, offspring of control dams fed ad libitum, cross-fostered to dams given the zinc-deficient diet. Asterisks indicate no IgM detectable. (B) Plaque-forming cell response to sheep erythrocytes in the F_1, F_2, and F_3 offspring. Response was determined 4 days after inoculation with 2×10^8 sheep red cells.

overall immunocompetence (*19*). Children who experienced protein malnutrition as early infants show deficient serum IgM production even after years of rehabilitation (*20*). The timing of the nutritional deficits—that is, whether malnutrition commenced prenatally or postnatally—plays a significant role in determining the severity and reversibility of the resulting immunodeficiency.

Our most notable observation, then, was the persistence of immunodeficiency in F_1, F_2, and F_3 offspring despite restitution with a diet adequate in all nutrients. Caloric restriction has been reported to have a similar impact on the immunocompetence of F_1 and F_2 progeny (*11*). A similar phenomenon was observed when dietary protein restriction altered brain DNA content and behavior and learning performance in F_2 progeny (*21*). The mechanism whereby zinc or other nutrients influence immune ontogeny in subsequent generations remains obscure. Germ cells obtained from zinc-deprived animals might be studied in vitro to identify the cause of these developmental defects.

This study has important implications for public health and human welfare, as the consequences of fetal impoverishment may persist despite generations of nutritional supplementation. Dietary supplementation beyond the levels considered adequate might allow for more rapid or complete restoration of immunocompetence.

RICHARD S. BEACH
Department of Internal Medicine, Division of Rheumatology/Clinical Immunology, and *Department of Nutrition, University of California, Davis 95616*

M. ERIC GERSHWIN
Department of Internal Medicine, Division of Rheumatology/Clinical Immunology, University of California

LUCILLE S. HURLEY
Department of Nutrition, University of California

References and Notes

1. R. M. Suskind, *Malnutrition and the Immune Response* (Raven, New York, 1977).
2. C. G. Neumann, G. J. Lawlor, E. R. Stiehm, M. E. Swendseid, C. Newton, J. Herbert, A. J. Ammann, M. Jacob, *Am. J. Clin. Nutr.* **28**, 89 (1975).
3. P. J. Fraker, S. M. Haas, R. W. Leucke, *J. Nutr.* **107**, 1889 (1977); G. Fernandes, M. Nair, K. Onoe, T. Tanaka, R. Floyd, R. A. Good, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 457 (1979).
4. T. Iwata, G. S. Incefy, T. Tanaka, G. Fernandes, C. J. Menendez-Botet, K. Pih, R. A. Good, *Cell. Immunol.* **47**, 100 (1979).
5. R. S. Pekarek, A. M. Hoagland, M. C. Powanda, *Nutr. Rep. Int.* **16**, 267 (1977).
6. R. S. Beach, M. E. Gershwin, L. S. Hurley, *Cancer Res.* **41**, 552 (1981).
7. ———, *J. Immunol.* **126**, 1999 (1981); *ibid.* **128**, 308 (1982).
8. M. H. N. Golden, B. E. Golden, P. S. E. G. Harland, A. A. Jackson, *Lancet* **1978-I**, 1226 (1978); R. S. Pekarek, H. H. Sandstead, R. A. Jacob, D. F. Barcome, *Am. J. Clin. Nutr.* **32**, 1466 (1979).
9. R. S. Beach, M. E. Gershwin, L. S. Hurley, *Dev. Comp. Immunol.* **3**, 725 (1979); R. S. Beach, M. E. Gershwin, R. K. Makishima, L. S. Hurley, *J. Nutr.* **110**, 805 (1980).
10. J. K. Chesters and M. Will, *Br. J. Nutr.* **30**, 555 (1973).
11. R. K. Chandra, *Science* **190**, 289 (1975).
12. P. B. Mutch and L. S. Hurley, *Am. J. Physiol.* **238**, E26 (1980).
13. R. S. Beach, M. E. Gershwin, L. S. Hurley, *J. Nutr.* **112**, 1169 (1982).
14. I. E. Dreosti, S. Tao, L. S. Hurley, *Proc. Soc. Exp. Biol. Med.* **127**, 169 (1968).
15. S. D. Davis, *Nature (London)* **251**, 548 (1974); B. M. Gebhardt and P. M. Newberne, *Immunology* **26**, 489 (1974); J. R. Prohaska and O. A. Lukasewycz, *Science* **213**, 559 (1981).
16. B. Maro and M. Bornens, *FEBS Lett.* **97**, 116 (1979).
17. J. B. Solomon, G. S. Riddell, D. F. Whyte, *Immunology* **22**, 219 (1972).
18. M. A. Kenney, C. E. Roderuck, L. Arnrich, F. Piedad, *J. Nutr.* **95**, 173 (1968); M. Mathur, W. Ramalingaswami, M. G. Deo, *ibid.* **102**, 841 (1972).
19. G. H. Aref, M. K. Badr El Din, A. I. Hassan, I. I. Araby, *J. Trop. Med. Hyg.* **73**, 186 (1970).
20. Z. Spirer, G. Hauzer, V. Zakuth, S. Diamant, A. Golander, *J. Clin. Lab. Immunol.* **6**, 111 (1981).
21. S. Zamenhof, E. Van Marthens, L. Grauel, *Science* **172**, 850 (1971); D. E. Bressler, G. Ellison, S. Zamenhof, *Dev. Psych. Biol.* **8**, 315 (1975).
22. We thank K. Makishima, K. Hernandez, and T. Rundgreu for their assistance. This work was supported by grant HO 14388 from the Public Health Service and by the Association for Children with Learning Disabilities.

10 May 1982; revised 28 July 1982

Surface Structures Involved in Target Recognition by Human Cytotoxic T Lymphocytes

Abstract. *Cloned human cytotoxic T lymphocytes and monoclonal antibodies inhibiting their function (anti-$T3_A$, anti-$T4_A$, and anti-$T8_A$) were used to elucidate the role of T cell surface glycoproteins in cell-mediated lympholysis involving individual classes of gene products of the major histocompatibility complex on target cells. The results indicate that several surface molecules are required for specific target recognition: T3 and T4 on T4+ cytotoxic T lymphocytes and T3 and T8 on T8+ cytotoxic T lymphocytes.*

Cell-mediated lympholysis (CML) is a process whereby T lymphocytes specifically destroy target cells to which they have been sensitized. This mechanism appears to be of importance in allograft rejection, tumor destruction, and lysis of syngeneic cells infected by virus. However, despite recent advances in understanding physiologic requirements and cellular processes of this effector function, the molecular mechnisms involved in target cell recognition and lysis are ill defined (*1*).

In man, cytotoxic effector cells are derived from either of the two major T cell subpopulations. These have been termed T4+ or T8+ on the basis of their uniquely expressed 62,000-dalton (62KD) (T4) and 76KD (T8) glycoproteins (*2–4*). More important, the target antigens recognized by individual subsets are the product of different gene regions of the major histocompatability complex (MHC). Thus, allosensitized T4+ T cells kill target cells bearing class II MHC antigens whereas T8+ T cells kill targets expressing class I MHC antigens (*4*). Similar observations regarding differences in cytotoxic T lymphocyte (CTL) specificity of individual T cell subsets have been observed in the murine system with the homologous Lyt2− and Lyt2+ populations (*5*).

This association between the surface phenotype (that is, surface glycoproteins) of CTL and the class of MHC molecules recognized implies that subset-restricted structures may be required to facilitate selective lysis of different target antigens. This view has been supported by recent findings showing that monoclonal antibodies to the T4 or T8 glycoproteins selectively inhibit cytotoxic effector function of T4+ or T8+ CTL clones (*4*). In addition, a 20KD T cell surface molecule, T3, present on all mature T lymphocytes, participates in cell-mediated lympholysis: antibodies to this structure block killing by both T4+ and T8+ CTL clones.

Whether such surface molecules serve as recognition elements, or alternatively, represent components of the lytic mechanism is unknown. To address this question, we utilized cloned populations of T4+ and T8+ CTL and examined the ability of monoclonal antibodies to the surface structures (T3, T4, and T8) to influence killing under various experimental conditions. Because appropriate concentrations of lectin can induce approximation of CTL and target cells in the absence of antigen recognition (*6*), it is possible to assess the intrinsic killing capacity of CTL clones even in the presence of monoclonal antibodies that inhibit cytolytic function. We reasoned that if the antibodies bind to a surface structure related to the lytic mechanism itself, then artificial approximation should not be capable of reconstituting effective lysis. However, if these anti-

0036-8075/82/1029-0471$01.00/0